PROGESTERONE RELATED CELLULAR CHANGE IN THE UTERINE CERVIX

WITH PARTICULAR REFERENCE TO PROGESTERONE-ONLY CONTRACEPTIVES

BY

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ABSTRACT

This study examines the effect of progesterone-only injectable contraceptives, and medroxyprogesterone acetate (Depo-Provera) in particular, on the cells of the uterine cervix.

Cervical and vaginal smears were taken before commencement of therapy and at 3 and 6 month intervals thereafter on 79 asymptomatic women attending a family planning clinic. Results of hormonal and cellular measurements before and after therapy were compared. The effect on menstrual cycling was also studied.

Methods used were hormonal maturation indices, image analysis measurements and microscopic observation of cellular features. The latter included anisocytosis, anisokaryosis, karyomegaly, plaque formation, cytoplasmic wrinkling, nuclear grooving, hypertrophy, atrophy, cytoplasmic moulding and density, retarded maturation and nuclear protrusions. Squamous, endocervical and metaplastic cells were examined.

Analysis of the results showed that progesterone-only contraceptives produce all of the above to a greater or lesser degree resulting in an increased relative nuclear area which may be confused with intraepithelial neoplasia. This is due to the production of a folate deficiency at target organ level which interferes with cell division and slows the maturation process. This effect enabled further observations to be made leading to the establishment of the origin and content of the nipple-like protrusions which occur in endocervical cells in response to hormonal activity.

Physiological effects included amenorrhoea and irregular menstrual cycling. Most women showed evidence of interference with normal cycling to a varying degree.

The documented cellular changes were shown to modify the expression of common inflammatory and neoplastic conditions of the uterine cervix. These included trichomoniasis, herpesvirus cervicitis, human papillomavirus infection, folate deficiency, cervical intraepithelial neoplasia and invasive carcinoma as well as multiple pathologies. The potential for diagnostic error was examined.

New diagnostic criteria were formulated based on the comparison of cellular features found in the presence of the contraceptive with those found under normal conditions. It is anticipated that these criteria will facilitate the cytological diagnosis of pathological conditions of the uterine cervix in users of depo-medroxyprogesterone acetate (DMPA), leading to increased accuracy and improved and better directed patient management.

In this research the statistical planning and analyses, and recommendations arising from these analyses, have been done with the support of the Institute for Biostatistics of the Medical Research Council.

PREFACE

This study represents original work by the author and has not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

The research described in this thesis was carried out in the Department of Anatomical Pathology, Cytology Unit, University of Natal Medical School, under supervision of Dr. V. Chrystal.

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CHAPTER 1

INTRODUCTION

1.1 MOTIVATION

1.1.1 Necessity for diagnostic criteria

The benefit of diagnostic cytology to the clinician and patient lies principally in the rapid identification of disease processes with the concomitant prompt institution of appropriate therapy. In order to achieve this, it is essential that diagnostic criteria be defined as accurately as possible in order to set definitive boundaries to the cellular changes encountered.

This permits accurate and consistent reporting of a variety of conditions with standardization of results on a national and international basis. The degree of reproducibility achieved, facilitates the maintenance of standards as well as simplifying the teaching of students.

Increasing diagnostic efficiency reduces the need for elaborate quality control measures and improves inter-disciplinary correlation i.e. histopathology and cytology, with a consequent positive effect on confidence levels. Implications for patient management are clear, in that by creating more reliance on the accuracy of cytology reports, better utilization of resources in terms of time, finance, staff and facilities may be achieved.

While the broader criteria for benign pre-malignant and malignant change may be undisputed, the evolution of new disease forms and the introduction of improved or novel drugs, requires an on-going re-adjustment of these boundaries to improve the sensitivity and specificity of the diagnostic criteria involved.

This study is concerned with the identification of cellular changes associated with the use of progesterone-only contraceptives and in particular with the injectable contraceptive, medroxyprogesterone acetate (MPA) marketed by Upjohn under the name Depo-Provera 150 (DMPA). It is used extensively in the Republic of South Africa and the Southern

African region particularly in the lower socio-economic strata.

In this Cytology Unit alone, ± 60 000 Pap smears are received annually from women attending Family Planning Clinics throughout the Natal region, approximately two-thirds of whom are receiving DMPA. It therefore warrants investigation as a drug having the potential for considerable influence on the cells of the female genital tract.

1.2 NATURE OF STUDY

Since endogenous progesterone has a powerful influence on the maturation and differentiation of a variety of cell types it was postulated that regular doses of exogenous progesterone might cause distinctive and identifiable changes, particularly in the squamous epithelium, in both its normal state and in pathological conditions. It was therefore decided to undertake a prospective study of the cytological effects brought about by long term progesterone-only contraceptive therapy and Depo-Provera in particular.

In order to provide a comparative basis for the assessment of cellular changes observed in the squamous and columnar cells of the vagina and endocervix in the presence of DMPA, normal values were established for each subject by examining vaginal and cervical smears taken prior to therapy. These were then compared with smears taken at intervals during use of the contraceptive. Considerable assistance was received from the staff of the Family Planning Clinics with the organisation of this part of the project.

A selection of cell types from squamous and columnar epithelium were assessed and the values obtained were compared with accepted norms and with the norms of individual subjects.

1.3 OBJECTIVES

There were two main objectives.

1.3.1 Normal range/values

The first objective was to establish the existence of a normal range of cellular change which could be directly attributed to DMPA.

1.3.2 DMPA related changes in abnormal conditions

The second objective was the identification of conditions of altered cellular behaviour in the presence of DMPA in abnormal conditions, with a view to demonstrating the modification of the cellular expression of such conditions by a strong progesterone effect.

Included in both objectives was the necessity for differentiating the progesterone effect from pre-malignant change.

A secondary aim was to determine, if possible, the length of time such changes persist and whether or not they are reversible.

CHAPTER 2

REVIEW OF LITERATURE

2.1 HISTORICAL REVIEW

Contraception is not a new idea. Aristotle in the fourth century B.C. recorded the practice of his times and Soranus in the second century A.D. wrote at length on contraceptive technique and theory. However, this knowledge was suppressed by generations subjected to puritanical domination with the result that active birth control measures and education have only been a reality since 1916 (Potts and Diggory 1983).

Long-acting progestogens were first synthesised in the 1950's. Medroxyprogesterone acetate was first tested as a contraceptive in the early 1960's and by the 1980's was being employed as a contraceptive in 80 developed and developing countries including the Republic of South Africa.

2.2 INTERNATIONAL REACTION TO DMPA

Although DMPA was rapidly adopted world-wide as a contraceptive measure, considerable controversy continued to surround this preparation regarding possible undesirable side-effects, such as an increase in breast and cervical carcinoma (Finkel and Berliner 1973, McDaniel and Pardthiasong 1973, Powell and Seymour 1971). In March 1978 the United States Food and Drug Adminstration (FDA) denied a request to market DMPA for contraception in the USA (Edelman 1979) on the grounds that there were unresolved questions pertaining to the safety of the drug.

As demonstrated in the Proceedings of an International Forum on DPMA in 1979, many of the problems related to the F.D.A's reluctance to approve the drug arose from the equivocal findings of poorly controlled studies, often with inappropriate animal models (Drill 1976). In an update on DMPA Schwallie (1984) gives a summary of the Public Board of Enquiry hearing held in January 1983 in Washington D.C. in which he reveals dissension and disagreement on the acceptability of this contraceptive, with considerable criticism of most surveys performed. Major defects in this respect appear to have been the presence of numerous co-variables and the absence of base-line studies.

In the course of this enquiry, fifty-one expert witnesses testified for the FDA Bureau of Drugs (USA) and the Upjohn Company, the manufacturers. Non-party participants were drawn from the World Health Organisation, the International Planned Parenthood Federation, the United States Agency for International Development, Family Health International, American College of Obstetricians and Gynaecologists Health Research Group, National Women's Health Network and eminent gynaecologists from prominent universities.

During this hearing the only mention of possible cytological effects was brief and inconclusive. References were made to possible DMPA stimulated mammary hyperplasia, a controversial adenocarcinoma in a female primate and an apparent absence of carcinogenicity demonstrated in a comparative study between a large group of women on oral contraceptives and a relatively small study of women on DMPA. There was criticism of poorly controlled investigative procedures and the persistent controversy surrounding this preparation was not resolved. Wied (1983) a participant in the hearing subsequently noted the importance of establishing base-line values for studies investigating the cytological influence of steroid hormone contraceptives.

2.3 REVIEW ARTICLES AND STUDIES PERFORMED

The first comprehensive review of DMPA was performed by Nash (1975) who concentrated on the use-effectiveness, acceptability and common side-effects of the drug. In a section dealing with possible carcinogenic potential there is criticism of the studies performed up until then with regard to the lack of matched controls and the failure to take into account factors such as the age of the group surveyed, age of first sexual experience and socio-economic factors. Nash also draws attention to the lack of scientific support for reports of increased carcinoma in situ in DMPA users, noting that some of these cases had been recorded during the first year of the study and that such findings would be inconsistent with the accepted pattern of pre-invasive malignant disease.

Interpretation of the results of the 20 studies analysed in this review is complicated by the fact that in eight of these, the women participating in the study were given oestrogen in varying amounts while they were receiving DMPA.

In 1978 a World Health Organisation (WHO) Scientific Group compiled a report (WHO Technical Report Series, No 619) which presented the views of an international group of experts on steroid contraception and the risk of neoplasia. The report focused principally on the clinical effects of contraceptives, including those containing progestogens only, and although reports of abnormal cytology are cited, no mention is made of specific cellular changes even though at least two members of this group were closely associated with diagnostic cytology units.

The group concluded that the methods used in the studies reviewed had serous limitations in terms of reliability, mainly due to insufficient attention being paid to confounding variable factors. In their conclusion it was recommended that properly designed studies be undertaken as soon as possible in order to estimate the risk of neoplasia to users of DMPA.

The failure of this group to recommend the identification of all cellular changes which might be related to progestogen therapy and thereby clarify the situation for further research, reflects the general lack of coordination in the approach to the problem as a whole. It also illustrates the relative obscurity of cytology at that time and the lack of appreciation of its potential as a diagnostic and research tool.

A review of injectable contraceptives by Fraser and Weisberg (1981) with special emphasis on DMPA, repeated the criticisms of the previous two reviews and failed to provide any further information on possible cytological effects. For this review over 800 references were consulted of which 381 are cited. The authors note that ethnic differences may influence drug effects and extrapolations from one race to another should be treated with caution. In this connection it is important to differentiate ethnic from socioeconomic factors which have also been identified as a potential source of error in study design and data interpretation.

In a memorandum published by WHO in October 1982, representatives of drug regulatory authorities, a toxicology review panel, the pharmaceutical industry and the steering committee of the WHO task force on long-acting agents for fertility regulation presented their findings on injectable hormonal contraceptives (Diczfalusy 1982). The consensus was that more research was required on the effects and physiological

consequences of long-term use of such drugs on carbohydrate and lipid metabolism as well as an examination of the risk of neoplasia among users.

Reference was once again made to the unsatisfactory studies performed using beagle (Briggs 1980), mouse (Reboud 1977) and primate subjects (Berliner 1974) with criticisms based on the unsuitability of the selected animal models, the inconclusiveness of results due to the premature death of primates from other causes and the use of drug doses 50 times higher than that used in humans. It was noted that the endometrial carcinomas occurring in two monkeys arose in an atrophic endometrium from a cell type not found in women and that the death rate in the control animals was higher than in those receiving the drug. It was also the opinion of this group that the studies conducted to examine the risk of cervical neoplasia had suffered from a variety of methodological problems.

This memorandum not only confirms the findings of the previous WHO report group but has very little additional material to contribute in terms of current research, with most of the significant references being a repetition of those found in the preceding review articles. On the subject of cellular change the report has nothing to add to the previous conclusions and makes no specific recommendations in this area.

In view of the recurrent theme of the unreliability of data in all assessments of studies performed on DMPA it is not suprising that a report of an international forum on DMPA (Edelman et al. 1979) should be sub-titled 'A continuing controversy'.

2.4 REFERENCES TO CYTOLOGICAL EFFECTS

Although over 800 scientific references to DMPA exist (Potts and Diggory 1983) surprisingly few of these deal with the cytological effects of this contraceptive, concentrating mainly on its physiological effects, potential carcinogenicity and its role in the treatment of adenocarcinoma of the breast and uterus (Tseng 1975, Gurpide 1977).

Several studies, as already noted, have investigated the incidence of cervical dysplasia in women receiving this drug but the results of most are equivocal due to unsatisfactory study controls and insufficient

cognisance of such factors as inappropriate animal models, differing levels of potency between progestogens (Phillips et al. 1987) and socio-economic conditions. Moyes (1962) noted a disparity in gland and stromal development in the endometrium after treatment with norethisterone acetate, a progestogen similar in action to DMPA, with progestational hyperplasia after long term therapy.

Powell and Seymour (1971) comment on the effect of DMPA treatment on cervical cytology and histology but do not describe specific cytologic changes and their findings are inconclusive. Dabancens et al. (1974) looked for evidence of increased cellular dysplasia but concluded that no significant difference could be found between women on DMPA and those on other forms of contraception.

The first suggestion that cellular changes observed in women on contraceptive therapy might be other than pre-malignant, came from Lindenbaum et al. (1975), who suggested that some of the 'dysplasia' observed might be attributable to megaloblastic change arising from a folate deficiency occurring as an end organ reaction. A later investigation of this hypothesis provided supportive evidence with a suggested mode of action by DMPA (McCallum 1982).

Nash, in his review of DMPA in 1975, maintained that there was no conclusive evidence that Depo Provera altered the normal incidence of cervical atypia either upward or downward. In 1983 Wied in his testimony to the American Food and Drug Administration hearing on Depo Provera emphasized the problem identified by Nash of objective interpretation of cervical cellular atypia and the necessity for establishing base-line values (Schwallie 1984). Drill (1976) in his examination of the effect of oestrogens and progestins of the cervix uteri, states that although the histologic changes produced by estrogens, progesterone, pregnancy and oral contraceptives are benign, they may at times resemble malignant change and be confused with cervical intraepithelial neoplasia.

Maqueo et al. (1966) undertook a histological survey of the cervix in woman exposed to high dosage progestins and recorded hyperplasia of the cervical glands similar to that found in pregnancy as well as frequent stromal oedema and squamous metaplasia occasionally accompanied by pseudo-decidual transformation. Only orally administered progestins

were used. No cytological comment was made. A later study of the effect of progestational compounds on the mouse cervix by Reboud (1977), confirmed the production of endocervical hyperplasia and hypersecretion similar to that found in women but did not include DMPA and did not specifically analyse cytological change.

Participants in an international forum on DMPA (Rall and van Niekerk 1979) noted that it had been found to have an inhibitory effect on tumour development and it was suggested that it might have a protective effect on the cervix uteri in this respect.

A study reporting dysplastic changes which reverted to normal after discontinuation of therapy (Engineer et al. 1980) again raised the question of interpretation of cellular atypia and must be regarded as inconclusive. Two other investigative reports on DMPA (Liang et al. 1983, Rosenfield et al. 1983) fail to mention cytological or histological changes in the cervix uteri but both recommended further studies.

Dallenbach-Hellweg later (1984 a) states conclusively that synthetic progestogens act differently on endometrial, endocervical and mammary glands. This author further claims that these drugs stimulate proliferation (mitoses) in endocervical and mammary glands, the proliferative effect increasing with their potency and duration of use, but suppress mitotic rates in endometrial glands.

In another paper (1984 b) the same author links the use of high potency progestogens to the development of endocervical adenocarcinoma although DMPA was not amongst the drugs used, all of which were administered orally. Phillips et al. (1987) comment on an apparent link between breast carcinoma and the use of oral contraceptives containing high potency progestogens, pointing out that the selected route of administration and target organ selectivity produce marked potency variation. Further emphasis is placed on the necessity for an improved understanding and appreciation of the differences between and activities of specific progestogens.

The situation current at the inception of this study was, therefore, such that considerable scope still existed for a thorough exploration of the cytological changes produced by DMPA in the uterine cervix.

CHAPTER 3

NORMAL CELLS OF THE VAGINA AND UTERINE CERVIX

The following cells referred to in this study are of epithelial origin and are derived from the stratified squamous epithelium of the vagina and ectocervix and the columnar epithelium lining the endocervical canal.

3.1 SQUAMOUS EPITHELIAL CELLS

3.1.1 Superficial cell

A relatively large ($\pm 1500 \mu m^2$) polygonal cell, with a small, central, dense, pyknotic nucleus (PLATE 1). The cytoplasm which is thin, flat and relatively transparent, stains eosinophilic or cyanophilic depending on maturity (Patten 1978).

3.1.2 Intermediate cell

A large polygonal cell similar to the superficial cell but having a vesicular nucleus which contains structures such as chromocentres and the sex/chromatin (Barr) body. The cytoplasm is thin and relatively transparent depending on maturity. Staining reaction is predominantly cyanophilic but may be eosinophilic (PLATE 1). In pregnancy, early intermediate or navicular cells may have nuclei displaced from the usual central location (Patten 1978).

For purposes of this study intermediate cells were divided into 2 categories, mature and immature, the former complying with the classic description and the latter similar but possessing a larger nucleus (PLATE 2).

3.1.3 Parabasal cells

Small oval or round immature squamous epithelial cells with a relatively large vesicular nucleus and a larger relative nuclear area than the intermediate cell (PLATE 3). The cytoplasm is dense and usually stains cyanophilic (Patten 1978).

PLATE 1 - upper

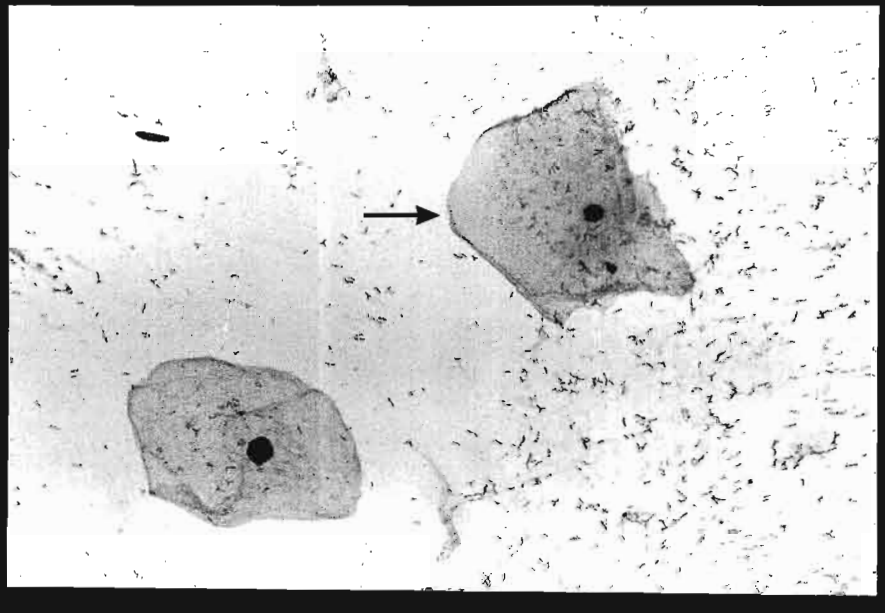
Mature superficial squamous epithelial cell (arrow) and an intermediate squamous cell with a vesicular nucleus (x100).

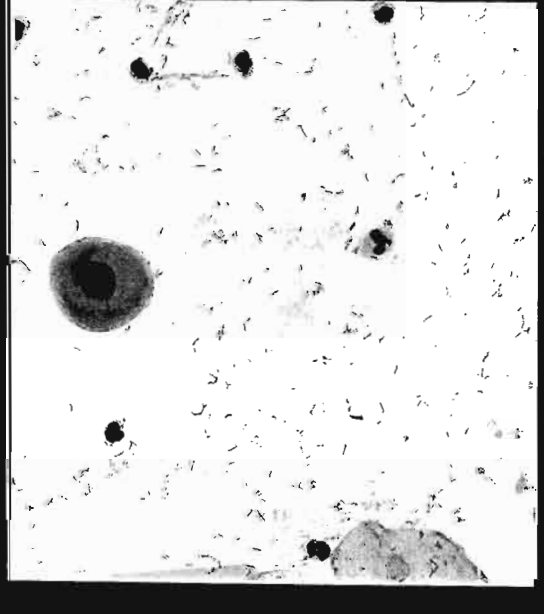
PLATE 2 - lower left

Immature intermediate squamous cells in pregnancy demonstrating cytomegaly (x100).

PLATE 3 - lower right

Parabasal squamous epithelial cell with dense cytoplasm and centrally placed vesicular nucleus (x400).





3.2 COLUMNAR EPITHELIAL CELLS

3.2.1 Endocervical cells

Derived from mucin secreting columnar epithelium lining the endocervical canal, these cells are found in sheets, strips or lying singly (PLATE 4). They may be elongated with an eccentric nucleus or round to oval with a central nucleus (PLATE 5). The cytoplasm varies from diffusely vacuolated to granular.

Cilia may be found arising from a terminal plate on well preserved cells, as well as one or more eosinophilic micronucleoli, depending on the biologic activity of the tissue. A protrusion of nuclear contents referred to as a "nuclear nipple" (PLATE 6), may occur at one nuclear pole (Reagan 1973).

3.3 METAPLASTIC CELLS

The origin of metaplastic cells is still subject to debate. They are found at the squamo-columnar junction in 8 out of 10 women of reproductive age. Fluhman (1961) states that these cells are of epithelial origin and arise above the basement membrane directly from columnar cells, while Patten (1978) maintains that evidence is lacking to exclude their origin from stromal cells.

The small basal or reserve cells of the endocervical epithelium have the potential of differentiating into either mucin-secreting endocervical cells or into squamous cells. Metaplastic cells are commonly accepted as arising from this basal cell layer and are not mucin-secreting (Koss 1979) but form increasingly mature squamous cells which replace the overlying endocervical epithelium.

The cells of relevance to this study arise from immature squamous metaplasia. These cells are small with a mean cellular area of $544\mu m^2$ (Reagan 1958) and round or oval to polygonal in shape depending on maturity. The cytoplasm is commonly dense and cyanophilic. The nuclei occupy a relatively large nuclear area, are round or oval and generally centrally situated. Nucleoli are not present (PLATE 7).

PLATE 4

Sheets and strips of endocervical columnar cells (x100).

PLATE 5

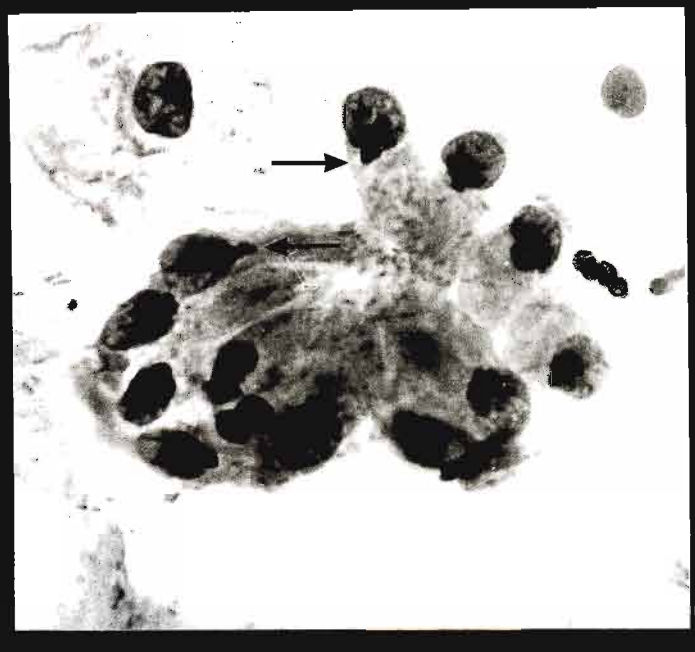
A sheet of endocervical cells in 'honey-comb' arrangement (x100).

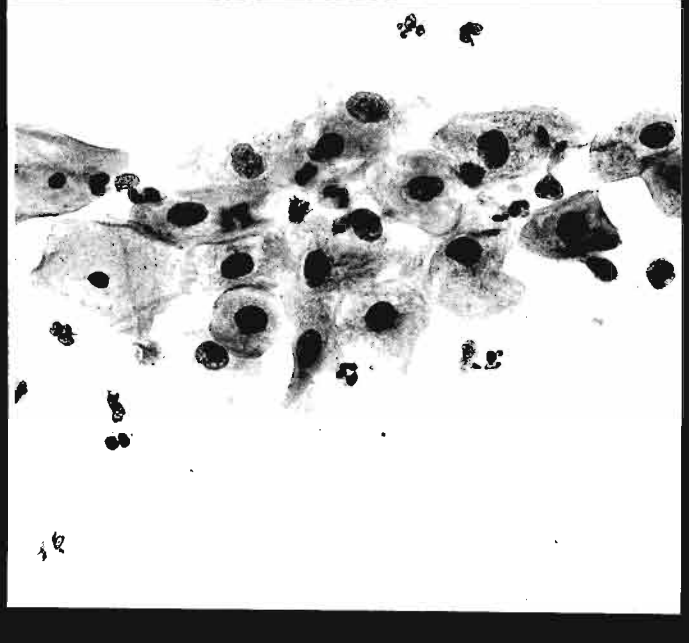
PLATE 6

An acinar arrangement of normal endocervical cells displaying nuclear protrusions or 'nippling' (arrowed) (x400).

PLATE 7

Normal squamous metaplastic cells with moderately dense cytoplasm and centrally placed nuclei (x100).





CHAPTER 4

PHYSIOLOGICAL AND CYTOLOGICAL INFLUENCE AND ACTION OF ENDOGENOUS PROGESTERONE

4.1 ORIGIN

The sex steroid hormone progesterone is the only naturally occurring progestogen (Lewis 1980). Its main source is the corpus luteum in the ovary but it is also produced in very small quantities by the adrenal cortex (Paterson 1983). It plays a major role in the functioning of the female reproductive system. In the plasma, steroid hormones are bound to proteins and in this form are biologically inactive. The concentration of the specific binding proteins is directly influenced by the fluctuations in the quantity and type of sex steroid hormones present. Target tissue metabolism is therefore regulated in a highly specific and exclusive manner by the action of the sex steroids on protein synthesis and on the stimulation of RNA production (Chan, O'Malley 1976).

4.2 SEX STEROID ACTIVITY AND THE REPRODUCTIVE PROCESS

The menstrual cycle in the adult human female is regulated by sex steroid hormones.

In the follicular phase during which oestradiol - 17β is the major steroid produced, very little progesterone is synthesized. After ovulation, the follicle differentiates to form the corpus luteum and progesterone is the principal steroid produced at this time, with smaller amounts of 17 α - hydroxy-progesterone, androstenedione and oestradiol - 17β also being synthesized.

In response to a midcycle surge of luteinizing hormone, conversion of ovarian granulosa cells to progesterone secreting cells i.e. luteinization, begins 24 to 36 hours before ovulation (McKenna 1982) and plasma progesterone remains high for about 10 days after ovulation (Robertson 1981).

During pregnancy large amounts of steroid hormones are produced by the

placenta from precursors in maternal and fetal plasma. These are chiefly oestriol and progesterone (Paterson 1983).

The action of the progesterone is to stimulate glandular secretory activity and to produce stromal changes prior to decidua formation. It also effects the production of a dehydrogenase which converts oestrogens from the potent oestradiol to the weak oestrone and because this interferes with oestrogen activity in the cell, progesterone is therefore a potent anti-oestrogen (Robertson 1981). This has considerable significance for cellular differentiation and metabolism and consequently is a factor of importance in this study.

4.3 CELLULAR EFFECT

4.3.1. In the non-pregnant state

Although under hormonal influence the numbers of the different cell types may vary at different points in the cycle, individual cells do not display marked change. The two most striking hormonal patterns produced in the course of a normal 28 day cycle are those of maximum oestrogen activity at mid-cycle, immediately prior to ovulation, which produces an abundance of flat eosinophilic, free-lying superficial cells with pyknotic nuclei, and the luteal phase pattern in which large, cyanophilic intermediate cells with vesicular nuclei predominate.

The progesterone effect observed during this phase is typified by crowding of the cells, crumpling of the cell membrane (PLATE 8) and loss of distinct cell borders (Meisels 1983).

4.3.2 In pregnancy

In pregnancy the corpus luteum persists and by the end of the first trimester the majority of vaginal smears will display the cytolysis, intermediate cell proliferation and reduction in maturation level typical of this state, indicative of and consistent with, a strong progesterone influence (PLATE 9). The cells frequently present in plaques due to the tendency of progesterone to increase cell cohesion (PLATE 10).

PLATE 8

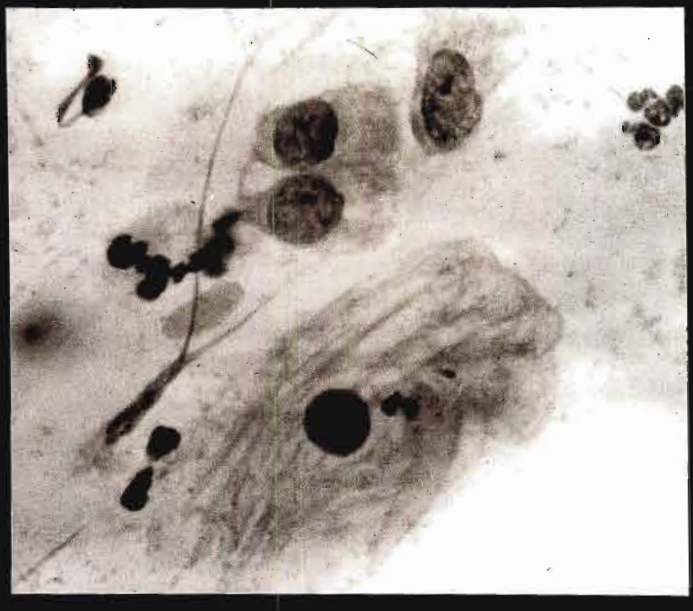
Immature intermediate cell showing the increase in cell size and cytoplasmic wrinkling associated with both the luteal phase of the menstrual cycle and pregnancy. (x400).

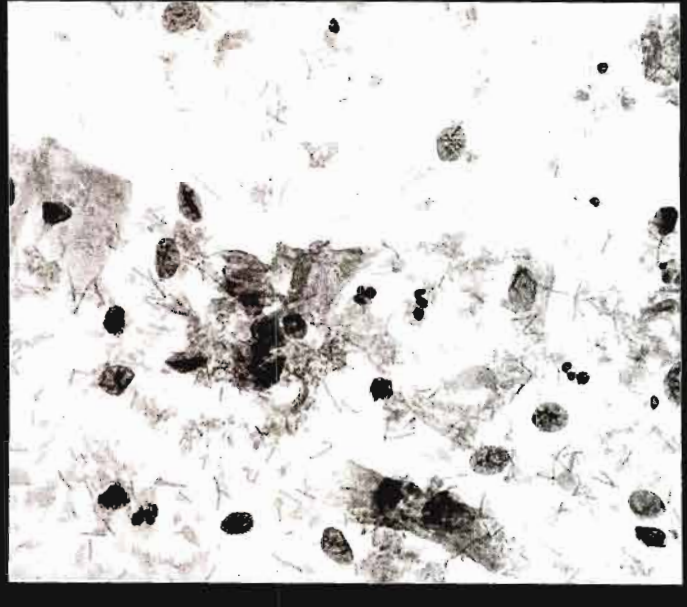
PLATE 9

Intermediate cells showing cytolysis with marked cytoplasmic disintegration (x100).

PLATE 10

Plaque formation and hypertrophic intermediate cells in pregnancy (x100).





CHAPTER 5

MATERIALS AND METHODS

5.1 SAMPLE SELECTION

5.1.1 Population studied and source of material

Material for this study was obtained in the form of vaginal and cervical smears from women of reproductive age attending family Planning Clinics in the Durban area. The smears were taken with an Ayre spatula from the upper lateral vaginal wall and from the external os of the uterine cervix, in the course of routine gynaecological examinations.
In accordance with routine established laboratory and clinic procedures, the smears were also screened for exclusion of neoplastic and other diseases.

5.1.2 Women with normal smears and menstrual cycles

Data pertaining to the menstrual cycles in these women was recorded to establish the extent of hormonal activity at the commencement of contraceptive therapy, in order to determine whether there was an alteration in menstrual cycling after therapy.

5.1.3 Women with normal smears and amenorrhoea

The majority of these women had been delivered during the previous six months. The cause of amenorrhoea was recorded and subsequent alterations in menstrual cycling were noted.

5.1.4 Women with abnormal smears

Women found to have pre-malignant lesions of the uterine cervix were excluded from that part of the study requiring pre- and post- therapy estimation of epithelial maturation levels and measurements of nuclear and cytoplasmic dimensions. Also excluded were smears showing evidence of viral infection and other conditions likely to alter radically the normal cellular parameters. These cases however were subsequently studied with a view to establishing the degree of distortion of the cytological expression of such conditions likely to be encountered in

the presence of a progesterone-only contraceptive.

5.1.5 Sampling intervals

Smears were taken at the initiation of contraceptive therapy and at intervals of three months, coinciding with subsequent injections, for three or more doses.

5.2 EXOGENOUS PROGESTERONE

5.2.1 Properties

Depo-Provera 150 is an aqueous suspension of Provera medroxyprogesterone acetate (DMPA) for intramuscular administration. It is a derivative of progesterone, freely soluble in chloroform and insoluble in water (Upjohn handbook).

Chemical name: 6 alpha methyl - 17 alpha hydroxyprogesterone acetate.

5.2.2 Metabolic effect

The use of progesterone contraceptives has been linked to a decrease in circulating levels of high density lipoprotein cholesterol and also to changes in carbohydrate metabolism (Diczfalusy 1982). Common side effects are amenorrhoea (Powell 1971, Nash 1975) and unpredictable bleeding. Hypo-estrogenicity at $\pm$50% below the pre-treatment level of oestrogen may occur, with mean serum estradiol levels being approximately equivalent to those in the early follicular phase of women with ovulating cycles (Nash 1975).

5.2.3 Contraceptive dosage and action

The exogenous progesterone received by the women in this study was chiefly in the form of the injectable contraceptive Depo-Provera. MPA is a long acting progesterone administered intramuscularly in a dose of a 150mg every 90 days. It has been documented as preventing contraception through:

Inhibition of the secretion of gonadotrophic hormones, especially the luteinizing hormone.

The alteration of secretory changes in the endometrium, thus preventing the implantation of the embryo.

Increasing the viscosity of the cervical mucous thus creating an unfavourable environment for the spermatozoa.

Unlike other progestational compounds DMPA has no androgenic or oestrogenic activity. The average duration of activity as documented by the manufacturers, is 17 weeks with a range of 13 to 41 weeks (Upjohn handbook).

5.3 STUDY DATA

5.3.1 Collection

Clinical history and information on each woman included in this study was obtained from routine cytopathology request forms completed by the clinical staff. Information submitted included the woman's age, parity, menstrual cycle details, contraceptive history and relevant clinical findings (See Appendix A).

5.3.2 Study participation identification.

All request forms submitted with smears from participating subjects were identified as such by the staff of the Family Planning Clinics. Adhesive green labels were used on all forms and patient cards to assist recognition.

5.3.3 Study data records

A card index system was used to record information transferred from the laboratory request forms. The smears were filed separately and given sequential numbers with the addition of A, B, C etc. for repeat specimens in addition to laboratory accession numbers.

5.4 VAGINAL AND CERVICAL SMEARS

5.4.1 Preparation and identification

Cellular material obtained from the vagina and cervical os was

transferred to separate glass slides and immediately fixed while wet with an aerosol cytological fixative containing polyethylene glycol. Each slide was marked with a name or clinic number.

5.4.2 Staining Method

The Papanicolaou staining technique employing Gill's progressive haematoxylin, Eosin Azure 65 and Orange G 6, was used to demonstrate nuclear and cytoplasmic detail (Gill et al 1974). Permanent preparations were made by mounting the smears with Entellan and were examined by light microscopy (See Appendix B).

5.5. CYTOLOGICAL MEASUREMENTS

5.5.1 Calculation of hormonal status by maturation index

The maturation index is a count of the parabasal, intermediate and superficial cells in a vaginal smear, which expresses the relationship between the cell types as percentages written in the form e.g. 0/90/10 which denotes an absence of parabasal cells, 90% intermediate cells and 10% superficial cells (Wied et al. 1983). According to the results obtained, a change in the level of cell maturity may be demonstrated i.e. a shift to the left denoting an increase in the number of immature cells present. Such indices are only meaningful when related to a previous index on the same patient.

Maturation indices were performed at the commencement of contraceptive therapy and compared with those obtained from subsequent smears. Each subject thus acted as her own control for this purpose.

5.5.2 Image analysis

Image analysis was performed on the vaginal smears. Cell images were visualised on a T.V. monitor and cellular parameters estimated using the VIDS II programme.

Nuclear and cellular areas were measured and the relative nuclear areas calculated in intermediate cells occurring in consecutive microscope fields. These measurements and calculations were performed on base-line smears (Wied et al. 1983) taken prior to contraceptive therapy and on

smears repeated at the time of the second or third dose. From these data, mean cell and nuclear areas were calculated.

5.6 CYTOLOGICAL ASSESSMENT

5.6.1 Light microscopy assessment of cells

Vaginal and cervical smears were examined microscopically at 100x and 400x magnification for changes and modifications of cellular components and features such as cellular arrangement, quality and structure of the cytoplasm, nuclear characteristics and staining reaction.

5.6.2 Assessment parameters

Worksheets were designed for recording the following features: anisocytosis, anisokaryosis, cytoplasmic density, plaque formation, nuclear grooving, cytoplasmic wrinkling, retarded maturation, flat sides (moulding), flat edges, increased numbers, nuclear position, metaplastic cell shape, hyperchromasia, nucleoli, hypertrophy and nuclear protrusions.

5.7 STATISTICAL METHODS

Hotelling's T^2 test was used to compare the frequency of each variable in the maturation indices (superficial squamous, mature intermediate, immature intermediate and parabasal cells), at the three different stages of the study i.e. prior to contraceptive use and three and six months thereafter.

The paired t-test was used to establish differences in other parameters at stages 1 and 2, using Barferroni's adjustment to permit comparisons between observations at all three stages.

The correlation between the occurrence of amenorrhoea at stage 3 and all other variables was achieved using the Chi-square or Fisher's exact test (in the case of small cell numbers) for categorical data, and t-tests for continuous data.

A binomial test was used to establish the significance of image analysis observations related to relative nuclear area.

CHAPTER 6

RESULTS

6.1 SAMPLE

Vaginal and cervical smears from a total of 79 women of similar socio-economic background formed the basis of the study. As it was necessary for base-line values to be established and that there should be sufficient follow-up to enable physiological and cytological changes to be assessed, a number of cases which did not fulfil all the required criteria had to be rejected. Some of the cases rejected for cytological assessment were nevertheless suitable for inclusion in the analysis of physiological change.

6.1.1 Sample cases accepted

6.1.1.1 Sample cases accepted for investigation of physiological change due to DMPA.

Of the 79 cases evaluated 45 cases were considered acceptable, having sufficient follow-up (three months or more) for an assessment to be made of the physiological effect of the contraceptive.

6.1.1.2 Sample cases accepted for cytological assessment.

A total of 35 cases was accepted as satisfying minimum criteria for cytological evaluation i.e. initial pre-treatment vaginal and cervical smears with follow-up smears at three months. Further follow-up was obtained at six months on 26 of these cases and on 12 of these at nine months (TABLE 1).

TABLE 1 Sample cases yielding follow-up smears suitable for cytological analysis

Clinic visit	Cases
1 (Initial)	79
2 (3/12)	35
3 (6/12)	26
4 (9/12)	12

6.1.2 Sample cases rejected

Forty seven cases were partly or wholly rejected from the study (TABLE II)

TABLE II Sample cases rejected from study

Reason	Number rejected
Inadequate menstrual history	2
Infection - Trichomoniasis	6
- Gardnerella vaginalis	1
- Candidiasis	1
Lost to follow-up	33
No initial vaginal smear	4
Total	47

6.2 PHYSIOLOGICAL RESULTS OF PROGESTERONE CONTRACEPTIVE ADMINISTRATION

The menstrual status of the 45 women included in the sample was examined for evidence of a change in status between that present at the initial clinic visit and that of subsequent visits.

Of the 45 women, 27 had normal regular cycles at the start of therapy, one had a normal but irregular cycle and 17 had amenorrhoea.

Nineteen of the women with regular cycles and the one with an irregular cycle developed amenorrhoea within the first year of therapy. A further 3 women of the remaining 7 in this group developed minimal menstrual flow with 'spotting' only.

Five women retained normal cycles at three months and of these, 4 were lost to further follow-up with the remaining one retaining a normal cycle. A definite change in menstrual cycling pattern was therefore demonstrated in 82% of the group.

Of the 17 women with initial amenorrhoea 15 were immediately postnatal. Three of these and 2 additional women had been given Micronovum, an oral progesterone contraceptive, prior to starting DMPA. None of these women resumed menstrual cycling during the period of the study.

There was therefore no change in menstrual cycle status in the second group.

6.3 CYTOLOGICAL MEASUREMENTS

6.3.1 Hormonal status and cell maturation

The hormonal status of individual women was established by performing a maturation index of vaginal smears before DMPA was first given and at three and six monthly intervals thereafter at the time of the second and third doses. A total of 35 cases was assessed, 34 at the first and second clinic visits, 21 at the first, second and third visits and one at the first and third visits only. 200 cells were counted on each vaginal smear taken at each clinic visit i.e. a total of 18 200 cells.

An estimation of the relative incidence of the four cell types, (superficial, mature intermediate, immature intermediate and parabasal) in each smear, enabled a comparison to be made of the cell differentiation patterns, resulting in the identification of directional maturation development.

Maturation indices for hormonal activity comparison (based on the relative prevalence of superficial, mature intermediate, immature intermediate and parabasal cells) were analysed using Hotelling's T^2 test to compare each variable, i.e. cell types, at different stages (0 with 3 months, 3 with 6 months and 0 with 6 months).

Results indicated significantly decreased levels of epithelial maturation at three months demonstrated by an increase in parabasal cells and a decrease in superficial squamous cells. At six months post therapy a maximum increase in immature intermediate cells was present with a significant increase over levels present at three months. A similar effect was observed in the incidence of mature intermediate cells.

The results confirmed an overall increase in immaturity directly associated with the administration of DMPA.

At three months 33 out of 34 cases demonstrated a decrease in maturity. The remaining case displayed a very immature cell picture at the

commencement of therapy which, while showing a 26% increase in maturity at three months and a further 48% at six months still displayed a complete absence of superficial cells (TABLE III).

TABLE III Alteration in cell maturity 3/12 after 1st dose

		Cell maturation	
Initial menstrual status	n	Increased	Decreased
Normal regular	20	-	20
Normal irregular	1	-	1
Amenorrhoea	13	1*	12

* This case had increased cell maturation but retained an immature cell maturation pattern. (See TABLES IV and V)

At six months the only other case to show an increase in maturity was a woman who had discontinued DMPA and had changed to an oestrogen/ progesterone contraceptive pill.

The results of hormonal evaluation performed at 6/12 are shown in TABLE IV.

TABLE IV Alteration in cell maturity at 6/12

		Cell maturation	
Initial menstrual status	n	Increased	Decreased
Normal regular	12	-	12
Normal irregular	1	-	1
Amenorrhoea	10	1*	9

*Same case as in TABLES III and V.

The average decrease in cell maturation in 17 women with initial normal cycles who showed post-therapy changes (irregularity, spotting, amenorrhoea) was 29,8%.
The average decrease in cell maturation in 4 women with initial normal cycles with no change in menstrual status was 22,5%.
The average decrease in cell maturation in all 13 women with initial amenorrhoea was 27,4%.

The overall decrease in cell maturation in all women using DMPA was 26,7%.

A total of 97,1% of all women on DMPA showed a decrease in cell maturation which was most marked in the presence of amenorrhoea (TABLE V).

TABLE V Changes in menstrual status after DMPA therapy and associated cell maturation activity

		Cell maturation				
Menstrual status	Change	n	% Increase	n	% Decrease	n
Normal cycle	Nil	4	-	-	22,5	4
Normal cycle	irregularity, spotting, amenorrhoea	17	-	-	29,8	17
Amenorrhoea	Nil	14	26	*1	27,4	13

*Although increased in maturity this case had a final maturation index of 55/36/9/0 indicating an overall immature cell pattern (See TABLES III and IV).

Of the 20 women with normal menstrual cycles at the inception of therapy, 14 developed significant menstrual irregularities including amenorrhoea, while 6 retained normal cycles. The difference in cell maturation initially and at three months is shown in Tables VI and VII. There was a marked decrease in superficial squamous cells and significant rise in the incidence of immature intermediate cells three months after commencement of progesterone therapy (TABLE VI).

TABLE VI Average distribution of 3 cell types in 14 women of 20 with normal initial cycles developing amenorrhoea and irregular cycles

	Clinic visit			
Cell type	1st	2nd	% Increase	% Decrease
% Superficial	19,3	12,4	-	35,7
% Mature intermediate	64,6	63,5	-	1,7
% Immature intermediate	13,5	23,8	76,3	-

In women retaining normal cycles, the most marked response was a decrease in superficial squamous cells (TABLE VII).

TABLE VII Average distribution of 3 cell types in 6 women retaining normal cycles

	Clinic visit			
Cell type	1st	2nd	%Increase	%Decrease
% Superficial	26,6	4,8	-	81,95
% Mature intermediate	60,2	69,2	15,0	-
% Immature intermediate	13,2	13,8	4,5	-

A similar study was performed on 12 women who had amenorrhoea at the start of the study, prior to the administration of MPA (TABLE VIII).

TABLE VIII Average distribution of cell types at 3/12 in 12 women with amenorrhoea prior to MPA therapy

	Clinic visit			
Cell type	1st	2nd	%Increase	%Decrease
% Superficial	20,9	12,3	-	41,0
% Mature intermediate	52,6	39,6	-	27,7
% Immature intermediate	20,7	26,4	31,5	-

Only 13,6% of cases had completely atrophic smears at the third clinic visit, compared with 5,7% at the first visit, making this a less common feature of the contraceptive therapy.

There was, however, a marked increase in the number of cases showing reduced oestrogen activity, from 22,9% at the first visit to 69.6% at the third. Supporting evidence for this was derived from the increase in immaturity reflected in the maturation indices.

All results of the investigations performed in this section confirmed the tendency of the squamous epithelium of the vagina to retain or revert to an immmature maturation pattern in the presence of the progesterone contraceptive, the majority of women responding within three months of commencing therapy.

6.3.2 Image analysis

A total of 1040 intermediate squamous cells were measured in 13 cases. Three months after commencement of therapy the average nuclear area had increased by 5%, the average cytoplasmic area reduced by 11,3% and the total cell area reduced by 8,4%. Eleven out of 13 cases showed a positive increase in relative nuclear area resulting from the slight increase in nuclear area and reduction in cytoplasm (TABLE IX).

A binomial test analysis of the measurements showed a statistically significant difference ($p = 0,011$) consistent with increased immaturity and an increase in relative nuclear area.

TABLE IX Results of image analysis of intermediate cells in 13 cases three months after commencement of therapy demonstrating percentage increase or decrease in average nuclear, cellular and relative nuclear area

	Average nuclear area		Average cell area		Relative nuclear area	
Case	Increase	Decrease	Increase	Decrease	Increase	Decrease
1	34,6	-	-	17,9	40,0	-
5	-	23,0	-	25,0	6,8	-
8	-	7,6	-	9,4	2,3	-
14	30,4	-	-	11,9	34,0	-
21	40,7	-	30,2	-	9,1	-
22	27,9	-	-	1,2	2,3	-
24	15,0	-	-	3,6	16,0	-
31	-	16,3	-	44,6	35,7	-
32	-	3,9	-	31,7	30,7	-
34	15,9	-	12,7	-	6,1	-
38	-	19,8	-	8,3	-	9,5
42	-	3,9	-	12,7	12,2	-
43	-	6,	51,8	-	-	50,0
Total	6	7	3	10	11	2

6.4 CYTOLOGICAL ASSESSMENT - MORPHOLOGY

The following features were assessed with the aim of identifying the relative prevalence of each at all stages of therapy. All observations are expressed as percentages.

6.4.1 Anisocytosis

Moderate or marked anisocytosis (PLATE 11) was present to a greater extent in all cell types at stage 2 (3 months), in intermediate cells and to a lesser extent endocervical cells at stage 3 (6 months) but not in metaplastic cells, which by this stage, had returned to approximately stage 1 levels (TABLE X).

TABLE X Percentage cases showing anisocytosis

	Stage		
Cell type	1	2	3
Intermediate	55,9	71,4	89,5
Metaplastic	41,4	70,4	37,5
Endocervical	21,4	45,9	30,8

6.4.2 Anisokaryosis

Moderate or marked anisokaryosis was present to a greater extent in all cell types at stage 2 and in nearly all intermediate cells at stage 3. It was raised but at a lower level in endocervical and metaplastic cells at this stage (TABLE XI).

TABLE XI Percentage cases showing anisokaryosis

	Stage		
Cell type	1	2	3
Intermediate	61,8	79,4	95,0
Metaplastic	24,1	57,6	35,3
Endocervical	17,9	37,5	30,8

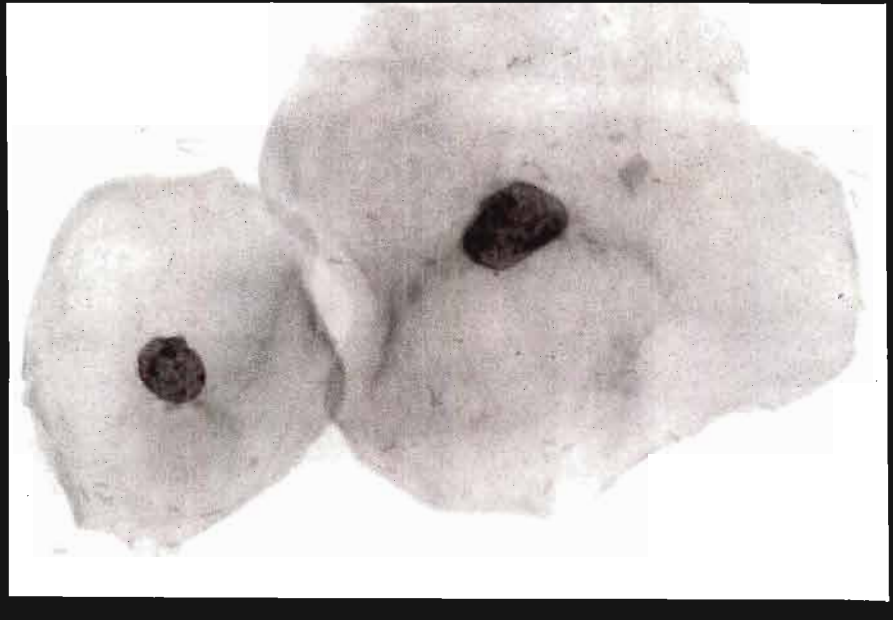
6.4.3 Cytoplasmic density

Intermediate cell cytoplasm was increased in density by 22,8% at stage 2 but was predominantly thin again at stage 3 showing a decrease of 15,7%. Metaplastic cells were predominantly dense at stage 1 and 2 but decreased in density by 39,9% at stage 3.

Endocervical cell cytoplasm was increased in density at stage 2 but decreased by 24,9% by stage 3 to a level slightly greater than stage 1.

PLATE 11

Variation in intermediate cell size as a result of progesterone therapy (x400).



The overall result was, therefore, an increase of density at stage 2 which was not maintained.
Cytoplasm of the squamous cells was predominantly basophilic in staining reaction with occasional amphophilia.

6.4.4 Plaque formation

This parameter applied only to intermediate and metaplastic cells both of which demonstrated an increase in plaque formation at stage 2 of 64,9% and 64,2% respectively which was maintained at stage 3 (PLATE 12).

6.4.5 Nuclear grooving

Maximum incidence of nuclear grooves in both intermediate and neoplastic cells was present at stage 3 (78,94% and 29,4% of cases respectively) (PLATE 13). No nuclear grooving was observed in the endocervical cells.

6.4.6 Cytoplasmic wrinkling

This parameter applied only to intermediate and metaplastic cells, the maximum incidence in both occurring at stage 2 and to a greater extent in the intermediate cells where it was associated with abundant thin cytoplasm (PLATE 12).

6.4.7 Retarded maturation

Evidence of retarded cellular maturation, in the form of dysynchronous nuclear/cytoplasmic development in metaplastic and immature intermediate cells was noticeably present at stage 2 and most marked at stage 3. Retention of columnar type nuclei by mature metaplastic cells showing squamous cytoplasmic differentiation was the most frequent finding (PLATE 14).

6.4.8 Flat sides (moulding)

With increased immaturity intermediate and metaplastic cells showed a tendency to develop thickened cell sides which, due to greater cell cohesion, produced prominent moulding between adjacent cells (PLATE 15). This was particularly evident in the metaplastic cells and most prominent at stage 2.

PLATE 12

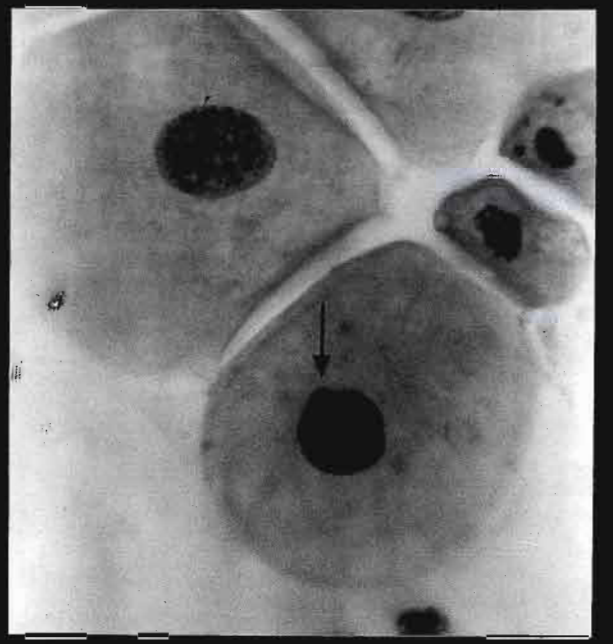
Intermediate cells displaying cytomegaly and plaque formation (x100).

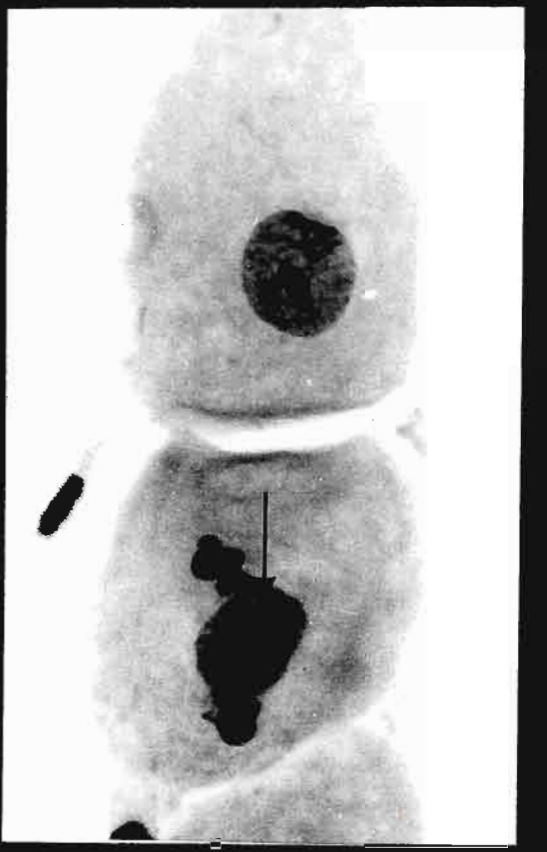
PLATE 13

Metaplastic cells displaying karyomegaly, cellular moulding, eccentric nuclei and the nuclear grooving (arrow) associated with folate deficiency (x400).

PLATE 14

Metaplastic cells displaying equivocal differentiation with one cell containing an endocervical type nucleus (arrow). Note flattened and thickened cytoplasmic borders (x400).





6.4.9 Flat edges

Due to retarded maturation, metaplastic cells were observed which retained the configuration of endocervical cells, particularly the palisade arrangement with flattened ends. This feature was most prominent at stage 2 where 88,5% of cases showed such cells and was still increased (68,7%) at stage 3 (PLATE 16).

6.4.10 Increased incidence

An increase in the overall incidence of both metaplastic and endocervical cells was noted at stage 2. At stage 3 this was still true for endocervical cells although the number of cases in which hyperplasia was observed dropped from 83,3% to 69,2%. A decreased incidence of metaplastic cells at stage 3 to stage 1 levels was balanced by an increase in immature intermediate cells.

6.4.11 Nuclear position

Immature metaplastic cells were examined in order to identify cells retaining the eccentric nuclear position and features of endocervical cell nuclei. Only five cases, all at stage 2, displayed this feature, which occurred as part of a process of equivocal differentiation. The majority of metaplastic cells at all stages had centrally placed nuclei (PLATE 15).

6.4.12 Metaplastic cell shape

The metaplastic cells at stage 1, prior to therapy, were less cohesive and more spherical in shape than those at stage 2 and 3 which displayed intercellular bridges and an increase in plaque formation.

6.4.13 Hyperchromasia

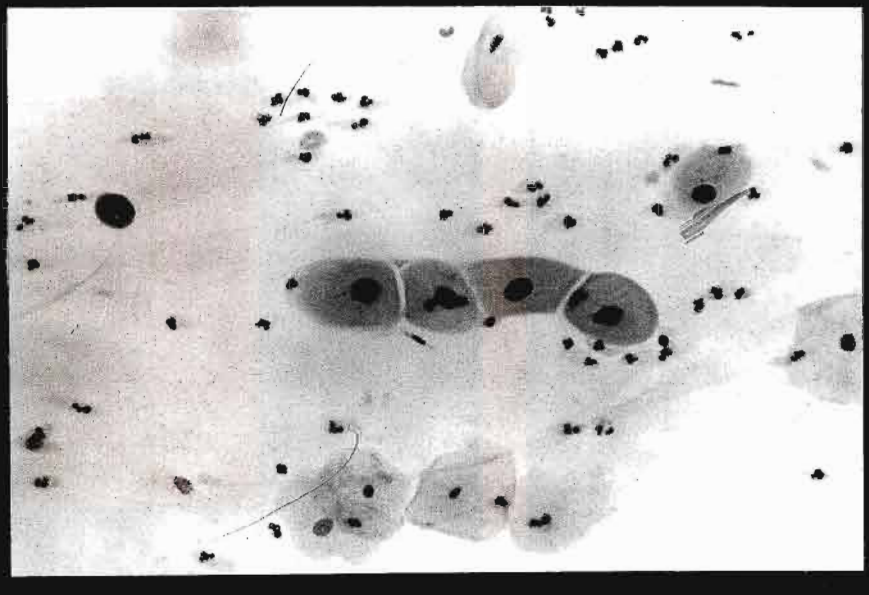
Neither intermediate nor metaplastic cells displayed any apparent degree of hyperchromasia. The endocervical cells showed hyperchromasia in 14,3% of cases at stage 1, 56,5% at stage 2 and 21,4% at stage 3.

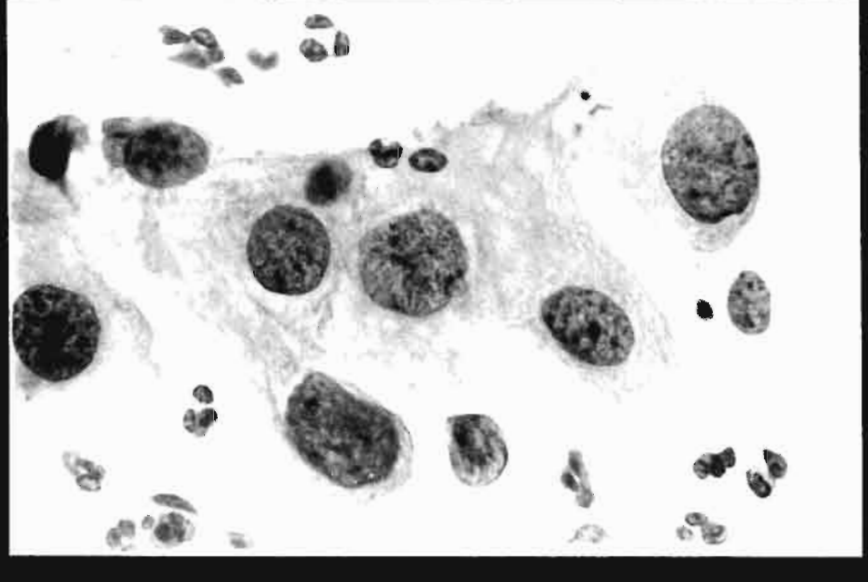
PLATE 15

Hypertrophic metaplastic cells with dense cytoplasm, thickened borders and moulding (x100).

PLATE 16

Group of endocervical cells showing metaplastic differentiation. Note presence of nucleoli, squamous cytoplasmic differentiation and retention of columnar type nuclear structure with a flat luminal surface (x400).





6.4.14 Endocervical nucleoli

The incidence and prominence of nucleoli in endocervical cells ranged from 53,6% at stage 1 to 100% and 92,3% at stages 2 and 3 respectively indicating an increase in biological activity.

6.4.15 Cellular hypertrophy

All three cell types showed significant cellular enlargement at stage 2 and varying degrees at stage 3. An incidence of 35,0% hypertrophic immature intermediate cells was considered significant and the figures show the total percentage of cases exceeding this level at each stage (TABLE XII).

TABLE XII Percentage of cases showing cellular hypertrophy.

	Stage		
Cell type	1	2	3
Immature intermediate	35,23	82,89	73,67
Metaplastic	21,4	64,0	41,2
Endocervical	46,4	97,7	69,2

6.4.16 Nuclear protrusions

The incidence of nuclear protrusions in the intermediate cells was greatest at stage 2 and 3, in metaplastic cells at stage 2 and in the endocervical cells at stages 2 and 3. The overall incidence was greatest in the endocervical cells (TABLE XIII).

TABLE XIII Percentage cases with nuclear protrusions

	Stage		
Cell type	1	2	3
Intermediate	8,8	31,4	31,6
Metaplastic	10,3	37,0	23,5
Endocervical	82,1	96,0	92,9
Endocervical with $\geq$ 20% protrusions	14,3	36,0	42,9

6.5 CYTOLOGICAL ASSESSMENT - CELL TYPES

In this section cellular changes are described which may be regarded as the norm for specific cell types of the uterine cervix epithelium, following the administration of DMPA.

6.5.1 Squamous cells

6.5.1.1 Superficial squamous cells

Maturation estimations in 32 women, three months after therapy showed a decrease in superficial cells in all cases. Superficial cells were present in 88% of vaginal smears prior to therapy, and in 79% and 75% at three and six months post therapy respectively. The total percentage present varied in the three instances from 0-53% to 0-38% and 0-31%. The relationship between the average incidence of superficial cells and the occurrence of menstrual disturbances is shown in TABLE XIV.

TABLE XIV Incidence of superficial cells and development of menstrual disturbances in women with an initial normal cycle

	% Average incidence		Menstrual cycle		Total cases
	Superficial cells	n	Amenorrhoea	Spotting	
Initial	26,7	20	-	-	20
3/12	9,4	6 (30%)	11 (55%)	3 (15%)	20
6/12	7,2	4 (20%)	13 (65%)	3 (15%)	20

Apart from the presence or absence of superficial squamous cells providing a reliable indicator of oestrogenic activity, there were no specific findings related to the administration of DMPA such as alterations in morphology or presentation.

6.5.1.2 Intermediate squamous cells

The mature intermediate cell was the most frequently encountered cell type in all cases, occurring at all stages of therapy but most prevalent at stage 3.

At stage 2 the following parameters showed a significant increase:

anisocytosis, anisokaryosis, cytoplasmic density, plaque formation, nuclear grooving, cytoplasmic wrinkling, retarded maturation, cellular moulding, hypertrophy (in the form of large immature intermediate cells) and nuclear protrusions.

Of these, cytoplasmic density, cytoplasmic wrinkling and cellular hypertrophy reached their highest levels at stage 2 and by stage 3 had decreased in prevalence but were still at levels higher than those of initial base-line smears. Apart from plaque formation which remained at similar raised levels in both stages 2 and 3 the remaining parameters achieved maximum observation levels at stage 3.

In 11 of 13 cases in which intermediate cells were subjected to image analysis an increase in relative nuclear area was demonstrated, which confirmed the observations made by light microscopy and analysis of maturation indices.

6.5.1.3 Parabasal cells

These cells were present in insufficient numbers for detailed analysis and apart from indicating, through their presence, a decrease in epithelial maturity, no specific changes could be identified in this cell type. However, since these cells form part of the continuum of squamous differentiation, the next stage of which would be the immature intermediate cell, they would be subject to the same influences as that cell and could be expected to respond in a similar manner.

6.5.2 Endocervical cells

Increased numbers of endocervical cells were present at stages 2 and 3, consistent with a mild to moderate degree of hyperplasia. This finding was supported by both a general and intranuclear increase in nucleoli at both stages.

Nuclear grooving was not found in the endocervical cells. Nuclear protrusions were significantly increased at stage 2 and showed slightly higher incidence at stage 3. All other parameters i.e. anisocytosis, anisokaryosis, cytoplasmic density, hyperchromasia and hypertrophy were found at maximum levels at stage 2 (PLATE 17). Cytoplasmic density and hyperchromasia at stage 3 were reduced to levels similar to those found

prior to therapy. Evidence of retarded maturation in the form of double nucleoli and nuclear protrusions was most prominent at stage 3 (PLATE 18).

6.5.3 Metaplastic cells

Metaplastic cells were most prevalent at stage 2. By stage 3 levels had dropped to those of stage 1.

At stage 2 the following parameters showed a significant increase: anisocytosis, anisokaryosis, plaque formation, nuclear grooving, cytoplasmic wrinkling, retarded maturation, cellular moulding, flat edges, hypertrophy and nuclear protrusions.

Of these, cytoplasmic wrinkling, cellular moulding, flattened cellular edges, hypertrophy and nuclear protrusions reached maximum levels at stage 2 and by stage 3 had decreased in prevalence although (with the exception of anisocytosis) were still at levels higher than those of base-line smears. Apart from plaque formation which remained at similar levels in stages 2 and 3 the only parameters to be found at a greater frequency at stage 3 were nuclear grooving and evidence of retarded maturation.

Cytoplasmic density showed a continuous decrease through both stages 2 and 3. The classic metaplastic cell shape was found more frequently in stage 2 and 3 smears, with cells tending to be more round prior to therapy. The nuclear position was predominantly central, although occasional eccentric nuclei were found at stages 2 and 3.

6.6 SUMMARY

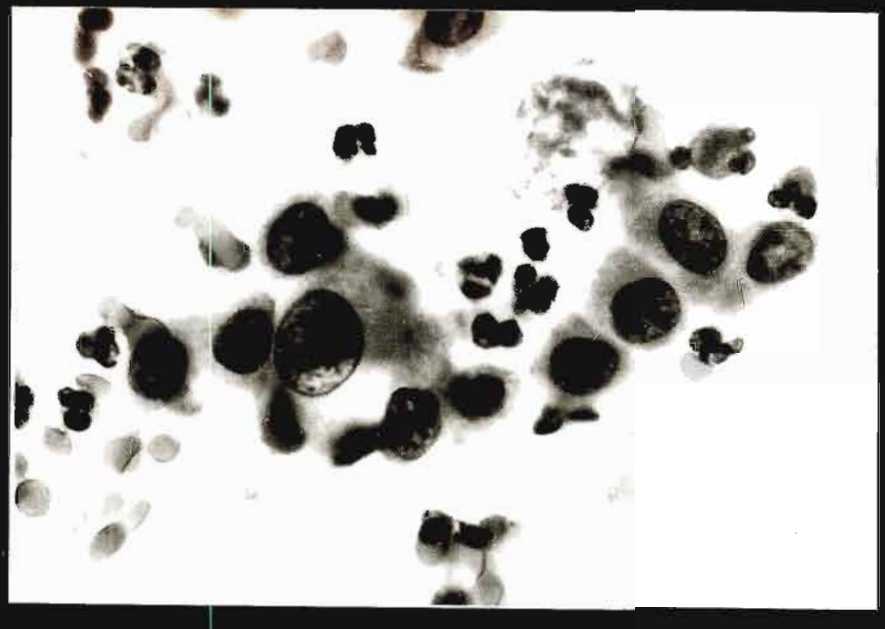
The most prominent and consistent cellular changes, all of which were observed within three months of starting DMPA contraceptive therapy, are summarised in TABLE XV.

PLATE 17

Endocervical hypertrophy due to delayed cell division. Two nucleoli are present in several of the cells. (x400).

PLATE 18

Influenced by progesterone, endocervical cells show evidence of retarded mitotic activity with nuclear protrusions representing aborted cell division. Cells still to divide contain two nucleoli (arrowed). In the remainder the second nucleolus is found in the nuclear protrusion (x400).



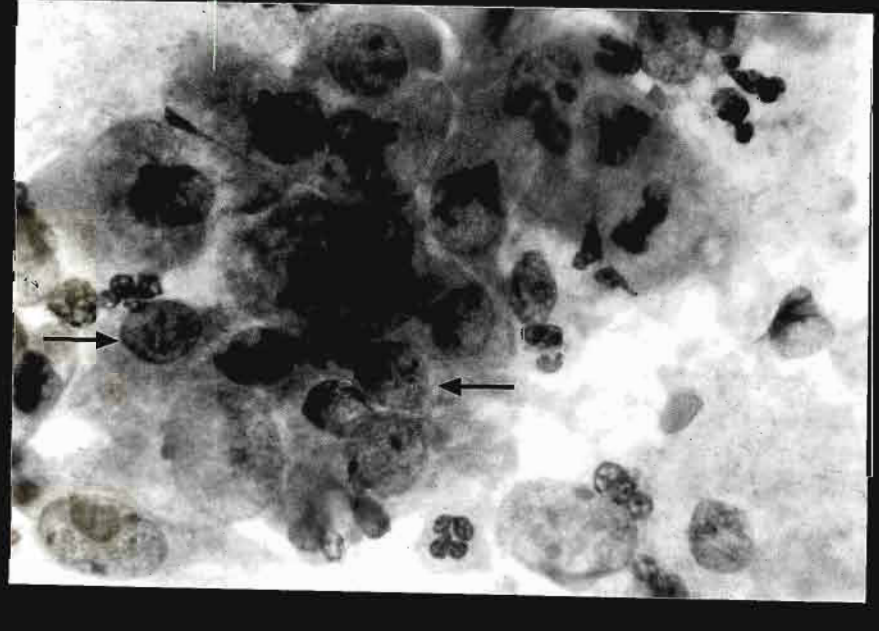


TABLE XV Cellular changes following DMPA contraceptive therapy

Observation	Cell type		
	Intermediate squamous	Columnar	Metaplastic
Variation in cell and nuclear size	+	+	+
Cytoplasmic density increased	+	+	+
Plaque formation	+	N/A	+
Nuclear grooving	+	-	+
Cytoplasmic wrinkling	+	N/A	+/-
Retarded maturation	+	+	+
Increased incidence	+	+	+
Cellular moulding	+	N/A	+
Hyperchromasia	-	+	-
Nucleoli	N/A	+	-
Hypertrophic cells	+	+	+
Flat edges	N/A	N/A	+
Nuclear protrusions	+	+	+

A few of these changes intensified after the second dose of DMPA but several decreased. These were: cytoplasmic density and hypertrophy in all cell types, variation in cellular and nuclear size in metaplastic and endocervical cells, cytoplasmic wrinkling in metaplastic and intermediate cells, hyperchromasia in endocervical cells and moulding, flat edges, nuclear protrusions and the overall incidence in metaplastic cells. The remaining features either retained the levels observed in stage 2 or increased in prevalence.

CHAPTER 7

DISCUSSION

7.1 SAMPLING

Obtaining adequate representative material posed more problems than had been anticipated. This was due partly to population mobility affecting follow-up and partly to inherent inadequacies in the material itself caused by poor preparatory technique.
Vaginal and cervical infections such as trichomoniasis and human papillomavirus (HPV) infection acquired during the course of the study, rendered smears unsuitable for inclusion but provided an opportunity for observing the effect of DMPA on the expression of cellular changes in these conditions.

7.2 MENSTRUAL STATUS AND CELL MATURATION

The well documented fact that DMPA frequently causes amenorrhoea (WHO Technical Report 1978) was supported by this study in which a change in cycling pattern was positively linked to a decrease in cell maturation levels. This decrease, as calculated from the drop in number of superficial cells, was most marked in women who had normal cycles at the beginning of therapy. However, those with initial amenorrhoea displayed the most extensive cell immaturity and the greatest deviance from accepted cell patterns. Since Depo Provera is frequently given immediately postnatal when the cervical and vaginal epithelium consistently displays immaturity and atrophy, this finding alone would justify the re-examination of the classic cytological criteria employed in the diagnosis of pathological conditions of the female genital tract.

Atrophic smears were not commonly found in the women in this study although reduced immaturity was well established within three months and was even more pronounced at six months.

Although there was some evidence to suggest that the effects of DMPA were more marked when given during the proliferative phase of the menstrual cycle, the numbers available for comparison were too small for a definite conclusion to be reached.

7.3 CELL IMAGING

The computerised imaging of the squamous cells successfully identified a sub-set of intermediate cells in which the relative nuclear area was increased. This finding confirmed the observations made by light microscopy and the analysis of maturation indices. Since an increase in the nuclear/ cytoplasmic ratio in favour of the nucleus is an accepted criterion for malignancy, it becomes evident that an adequate contraceptive history and appreciation of the cellular effects, especially in the case of DMPA, is essential for the accurate assessment of cytological atypia. It should be noted that the increase in relative nuclear area arose mainly through a decrease in cytoplasmic volume, rather than an increase in nuclear size, which indicates the production of a less mature cell type.

In spite of the potential for observer inconsistency and subjectivity the examination of smears by light microscopy proved remarkably sensitive and reliable and the results reproducible as demonstrated by comparison with the other methods employed.

7.4 CYTOLOGICAL EFFECTS OF DMPA ADMINISTRATION

It is clear from the range of parameters studied that the administration of an injectable progesterone contraceptive produces epithelial alteration similar to that found in normal progesterone states. However, the changes appeared in many instances exaggerated compared with those seen under normal conditions.

Superficial squamous cells, apart from providing a useful indicator of maturity were found unsuitable for detailed observations because of the relatively small size of the nuclei which prevented accurate assessment. Because of an overall decrease in cell maturity it is also possible that some superficial cells may have been regarded as intermediate cells as a result of an increase in nuclear size.

Parabasal cells occurred in small numbers which were insufficient for meaningful conclusions to be drawn and were therefore also excluded, apart from maturation index estimations.

The remaining three cell types, intermediate squamous cells,

endocervical cells and metaplastic cells were all found to occur in increasing numbers after commencement of therapy. Immature intermediate cells in particular, showing hypertrophy and increased relative nuclear area similar to those found in pregnancy (PLATE 2) were most prevalent at stage 3, at which both endocervical and metaplastic cell numbers, although still raised, had decreased compared with stage 2 levels.

Since the maturation of the squamous epithelium and position of the squamo-columnar junction are largely dependent upon hormonal influences (Jensen and Jacobson 1973, Mercer 1965, Quarmby and Korach 1984) it is likely that the apparent increase in immature intermediate cells would represent an increase in metaplastic maturity with a move to squamous differentiation. The fact that maximum levels of cell hypertrophy were reached at stage 2 (Table XII) would support this hypothesis and would indicate either an increased tolerance, after six months, to the effect of the progesterone, or an adjustment of normal hormone production levels.

Other parameters associated with a progesterone effect such as cytoplasmic density, cytoplasmic wrinkling, endocervical cell hyperchromasia and endocervical and metaplastic anisokaryosis and anisocytosis all decreased significantly at six months, favouring evidence for a decrease in cellular activity at this stage.

Cellular changes were most marked and deviated most noticeably from established norms at stage 2. Changes varied from an increased prevalence of a progesterone related effect to the appearance of such effects at an inappropriate stage in the maturation and development of a cell. Immature intermediate cells, frequently twice the normal size, presented with thin, abundant, markedly wrinkled cytoplasm and, according to the level of immaturity, displayed increased cell adhesion and plaque formation. Several early forms were found containing endocervical nuclei indicating marked interference with the normal maturation process.

7.5 RETARDED MATURATION

The persistent occurrence of such features as cellular hypertrophy, increased cellular adhesion, endocervical cell nuclei in metaplastic and intermediate cells, double nucleoli in endocervical cells, nuclear

protrusions, cellular moulding and retention by metaplastic cells of the flattened cell ends characteristic of endocervical cells, is consistent with interference with cell division i.e. an extended telophase period of the mitotic cycle (PLATE 19).

Progesterone is known to depress nucleic acid synthesis, which is necessary for cell division (Nordqvist 1972, Rodriquez et al. 1979). A protein synthesis inhibitor such as progesterone (Wiqvist and Linde 1987), will also lengthen the G_1 phase of the mitotic cycle, thus slowing cell replication (Hiramoto 1981, Wheatley 1982).

The effect of this inhibitory action is particularly well demonstrated by the phenomenon of nuclear "nippling" in columnar epithelial cells. This has been described in various anatomical sites including breast, bronchus and the uterine cervix (Papanicolaou 1954, Koss 1979, Taylor 1984) but was linked by these authors and others (Zinca 1971) to peak oestrogenic activity.

This view-point is now open to question as these nuclear protrusions have been observed in pregnant and post menopausal women, males and in patients on drugs containing antimitotic compounds (Burdon 1973). In the course of this study nuclear protrusions were, for the first time, shown to be an abortive attempt at nuclear division at the end of the mitotic cycle (McCallum 1988), the nuclear protrusion containing eosinophilic material derived from one of two nucleoli (PLATE 20).

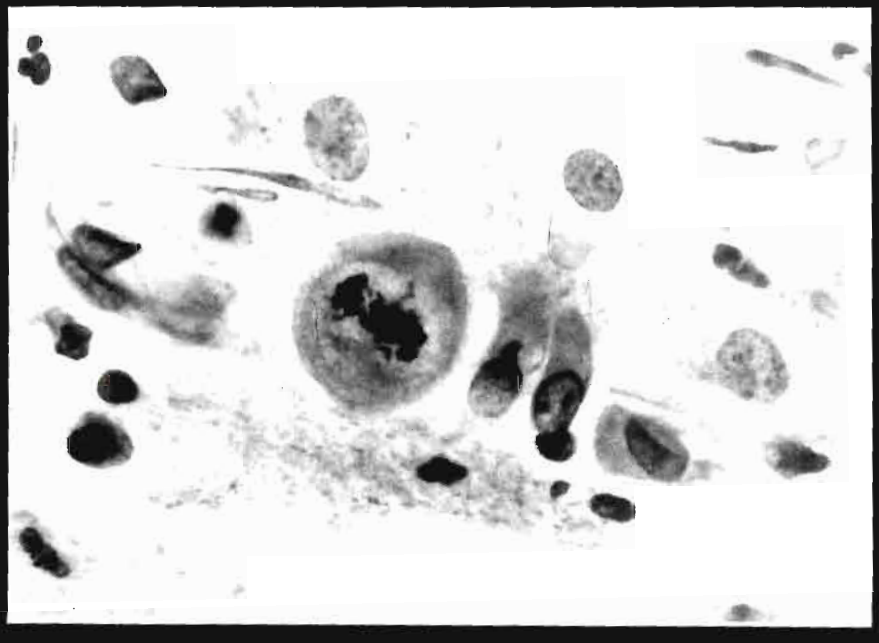
Studies of the endocrinology of the female genital tract (Bergqvist et al. 1985) have shown that the highest values of both progesterone and oestrogen cytosolic receptors occur in the late proliferative phase: the tenth to fifteenth days of the menstrual cycle (Tseng and Gurpide 1975, Gurpide et al. 1977, Demopoulos 1982). During the menstrual cycle the progesterone level rises prior to ovulation, mainly in the form of 17-hydroxyprogesterone. This induces an increase in the enzyme estradiol dehydrogenase, which oxidises estradiol to the less active form, estrone. Kitawaki (1987) showed that progestogens cause early secretory change in proliferative endometrium and that DMPA had the greatest effect on the stimulation of estradiol dehydrogenase activity. The stimulating properties of oestrogen in promoting nuclear and cellular division (Kobilcová et al. 1985) is therefore inhibited by both endogenous and exogenous progesterone (Tachi et al. 1972), bringing

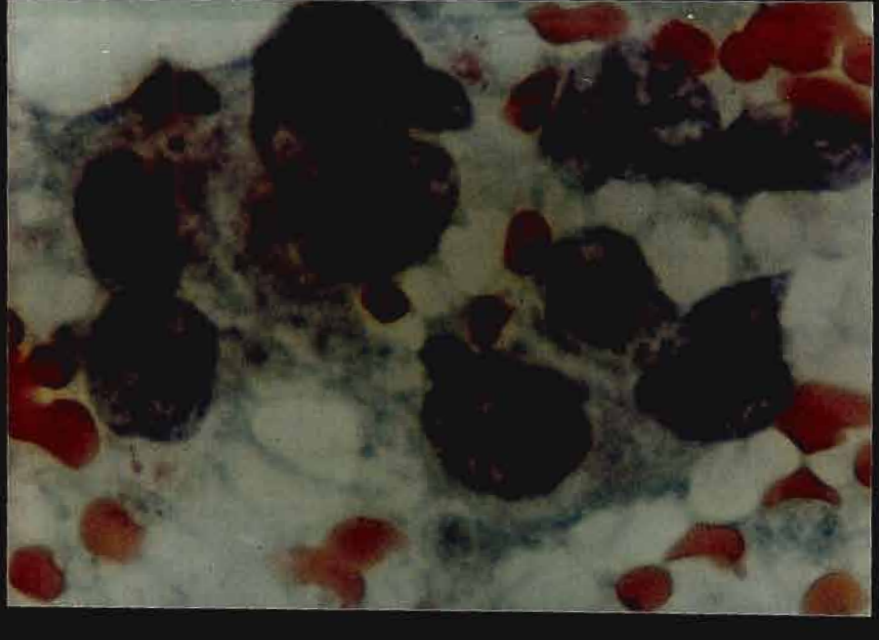
PLATE 19

One endocervical cell displays delayed mitotic division, the presence of a nuclear protrusion in a second indicates abortive nuclear division and the third contains two nucleoli indicating inability to proceed with the formation of the structures required for constriction and division (x400).

PLATE 20

Abortive nuclear division demonstrated by prominent nuclear protrusion (x400).





about a rapid change in directional differentiation (PLATE 21) and producing distortions in the dividing nuclei. The extent of resultant nuclear "nippling" provides an indication of the amount of progesterone activity present with an increased incidence pointing to the possible use of a progesterone-only contraceptive.

In women on DMPA nuclear protrusions may also be found in cells with advanced metaplastic and squamous differentiation (PLATE 22). This is consistent with the finding (Mercer 1965) that certain female sexual epithelia in primates may oscillate between producing keratin and mucin in response to a rise or fall of sex hormones and cyclic production of different cell types from the same stem cell may be encountered in metaplastic cycles under hormone control. It also demonstrates the observation by Truman (1974) that the nucleus does not undergo irreversible change during cell differentiation but retains totipotentiality.

The occurrence of maximum cellular hypertrophy at stage 2 is also consistent with slower cell division and a retarded maturation process. Wilborn et al. (1983) found that ciliogenesis was suppressed by progesterone, cell apices became flattened and the cytoplasm of stromal cells increased in volume thus producing hypertrophy. Implicated in this effect is the fact that progesterone is an oestrogen antagonist and that oestrogen not only facilitates calcium absorption essential for cell cleavage (Hiramoto 1981) but is also necessary for the utilisation of folate required for the methyl synthesis of thymydilic acid, a further prerequisite for cell division (Van Niekerk 1966, Laffi et al. 1972).

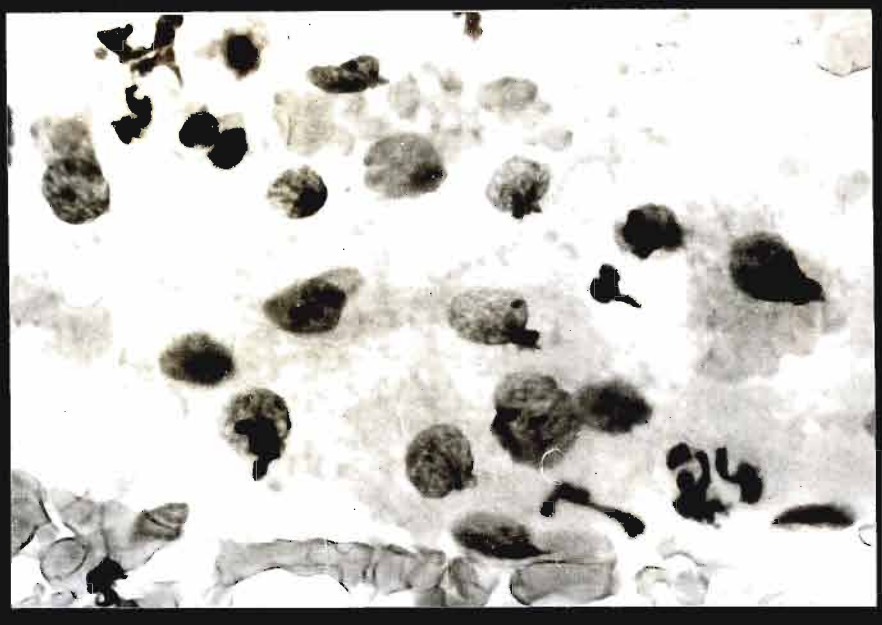
As a result of the complementary functions of oestrogen and folic acid (Jensen and Jacobson 1973, O'Malley and Means 1974), the suppression of oestrogen interferes directly with the action and absorption of folate, converting it to a storage form, in which it is unavailable for utilisation by the tissues (Eichner et al. 1975, Lindenbaum et al. 1975). As a target organ, the uterus, and more specifically, the uterine cervix, is vulnerable to this deficiency especially in areas of constant proliferation and hence greatest demand (Zanartu et al. 1970). This may cause localised folic acid deficiency with cellular changes which mimic metaplastic dysplasia with the attendant pitfall of over-diagnosis (Lindenbaum et al. 1975). The occurrence at stage 2 of a

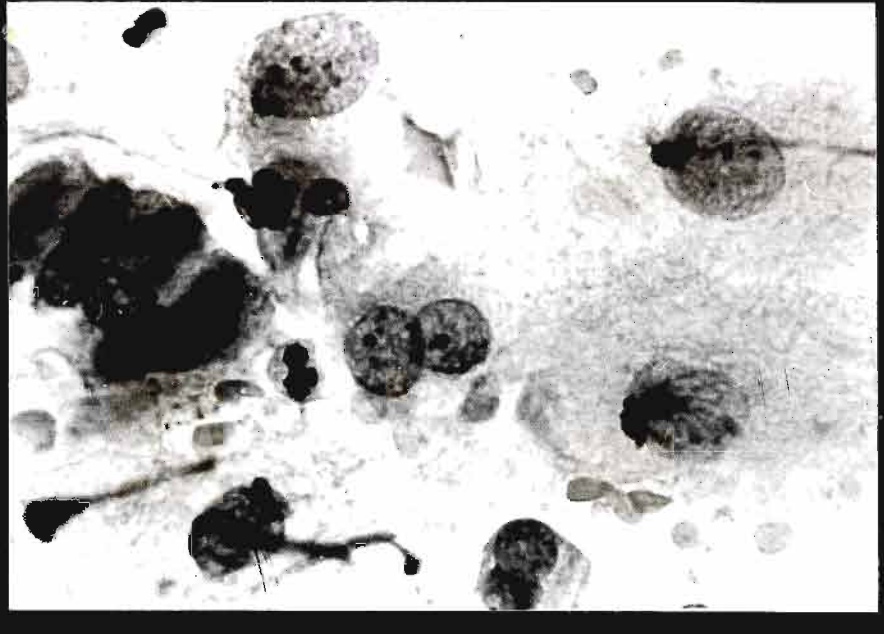
PLATE 21 (a and b)

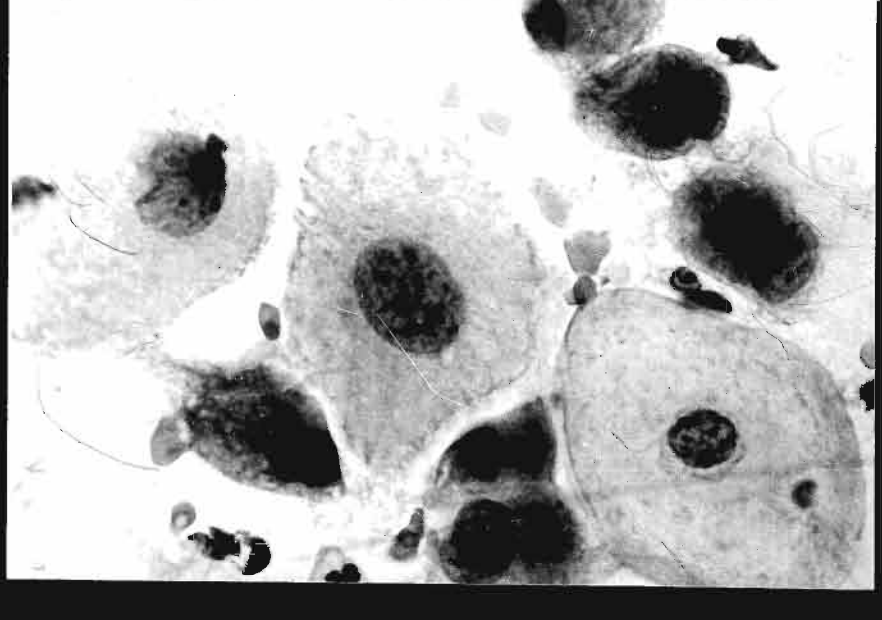
(a) - upper
Endocervical cells with abundant cytoplasm inconsistent with columnar differentiation (x400).

(b) - centre
Endocervical cells with metaplastic features indicating equivocal differentiation (x400).

PLATE 22 - lower
Metaplastic cells displaying a continuum of differentiation from columnar to squamous (x400).







greater incidence of nuclear grooving, which has been identified as an additional diagnostic parameter for folate deficiency (Klaus 1971), further supports the evidence for the significant role which may be played by this condition as an end organ reaction.

The possibility that the folate deficiency associated megaloblastic change is due largely to the anti-oestrogenic effect of large doses of progesterone is supported by the improvement in the cytological picture produced by the administration of oestrogen (McCallum 1982) and the failure of folate therapy alone(Hibbard 1964, Martin and Davis 1966).

In an underprivileged population the additional burden of oestrogen insufficiency coupled with increased folate demand may precipitate folate deficiency. Occult folate malabsorption may also become apparent under these conditions (Shojania and Hornady 1973, Olson 1975). In the assessment of cellular hypertrophy in cervical smears it is therefore essential that the contraceptive history of the patient is available and the implications and potential pitfalls fully understood.

The image analysis finding that a significant proportion of cells displayed an increase in relative nuclear area is a further indication of increased immaturity. The variation in cell size and the production of occasional exceptionally large cells probably reflects the level of mitotic activity in a particular cell line and the stage reached by cells within the mitotic cycle at the time of obtaining the smear and cell sample.

7.6. IMPLICATIONS

Having established certain features as characteristic of the cellular effects of long term progesterone administration, existing diagnostic criteria for a variety of conditions need to be re-assessed in the light of these changes. In particular, a distinction needs to be made between progesterone related change and the atypia of benign and pre-malignant conditions of the uterine cervix.

Anticipated effects of particular significance include larger than normal nuclei, a retardation of cell division resulting in cellular hypertrophy and an overall reduction of cell maturity. It is evident that this picture superimposed on conditions such as human

papillomavirus infection and cervical intraepithelial neoplasia for example, would considerably alter their expression and microscopic appearance.

An awareness of the modifications produced by long-acting progesterone is therefore essential for the correct interpretation and reporting of smears.

CHAPTER 8

APPLICATION OF FINDINGS

8.1. INTRODUCTION

The most consistent and best defined effects of medroxyprogesterone acetate (MPA) on the cells of the uterine cervix are, as previously described, a reduction in overall cell maturity with accompanying basophilia and an increase in nuclear and cell size.

By applying these findings in the course of screening cervical smears, readily identifiable alterations were observed in the expression of cellular changes and deficiencies were detected in existing diagnostic criteria associated with a wide variety of conditions. These conditions included inflammatory states (Gupta 1991), viral infections (Meisels et al. 1983, Chapman 1989) folate deficiency (van Niekerk 1966), cervical intraepithelial neoplasia and malignancy (Patten 1978, Koss 1979, Pacey 1991). The most frequently encountered and clinically significant of these are described further with regard to existing criteria, the modifications resulting from MPA therapy and the potential for diagnostic error due to misinterpretation of cellular features.

8.2. INFLAMMATORY CONDITIONS

8.2.1 Trichomoniasis

8.2.1.1 Diagnostic criteria

Prominent cytoplasmic eosinophilia unrelated to maturity. Formation of limited, clearly defined perinuclear clear zones or 'haloes' in the immediate area surrounding the nucleus of superficial squamous epithelial cells (PLATE 23).

Presence of small pear shaped trichomonads each possessing a single, crescent shaped, eccentric nucleus (PLATE 24).

8.2.1.2 Effect of MPA therapy

Cell maturity decreases with a consequent increase in basophilia.

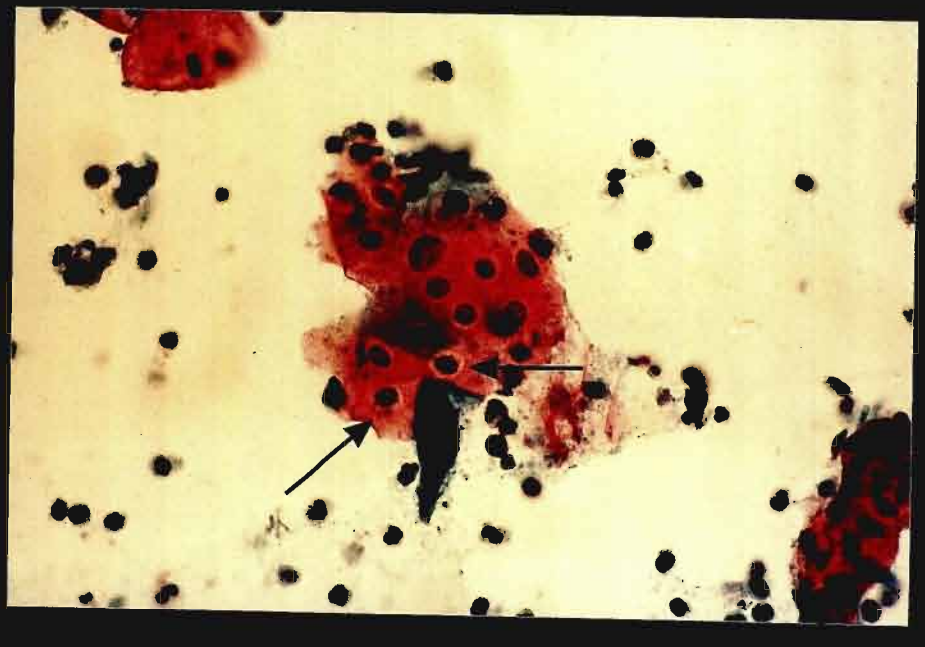
Perinuclear 'haloes' occur in intermediate cells and parabasal cells and are proportionate to the larger nuclear

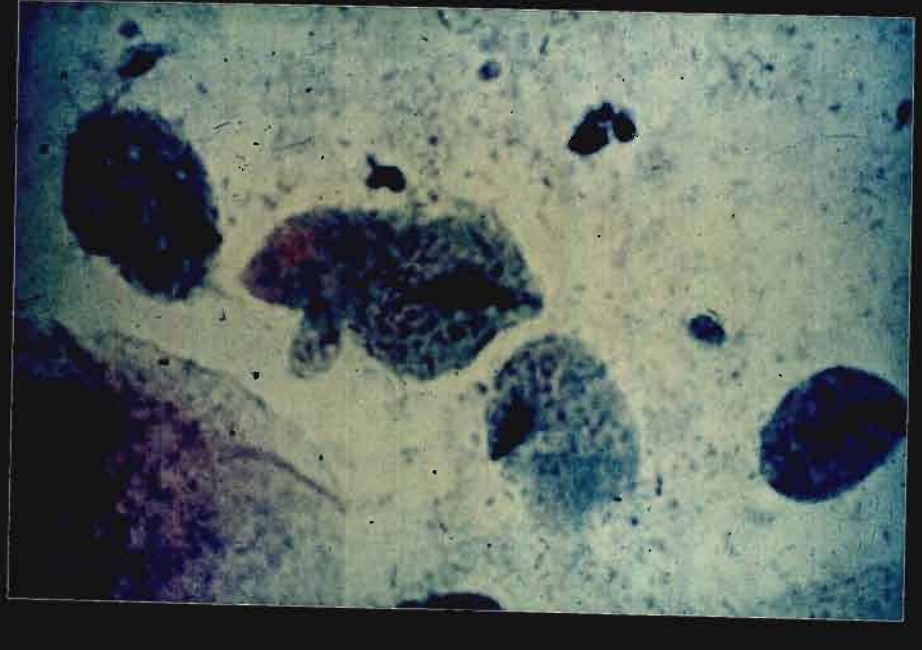
PLATE 23

Perinuclear 'haloes' in superficial squamous cells (arrowed) (x400).

PLATE 24

Trichomonads with crescent shaped eccentric nuclei (x1000).





size (PLATE 25).

The trichomonas organism itself may be affected and exhibit retarded maturation displaying multinucleation and incomplete division (PLATES 26 and 27).

A summary of effects is presented in TABLE XVI

TABLE XVI Effect of progesterone therapy on cellular features associated with trichomoniasis.

CELLULAR FEATURE	EFFECT OF PROGESTERONE
Eosinophilia	Increased basophilia
Mature cell pattern	Less mature cell pattern
Small, perinuclear 'haloes' in mature cells	Larger perinuclear 'haloes' in less mature cells, increasing in size with immaturity.
Single trichomonads with one nucleus	Multinucleate forms representing incomplete division.

8.2.1.3 Potential for diagnostic error

Errors in interpretation may lead to an incorrect diagnosis of human papillomavirus infection through the assessment of immature cells with perinuclear 'haloes' as koilocytes associated with HPV infection.

8.3 VIRAL INFECTIONS

The two viral infections most frequently encountered in cervical smears are caused by herpes simplex genitalis virus and human papillomavirus.

8.3.1 Herpes simplex genitalis.

8.3.1.1 Diagnostic criteria

Multinucleation, comprising the formation of many small nuclei which are closely packed, may exhibit moulding and share a common cytoplasm.

Disappearance of chromatin structure causing a 'ground-glass' appearance to the nucleus. Formation of numerous small basophilic nuclear inclusion bodies or single, large

PLATE 25

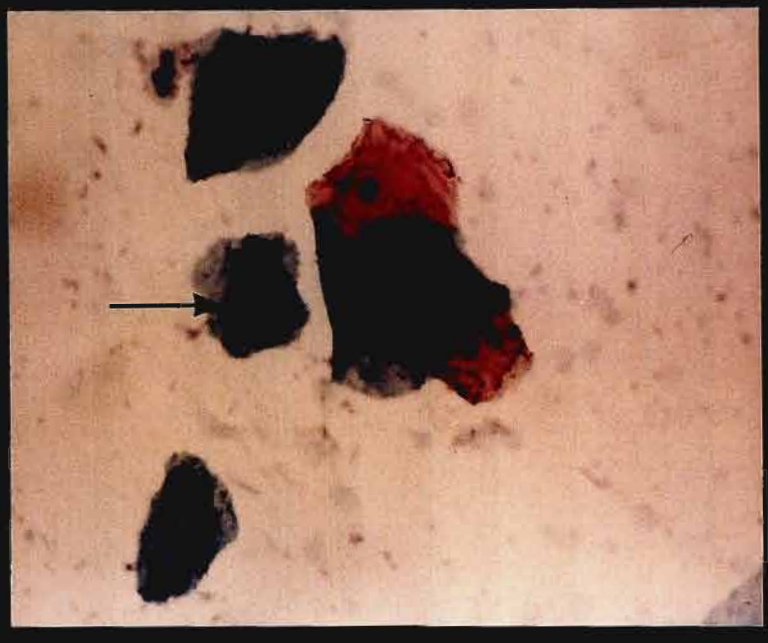
Intermediate cell showing enlarged perinuclear 'halo' (arrow) in the presence of progesterone (x100).

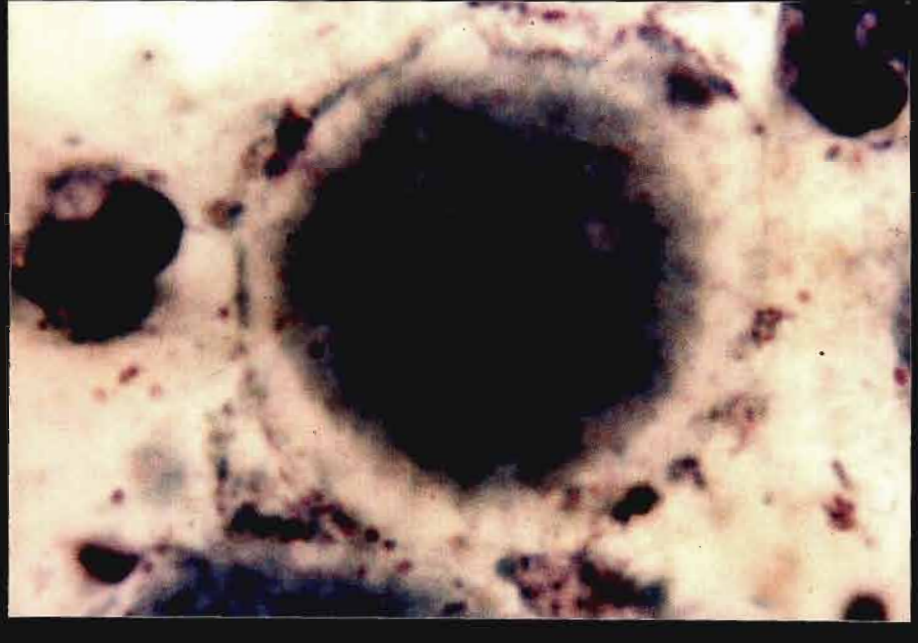
PLATE 26

Trichomonad showing multinucleation due to incomplete division. Three crescent shaped nuclei are visible (x1000).

PLATE 27

Trichomonad showing delayed division in the presence of MDPA (x1000).





eosinophilic inclusions (PLATE 28).

8.3.1.2 Effect of MPA therapy

Influenced by progesterone the nuclei fail to divide normally leading to the formation of single cells possessing large single nuclei which have a dark cloudy appearance and scanty densely basophilic cytoplasm (PLATE 29).

Small compact multinucleated cells (PLATES 30 and 31) and cells showing smudged nuclei and incomplete cytoplasmic division are also found (PLATE 32). Nuclei are predominantly round to oval as compared with the marked moulding usually observed in this condition. This is presumably due to the slowed rate of nuclear division caused by the progesterone (TABLE XVII).

TABLE XVII Effect of progesterone therapy on cellular features associated with herpesvirus infection

CELLULAR FEATURE	EFFECT OF PROGESTERONE
Multinucleation with numerous small nuclei	Fewer and smaller nuclei and frequent single cells with one or two nuclei
Nuclei show moulding	Nuclei round to oval with little or no moulding
Inclusions visible	Inclusions seldom visible but may occupy a large proportion of the nucleus in single cells
Inclusions basophilic and eosinophilic	Inclusions when present generally basophilic
'Ground-glass' nuclear appearance	Cloudy appearance of nuclei common
Cytoplasm has normal density and staining	Cytoplasm often dense and basophilic

8.3.1.3 Potential for diagnostic error

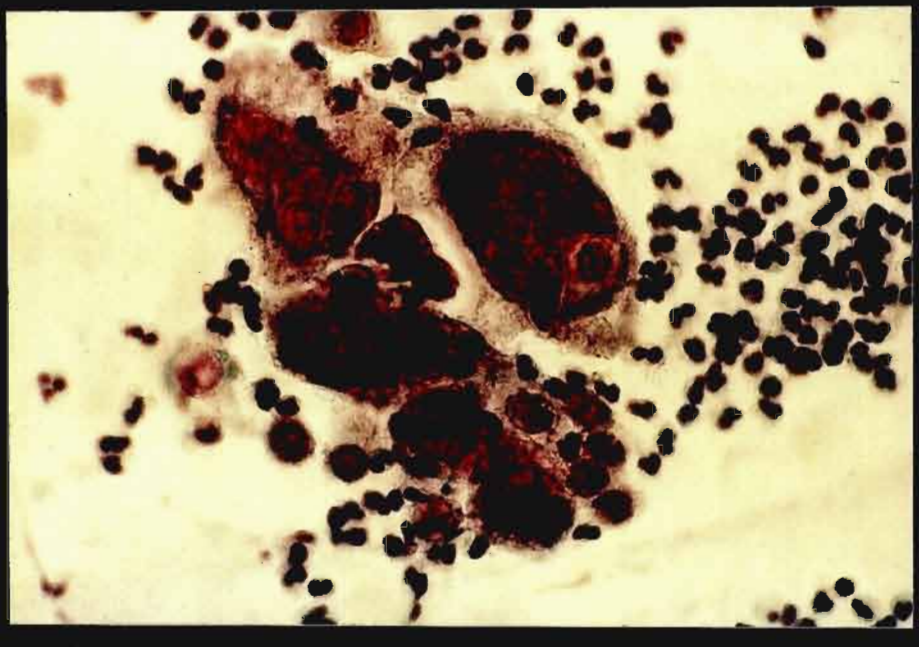
Errors in interpretation due to incorrect assessment may range from non-specific inflammatory atypia and a different type of virus, to dysplasia and even carcinoma in situ (CIS) due to the apparent disturbance in the nuclear/cytoplasmic ratio.

PLATE 28

Herpesvirus cervicitis - moulding and multinucleation associated with herpesvirus infection (x100).

PLATE 29

Two cells infected by herpesvirus with one showing a single large nucleus and viral inclusion (x400).



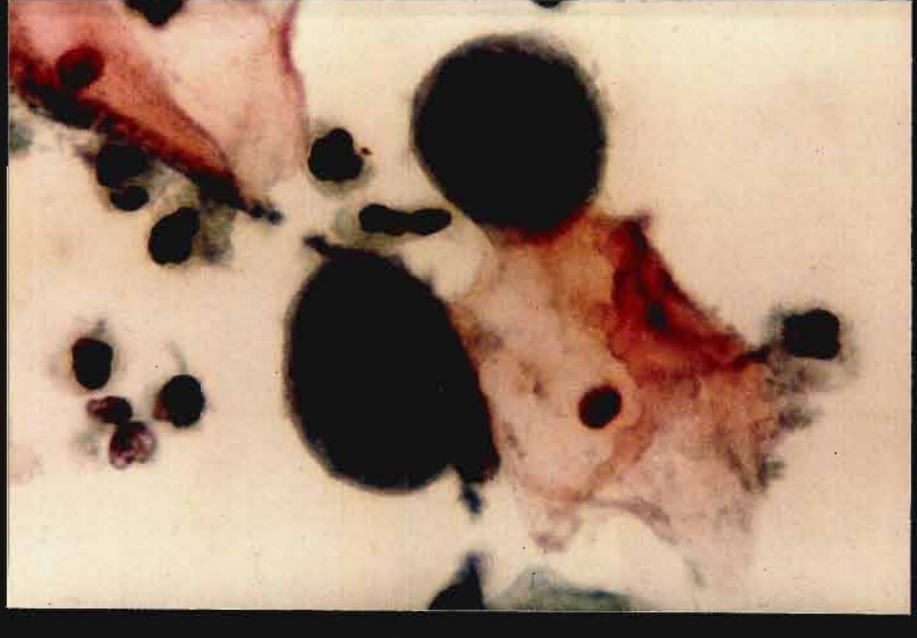


PLATE 30

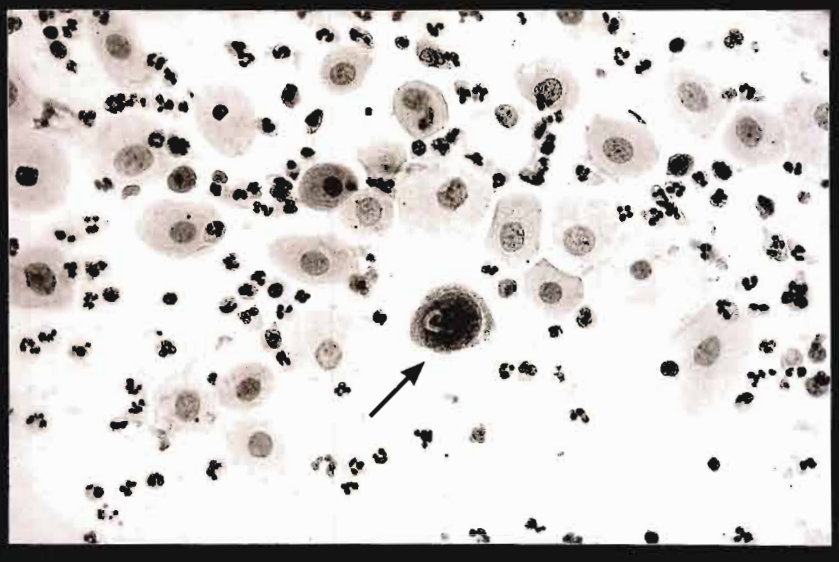
A single small immature cell infected by herpesvirus (arrow) (x100).

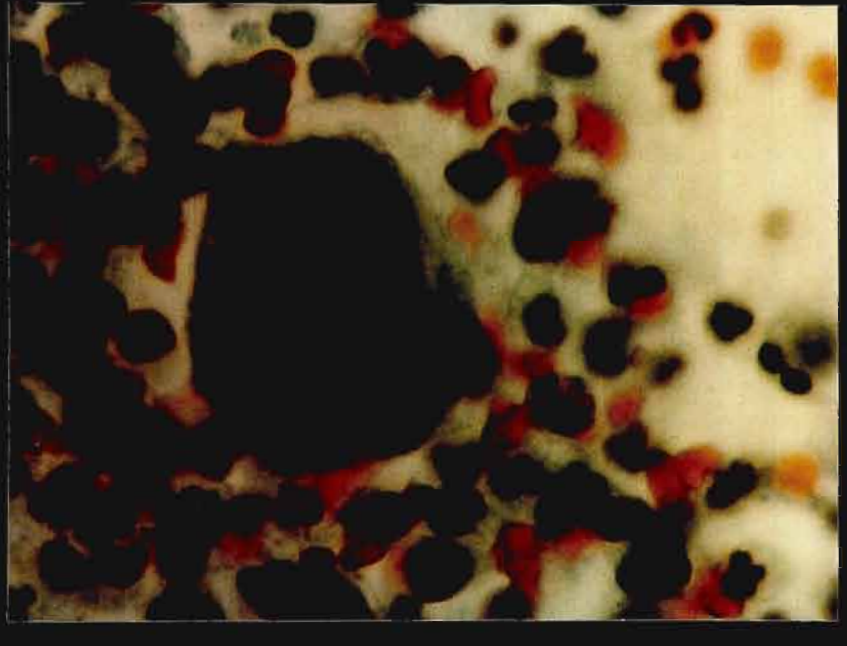
PLATE 31

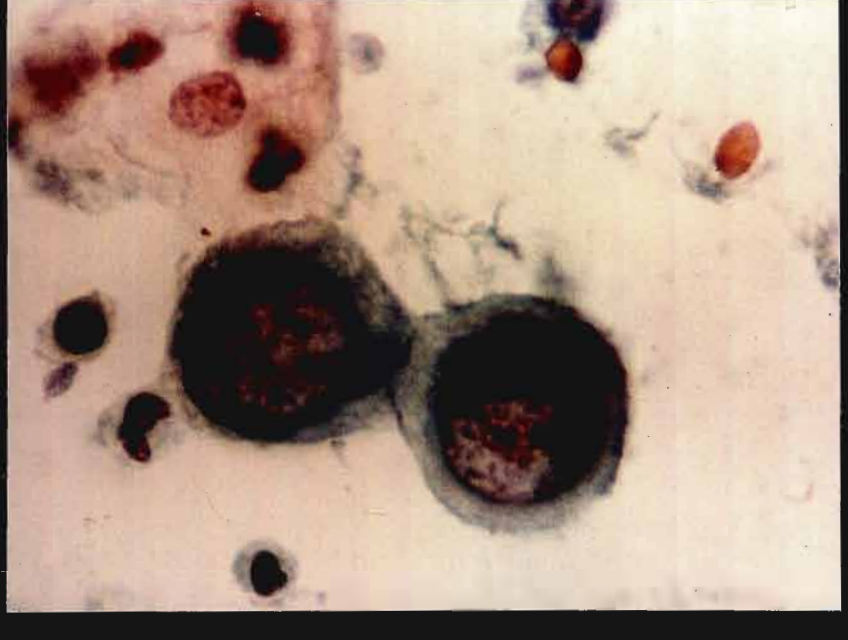
An immature cell picture produced by progesterone therapy with a single parabasal cell showing multinucleation due to herpesvirus (x400).

PLATE 32

Herpesvirus infected cells and retarded cell division due to progesterone (x400).







8.3.2 Human papillomavirus

8.3.2.1 Diagnostic criteria

Cytoplasm exhibits marked eosinophilia but orangeophilic or amphophilic cytoplasm may be present.

Presence of koilocytes, mature cells with relatively small, hyperchromatic or pyknotic nuclei surrounded by a large area of clear and transparent cytoplasm.

Binucleation is common (PLATE 33).

Tightly packed sheets of dyskeratocytes, small eosinophilic or orangeophilic cells which have small, condensed, hyperchromatic nuclei (PLATE 34).

Keratohyaline granules are common in the superficial cells.

8.3.2.2 Effect of MPA therapy

With the reduction of cell maturity basophilia replaces the eosinophilia. An interesting feature is the concentration of residual keratisation in the immediate perinuclear area with the rest of the cytoplasm retaining basophilic staining (PLATE 35).

The cytoplasmic clearing typically found in HPV infected cells is present but greatly decreased (PLATE 36). This finding is directly associated with cytoplasmic immaturity and density as well as with cellular hypertrophy, the larger nuclei being surrounded by a relatively smaller area of clearing. This feature is also present in the binucleate cells (PLATE 37).

Parabasal and intermediate cells frequently show inconspicuous subnuclear clearing in varying degrees (PLATE 38).

Nuclear pyknosis is not found.

Slightly crowded sheets of intermediate cells showing mild nuclear enlargement and atypia with subdued eosinophilia replace the tightly packed aggregates of dyskeratocytes (PLATE 39).

Keratohyaline granules are commonly found in both intermediate and parabasal cells (PLATE 40).

A comparison of cellular features in HPV infection and MPA therapy is given in TABLE XVIII.

PLATE 33

Classic HPV changes in mature epithelium (x400).

PLATE 34

Tightly packed sheets of dyskeratocytes (x100).

PLATE 35

HPV infected cell showing limited endoplasmic keratinisation (x400).

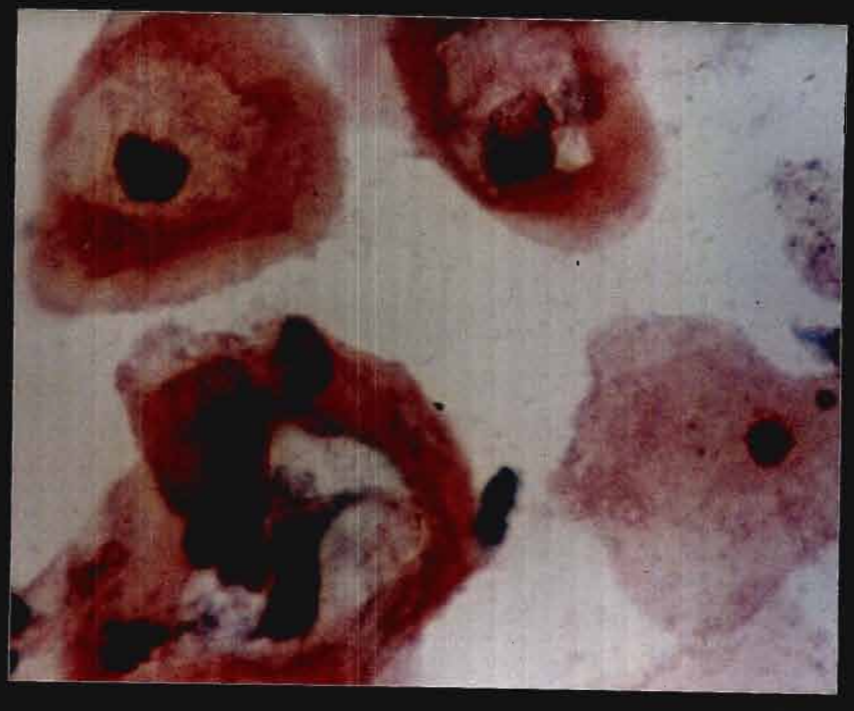


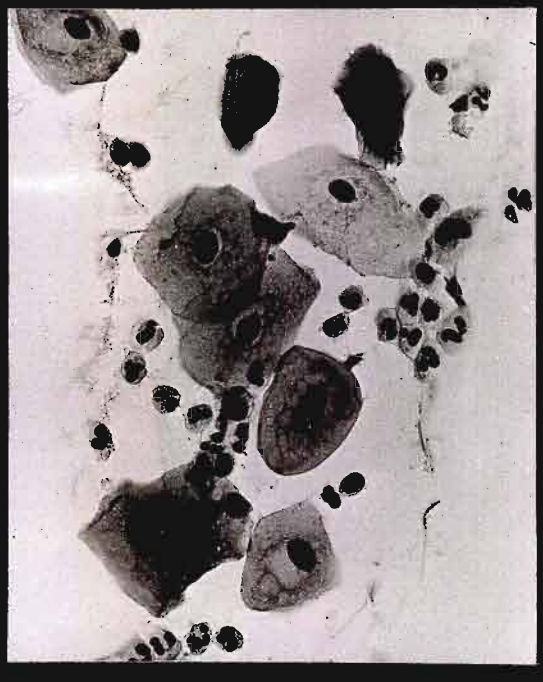
PLATE 36 - (upper left)
Intermediate cells showing minimal perinuclear clearing (x100).

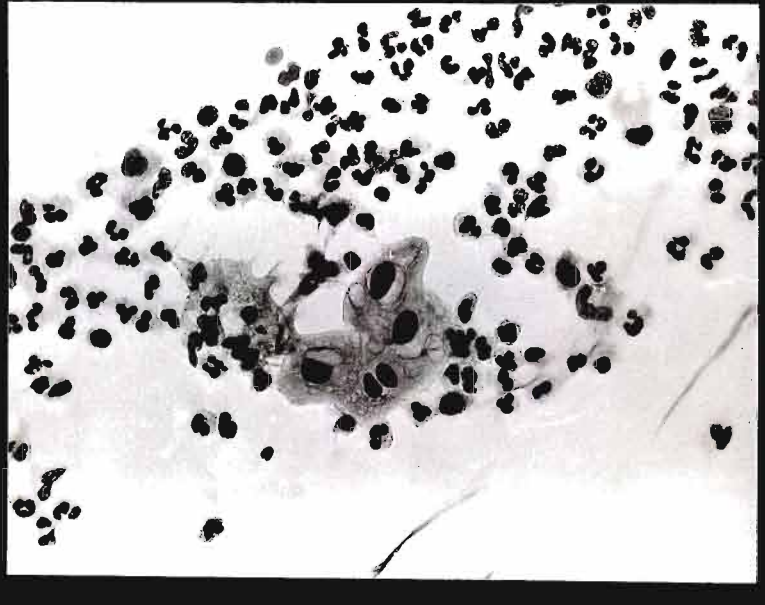
PLATE 37 - (upper right)
HPV change in immature cells with reduced perinuclear clearing and bi-nucleation (x100).

PLATE 38 (a and b)

(a) - centre
Parabasal cells displaying cytoplasmic vacuolation and subnuclear clearing (x100).

(b) - lower
Am immature intermediate cell with subnuclear clearing and cytoplasmic vacuolation (x400).





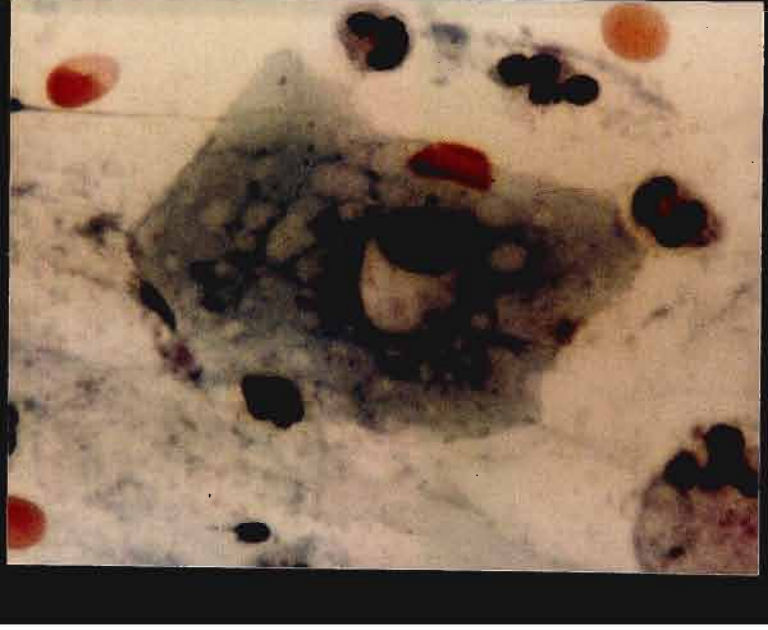
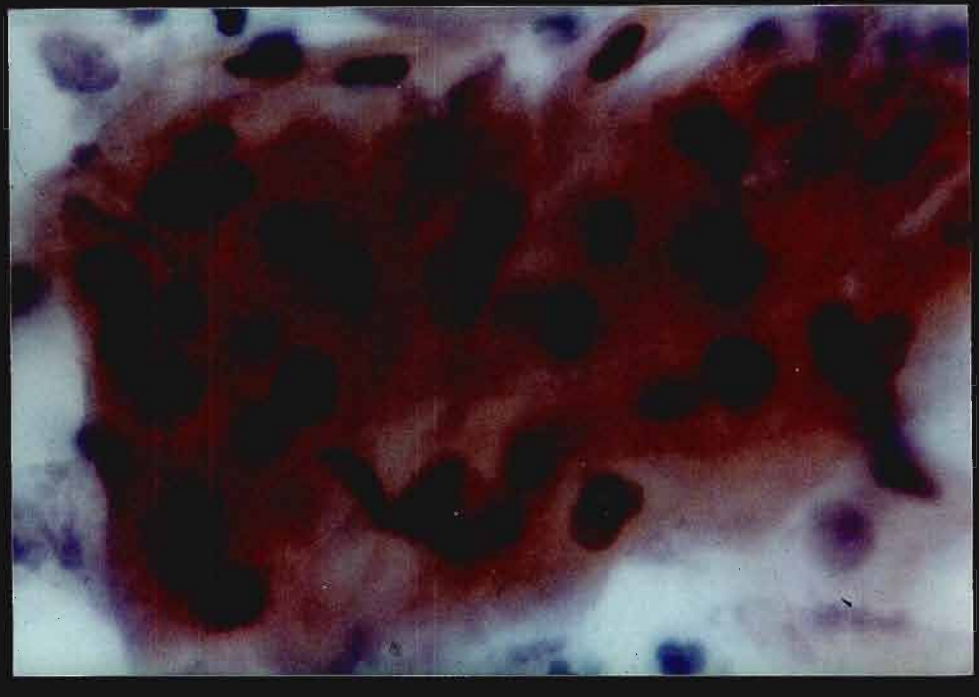


PLATE 39

In the presence of progesterone, dyskeratocytes show mild eosinophilia, more abundant cytoplasm and an increased relative nuclear area due to immaturity (x400).

PLATE 40

Minimal subnuclear clearing, bi-nucleation and numerous kerato-hyaline granules in immature squamous cells (x400).



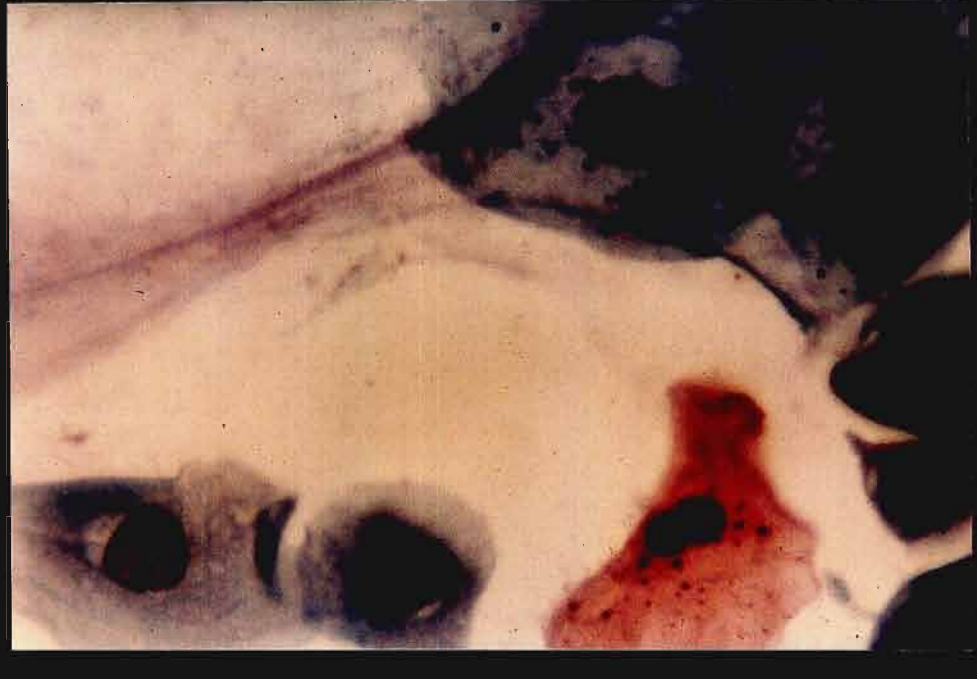


TABLE XVIII Comparison of cellular features in HPV infection and MPA therapy, separately and in combination.

Cellular feature	H P V	M P A	H P V + M P A
Predominant cell type	Superficial and intermediate	Intermediate	Intermediate
Cytoplasm			
Staining	Marked eosinophilia, some cyanophilia	Predominantly cyanophilic	Predominantly cyanophilic, some eosinophilia
Wrinkling	No	Yes	Yes
Hyperkeratosis	Marked	No	Not prominent
Amphophilia	Yes	No	Mainly confined to perinuclear area
Kerato-hyaline granules	Yes	No	Yes
Perinuclear clearing	Marked, with irregular border	No	Minimal, often subnuclear with smooth borders
Nucleus			
Pyknosis	Yes	No	Infrequent
Binucleation	Common in more mature cells	In endocervical cells	Common in less mature cells
Enlarged	Variable	Yes	Yes, often marked
Chromatin	Moderate to marked hyperchromasia	Finely granular, no hyperchromasia	Moderate hyperchromasia
Dyskeratocytes	Common, mature. Scanty orangeophilic cytoplasm	No	Infrequent, less mature. Adequate moderately eosinophilic cytoplasm
Cellular hypertrophy	Occasional	Frequent	Often marked
Plaque formation	No	Frequent	Frequent

8.3.2.3 Potential for diagnostic error

Although the potential exists for the incorrect assessment of HPV in immature cells as dysplasia, a more frequent source of error is the failure to recognise the cellular features of HPV in the presence of a marked progesterone effect.

The reduction of the visibly conspicuous viral manifestations in an immature epithelium to minimal cytological changes may result in the condition being overlooked or dismissed as inflammatory change only. Since there is an association of certain HPV types with the stimulation of cervical carcinoma (Meisels et al. 1983) this type of error holds serious implications for the patient.

8.4 FOLIC ACID DEFICIENCY (FAD)

8.4.1 Diagnostic criteria

Changes commonly found in intermediate squamous cells.

Cellular hypertrophy involving both nucleus and cytoplasm with retention of a normal nuclear/cytoplasmic ratio.

Cytoplasmic polychromasia.

Multinucleation.

Cytoplasmic inclusions (PLATE 41) and nuclear grooving (PLATE 42).

8.4.2 Effect of MPA therapy

A reduction in epithelial maturity results in FAD changes appearing in immature metaplastic cells (PLATE 43).

Due to the inhibition of nuclear division, single large cells containing a very large nucleus may occur although careful assessment will establish the presence of a normal nuclear/cytoplasmic ratio (PLATE 44).

Cytoplasm is dense and staining is likely to be predominantly cyanophilic.

Binucleation is more frequent than multinucleation due to delayed nuclear division.

Ingestion of polymorphs and small squamous cells remains a consistent diagnostic feature.

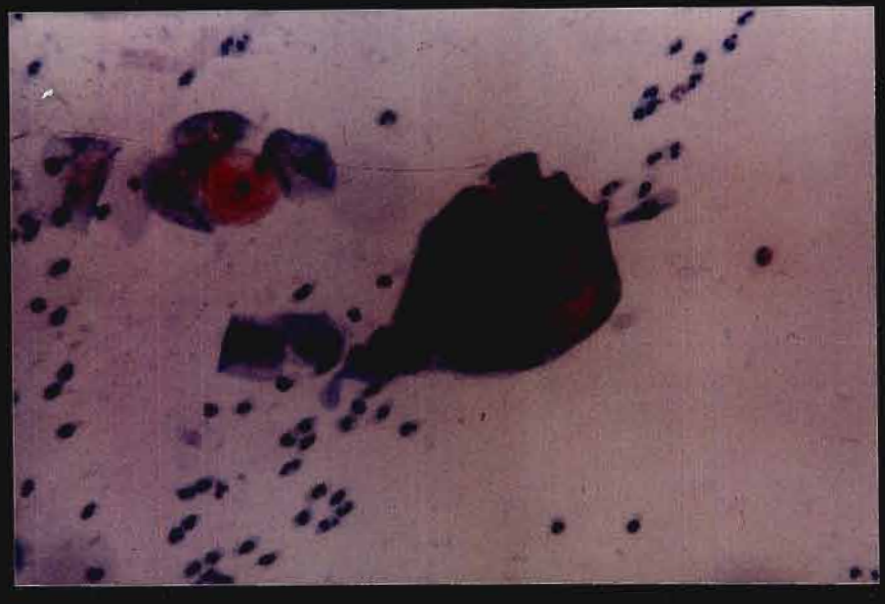
Incomplete cellular division may be seen, ranging from a common cell wall to the retention of an intercellular bridge.

PLATE 41

Folate deficiency in pregnancy. An intermediate cell shows megaloblastic change, a normal relative nuclear area and phagocytosis (x100).

PLATE 42

Nuclear groove (arrow) in an immature intermediate cell (x100).



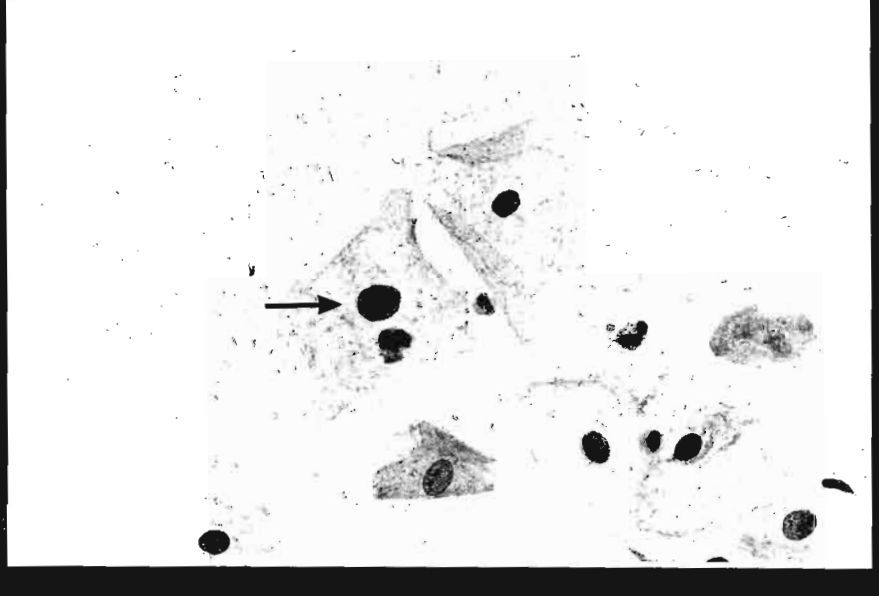
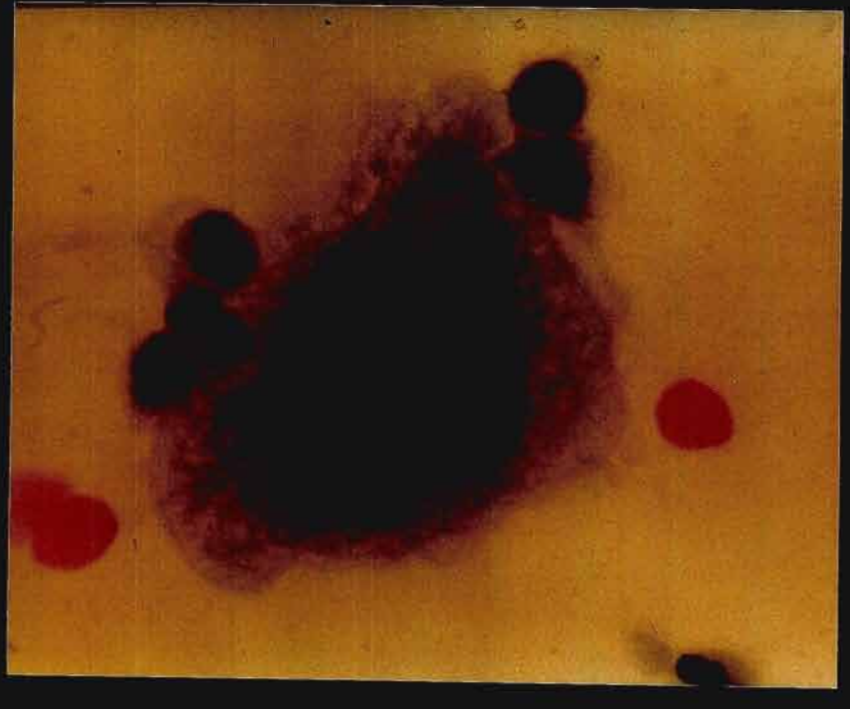


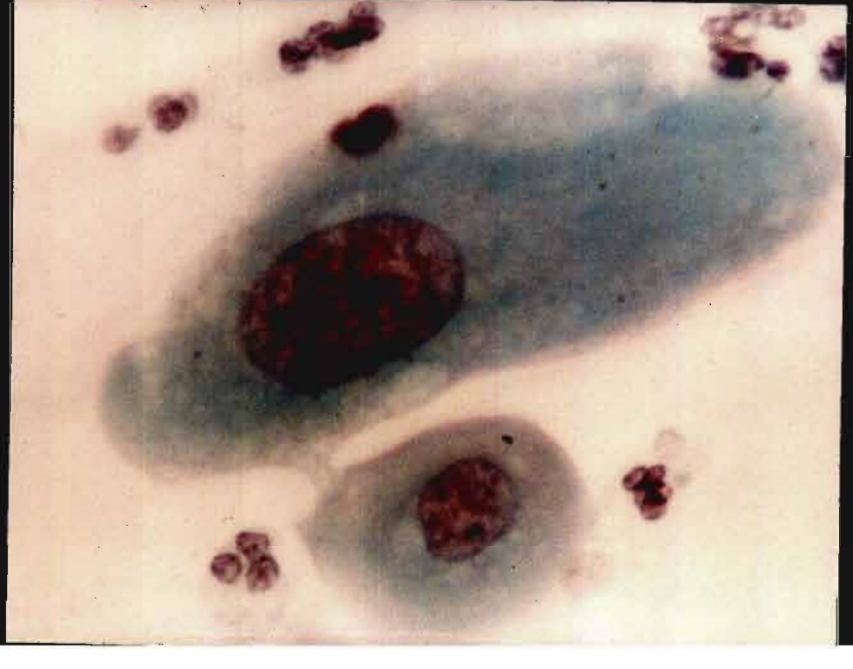
PLATE 43 (a and b)

(a) - upper
Folate deficiency change in metaplastic cells. Multiple nucleoli and increased nuclear size indicate retarded cell division. Phagocytosis is prominent (x400).

(b) - centre
Hypertrophic cell with bi-nucleation. Nuclei still retain endocervical features (x400).

PLATE 44
Incomplete cell division results in occasional giant forms such as the larger of these parabasal cells (x400).





8.4.3. Potential for diagnostic error

The most likely difficulty to be experienced is the differentiation of a folate deficiency from cervical intraepithelial neoplasia (CIN). The retention of a normal relative nuclear area despite the size of the cell, the finely granular chromatin and the presence of cytoplasmic inclusions are useful diagnostic pointers.

A further problem which compounds the difficulty is that whereas the condition responds rapidly to appropriate treatment, with the regression of cellular change in most cases, this does not apply to women on MPA therapy as the progesterone has an anti-oestrogenic action which interferes with the absorption of folate (McCallum 1982). However, change to a different form of contraception is frequently sufficient, with or without folate therapy, to effect a major improvement in the cytological picture. The use of one of these methods may therefore assist in the identification of the precise nature of the cytological changes.

Occasionally folate deficiency changes may be confused with HPV infection but the identification of intracytoplasmic inclusions viz. polymorphs or small parabasal cells, is a useful means of differentiating these two conditions as phagocytosis is not a feature of condylomatous change. In women on MPA, when these two conditions occur simultaneously, hypertrophic cells with large nuclei and a narrow ring of perinuclear clearing are seen. The structure of the chromatin in such cases depends on the severity of the condylomatous change and in the atypical condyloma may be hyperchromatic and moderately granular indicating dysplastic alteration .

The effects of progesterone administration on changes associated with folate deficiency as well as the differential diagnostic features of HPV infection and FAD are shown in Tables XIX and XX.

TABLE XIX Effect of progesterone therapy on cellular features associated with folate deficiency

CELLULAR FEATURE	EFFECT OF PROGESTERONE
Cellular hypertrophy	Marked cellular hypertrophy
Relative nuclear area is normal	Relative nuclear area may be increased
Multinucleation is common	Single very large nuclei and binucleate forms may be found due to incomplete division
Cytoplasmic polychromasia	Cytoplasmic basophilia more common
Changes common in intermediate cells	Changes occur in intermediate, parabasal, metaplastic and endocervical cells
Ingestion of small squamous cells and polymorphs in intermediate cells.	Ingestion of other cells by intermediate and parabasal cells
Cells usually single	Incomplete cellular division ranging from common cell wall to inter-cellular bridge and sheets may be found

TABLE XX Differentiation of HPV infection and folate deficiency (FAD) in the presence of progesterone effect

HPV	FAD
Perinuclear clearing sharply defined.	Infrequent and diffuse if present.
Binucleation common	Single, binucleate and multinucleate cells
Diffuse, centrally situated keratinisation	No keratinisation but amphophilia may be found.
Kerato-hyaline granules	Not found
Not found	Ingested cells
No nuclear grooving	Nuclear grooving

8.5. CERVICAL INTRAEPITHELIAL NEOPLASIA (CIN)

8.5.1 Diagnostic criteria

8.5.1.1 CIN 1

Mature squamous cells of superficial and intermediate type with enlarged slightly hyperchromatic nuclei (Koss 1979) (PLATE 45).

8.5.1.2 CIN 2

Above features with the addition of parabasal and metaplastic cells. Nuclear features include enlargement, slight irregularity of outline and increasing chromatin granularity with consequent moderate hyperchromasia.

An increased nuclear/cytoplasmic ratio is observable in the smaller cells (Koss 1979) (PLATE 46).

8.5.2 Effect of MPA therapy

Reduction of epithelial maturity results in the CIN changes occurring in immature squamous cells. Large cells of intermediate and parabasal type are found which contain moderately hyperchromatic enlarged nuclei which nevertheless retain an entire nuclear border (PLATES 47 and 48).

8.5.3 Potential for diagnostic error

The presentation of dysplastic changes in immature cells may result in an erroneous diagnosis of CIS/CIN3. The problem is the classic one of dysplasia in an atrophic or immature epthelium and may be resolved through further evaluation of the cytological changes after topical administration of oestrogen. The critical diagnostic features are the composition of the chromatin which should not display coarse granularity or parachromatin clearing and the absence of an interrupted nuclear border which is characteristic of CIS. The changes may also be confused with those arising from a non-keratinising squamous carcinoma. In this case, the absence of nucleoli and granular chromatin will serve to differentiate CIN from an invasive carcinoma.

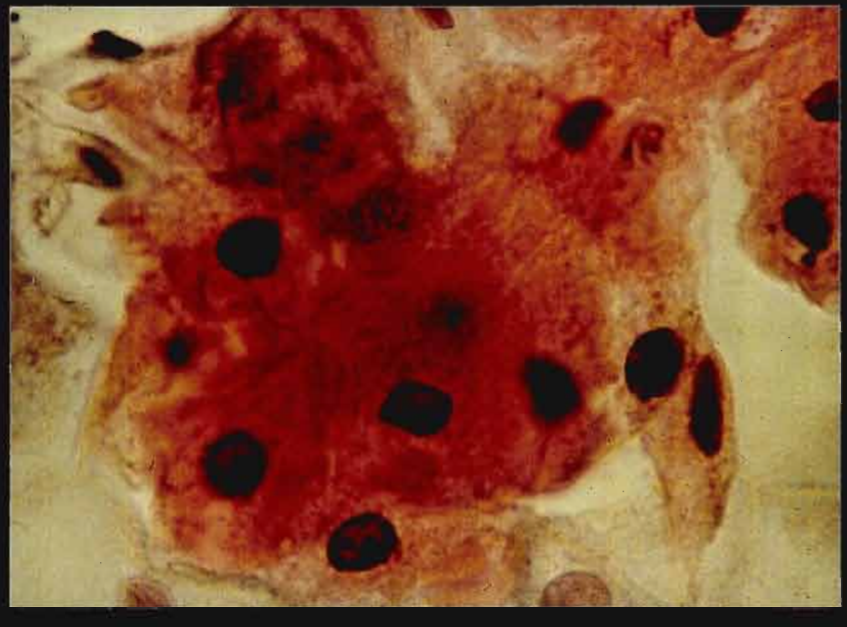
One of the major diagnostic pitfalls is the incorrect classification of dysplastic basal cell hyperplasia in the presence of MPA therapy. Large sheets of proliferative, small, hyperchromatic cells with scanty cytoplasm are found which may mimic CIS, adenocarcinoma, or invasive squamous carcinoma, being slightly larger than normal. These sheets

PLATE 45

Mature squamous cells showing features of CIN 1 (x400).

PLATE 46

Squamous intermediate cells derived from CIN 2 (x400).



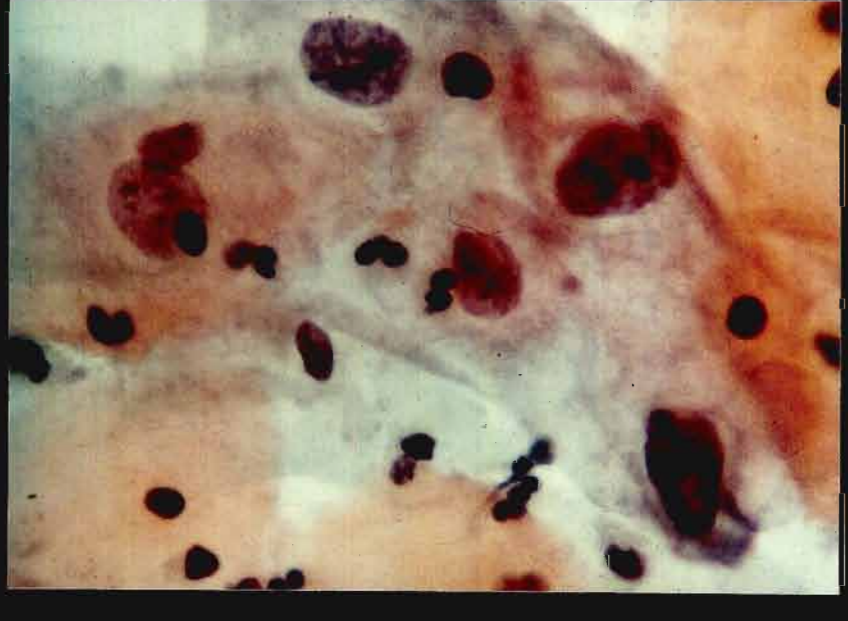
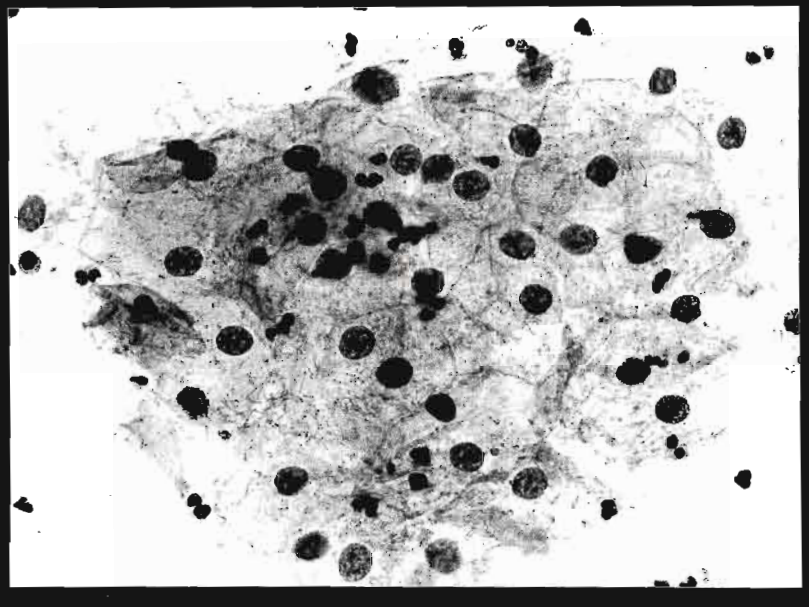


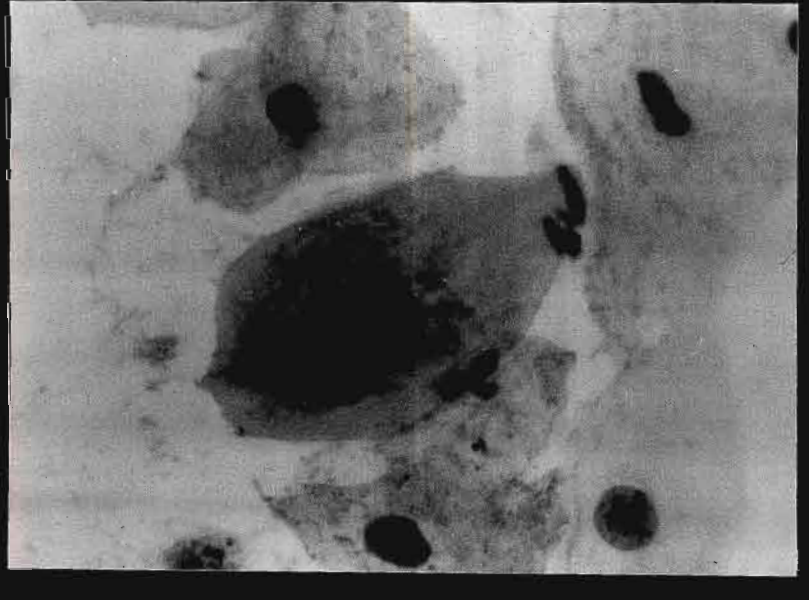
PLATE 47

Cellular changes of CIN 1 modified by progesterone (x100).

PLATE 48

An intermediate cell showing progesterone related change and features of CIN 2 (x400).





should be carefully examined for evidence of progression to squamous differentiation which will allow a correct assessment to be made (PLATE 49).

8.6 CARCINOMA IN SITU (CIS/CIN 3)

8.6.1 Diagnostic criteria

Absence of differentiation.

Coarsely granular chromatin.

Irregular nuclear outline with marked indentations and interrupted nuclear membrane.

Small cell size comparable to that of immature squamous metaplasia.

Scanty cytoplasm and markedly increased relative nuclear area.

Cells may present in a syncytial arrangement (PLATE 50).

8.6.2 Effect of MPA therapy

This is similar to that found in lesser degrees of dysplasia. In addition, because of the intrinsic lack of differentiation in this condition, delayed maturation caused by progesterone results in transitional forms which may give rise to diagnostic problems. Cells may also be larger and possess more cytoplasm than usual.

8.6.3 Potential for diagnostic error

The equivocal differentiation may result in this condition being classified as an endocervical adenocarcinoma. Retarded maturation results in many nuclear columnar epithelium characteristics being retained in the dysplastic metaplastic cells (PLATES 51 and 52). Significant differential diagnostic features are nuclear indentation, cytoplasmic features consistent with a squamous origin and chromatin condensation.

8.7 INVASIVE CARCINOMA.

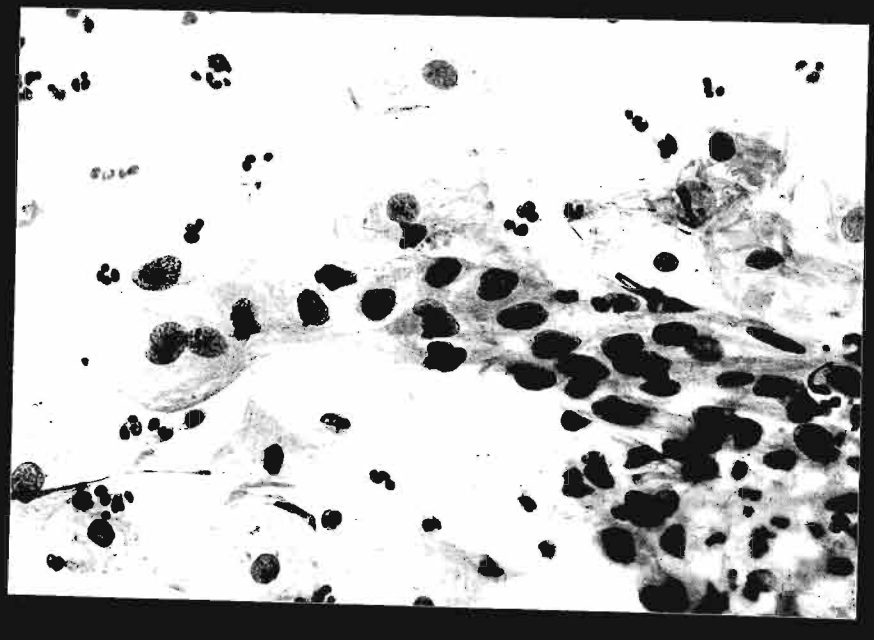
The three types of invasive carcinoma most commonly encountered in women of the age group under study are keratinising and non-keratinising squamous cell carcinoma and adenocarcinoma of the endocervix.

PLATE 49

Basal cell hyperplasia showing dysplastic change equivalent to CIN 2 in the presence of MPA (x100).

PLATE 50

Cells derived from squamous carcinoma in situ (x100).



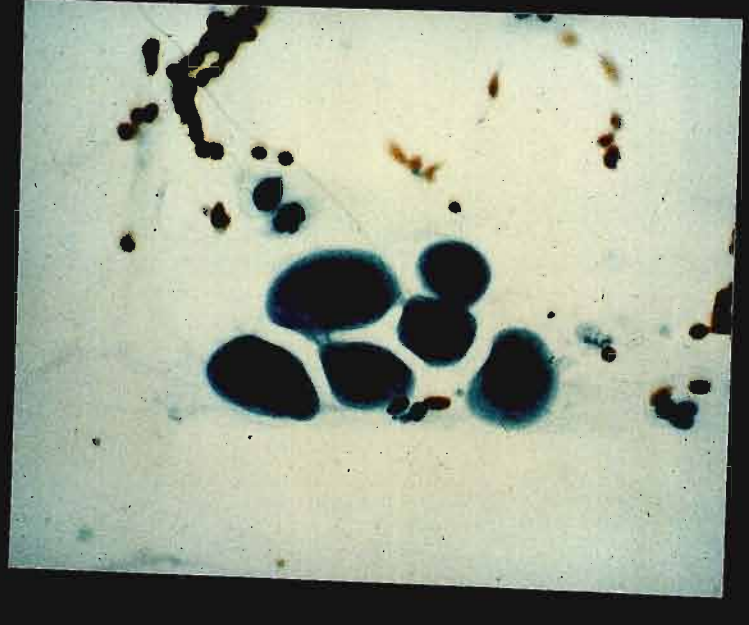
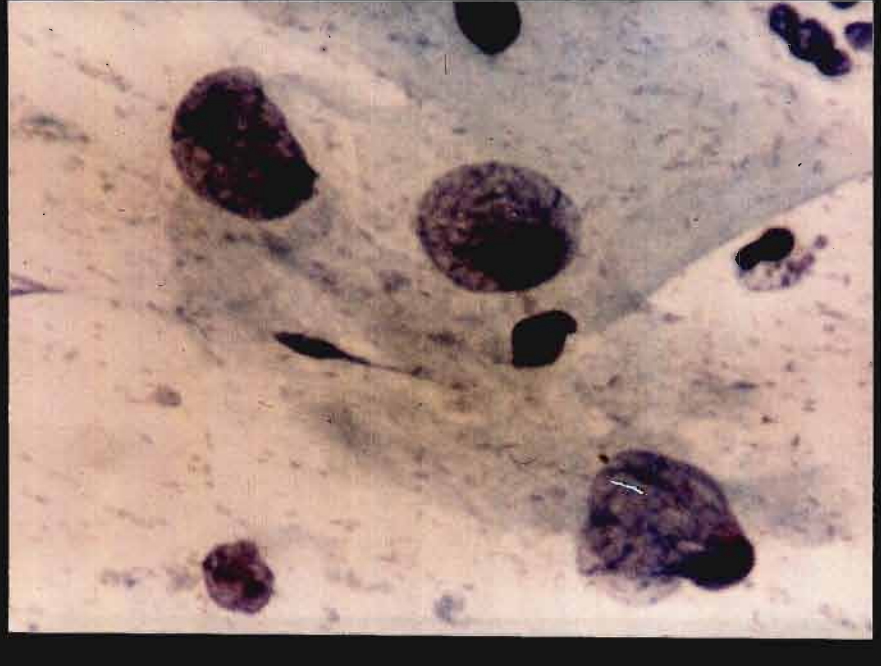


PLATE 51

Cells derived from squamous carcinoma in situ showing retarded differentiation and progesterone modification (x400).

PLATE 52

Cells derived from squamous carcinoma in situ showing equivocal differentiation (x400).



8.7.1 Diagnostic criteria

8.7.1.1 Keratinising squamous cell carcinoma (KSCC)

Orangeophilic cytoplasm.
Opaque, hyperchromatic nuclei.
Increased relative nuclear area.
Bizarre forms eg. caudate.
Marked variation in cell size but predominantly large.
Mainly isolated cells.

8.7.1.2 Non-keratinising squamous cell carcinoma (NKSCC)

Cyanophilic cytoplasmic staining reaction.
Large nuclei with coarsely granular chromatin.
Multiple large eosinophilic nucleoli.
Cells occur singly or in syncytia.
Cytoplasmic lysis is common.
Tumour diathesis is commonly found.

8.7.1.3 Adenocarcinoma

Predominantly eosinophilic cytoplasm which is often granular in texture with occasional vacuolisation.
Enlarged oval or round nuclei with multiple micronucleoli or macronucleoli.
Eccentrically placed nuclei.
Finely or coarsely granular chromatin which may be irregular.
Rosettes, cell masses, sheets, papillary formations and syncytia with evidence of overcrowding due to a decrease in cytoplasm.
Occasional palisade cell arrangement.
Dissociation of cells and numerous stripped nuclei.

8.7.2 Effect of MPA therapy

The effect on malignant cells from invasive tumours is principally to distort the abnormal forms commonly encountered i.e. cells will be larger, may ingest polymorphs and other cells, show equivocal differentiation and staining and possess massive nucleoli due to inefficient cell division.
Increased cell adhesion may be a confusing fact in those malignancies in which dissociation is a feature.

8.7.3 Potential for diagnostic error

Due to the merging of differential features caused by the progesterone the potential for error in invasive carcinomas lies mainly in the typing of tumours rather than the diagnosis of malignancy.

Due to occasionally gross cellular enlargement, bizarre forms may suggest a sarcoma or a sarcomatous component (PLATES 53 and 54). This is commonly observed in a keratinising squamous carcinoma in which the absence of nucleoli may be a helpful diagnostic criterion. The absence of orangeophilic cytoplasmic staining due to increased immaturity may also cause this tumour type to be incorrectly classified.

Non-keratinising large cell squamous tumours frequently display columnar nuclear features which may lead to an incorrect diagnosis of adenocarcinoma (PLATE 55).

The presence of centrally placed nuclei and characteristically finely granular chromatin distribution will assist diagnosis.

Adenocarcinomas of the endocervix are most likely to be confused with non-keratinising squamous carcinomas. One of the most useful differential features is the eccentric position of the nuclei (PLATE 56). An added difficulty is the large size of the cells especially when these are found singly. Nuclear protrusions are common and nucleoli may adopt bizarre forms but are not specific to this tumour type.

8.8 MULTIPLE PATHOLOGIES

Because of the increasing prevalence of HPV infection and the potential for folate deficiency to be triggered by MPA at end organ level, a combination of one or more of these conditions may be found in association with pre-malignant and malignant disease processes.

Many of these cases will pose diagnostic difficulties as already described and when complicated by co-existing pathologies great care should be taken in analysing the cellular changes seen.

8.8.1 HPV infection and folate deficiency

The combination of HPV infection and folate deficiency in the presence of progesterone may produce giant cells with ingested polymorphs, multinucleation, abundant cytoplasm and perinuclear vacuolation (PLATE 57). Immature cells displaying minimal HPV changes in the presence of incomplete cell division are also found (PLATE 58).

PLATE 53

Bizarre malignant cell derived from a non-keratinising squamous carcinoma in a progesterone modified epithelium (x400).

PLATE 54

Giant malignant cell with basophilic cytoplasm derived from a well differentiated squamous cell carcinoma (x100).

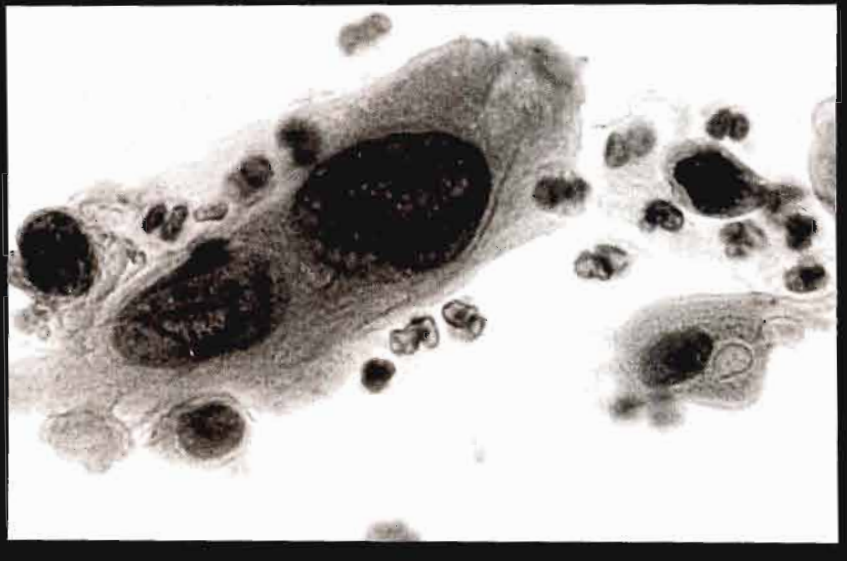


PLATE 55

Malignant cells from a non-keratinising squamous cell carcinoma display columnar nuclear features (x400).

PLATE 56

Cells from adenocarcinoma of the endocervix show typical eccentric nuclear position as well as cellular hypertrophy and evidence of retarded cell division (x400).

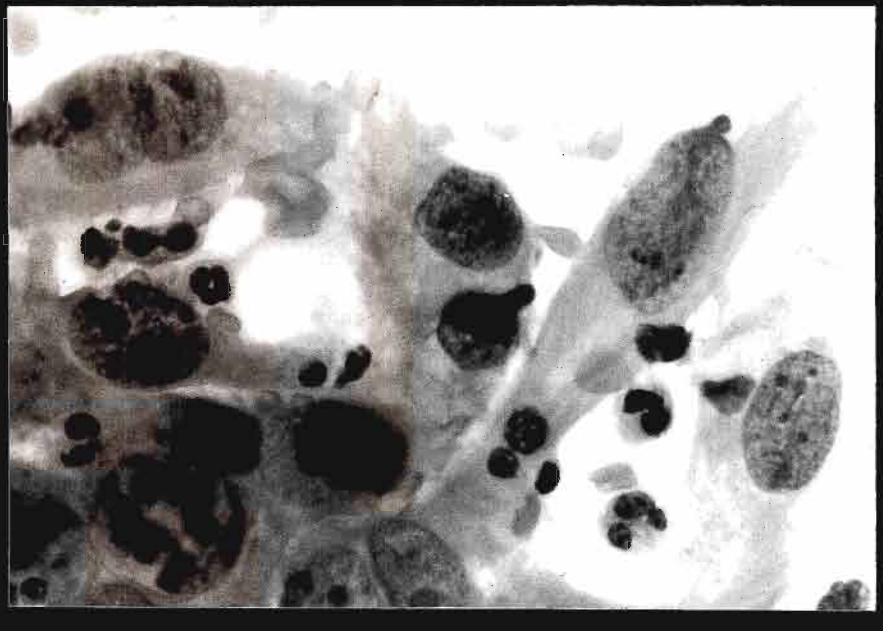


PLATE 57

In the presence of progesterone cells display hypertrophy, phagocytosis, multinucleation and perinuclear vacuolation due to HPV infection and folate deficiency (x100).

PLATE 58

Incomplete immature intermediate cell division due to progesterone, with associated HPV infection change (x400).

8.8.2 HPV infection and CIN 2

Immature cells are present displaying the classic features of HPV infection i.e. binucleation and perinuclear clearing as well as moderate hyperchromasia. There is an increase in nuclear chromatin density and clumping accompanied by a moderate increase in the relative nuclear area and mild irregularities of the nuclear border. Because of the overall decrease in maturity these changes may be incorrectly assessed as CIN 3 or carcinoma in situ (PLATES 59 and 60).

8.8.3 HPV infection, folate deficiency and CIN 2

In this instance giant cells may be encountered containing ingested small cells and/or polymorphs. Multinucleation is common and nuclei show hyperchromasia, chromatin clumping, slight nuclear border irregularity and perinuclear clearing. There is abundant cytoplasm and the relative nuclear area is slightly to moderately increased (PLATE 61).

Bizarre, binucleate or multinucleated cells may be found which contain hyperchromatic nuclei with irregular chromatin clumping. The abundant cytoplasm may show partial keratinisation typical of HPV infected cells in the presence of a high progesterone level (PLATE 62).

8.8.4 HPV infection and CIN 3

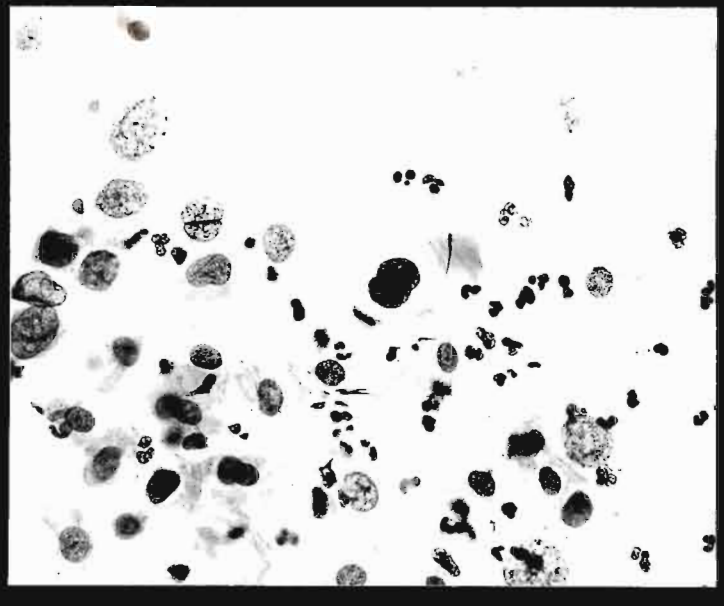
Syncytial groups commonly associated with CIN 3 will display more cytoplasm than usually found. Nuclear border irregularities and anisokaryosis remain useful diagnostic features. Perinuclear clearing is minimal and may be subnuclear. Nuclear grooving when present indicates slowed cellular division although there are indications of crowding and overlapping (PLATE 63).

PLATE 59

CIN 2 and HPV changes in immature squamous cells (x100).

PLATE 60

Immature squamous cells in CIN 2 and HPV infection showing increased relative nuclear area due to immaturity resulting from increased progesterone activity (x100).



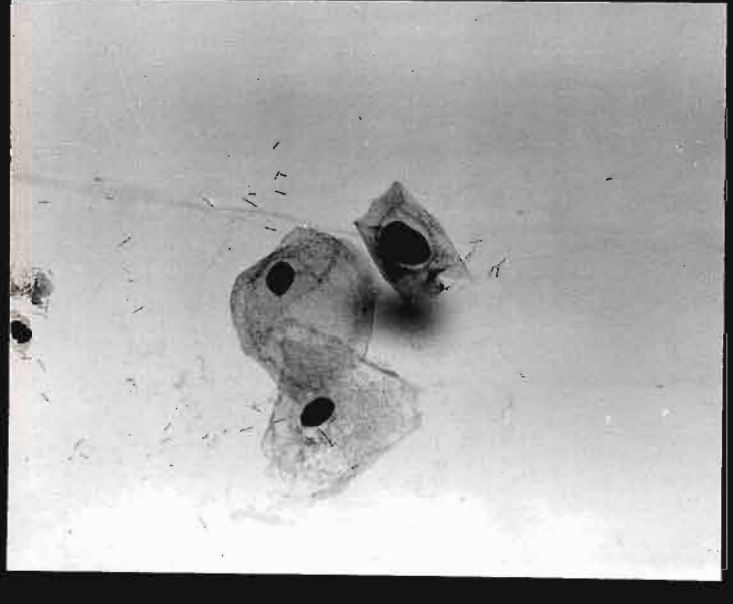
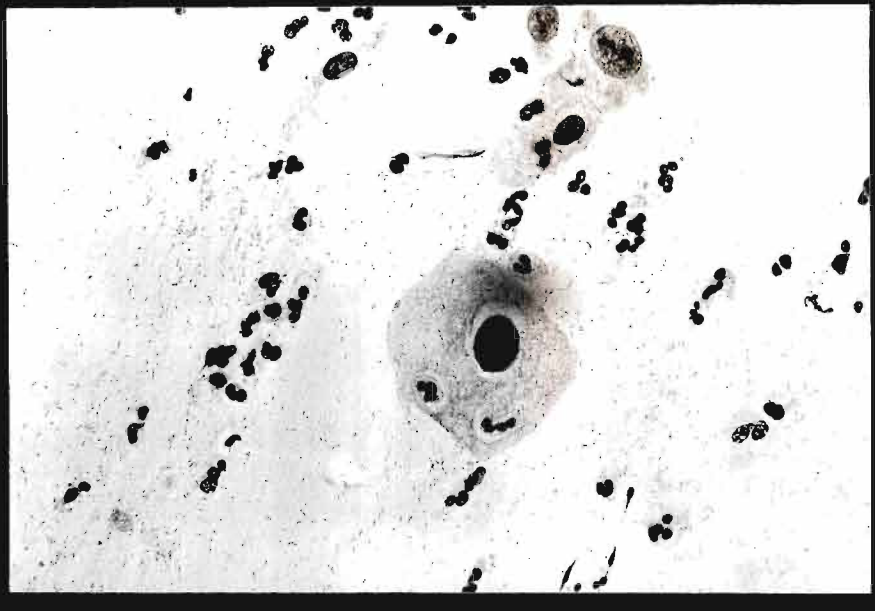


PLATE 61

Hypertrophic squamous cell with perinuclear clearing, moderate hyperchromasia, nuclear border irregularity and phagocytosis in HPV infection associated with CIN 2 and folate deficiency in the presence of raised progesterone levels (x100).

PLATE 62

Partial keratinisation, hypertrophy and nuclear irregularity in HPV infection associated with CIN 2 and folate deficiency in the presence of increased progesterone (x100).



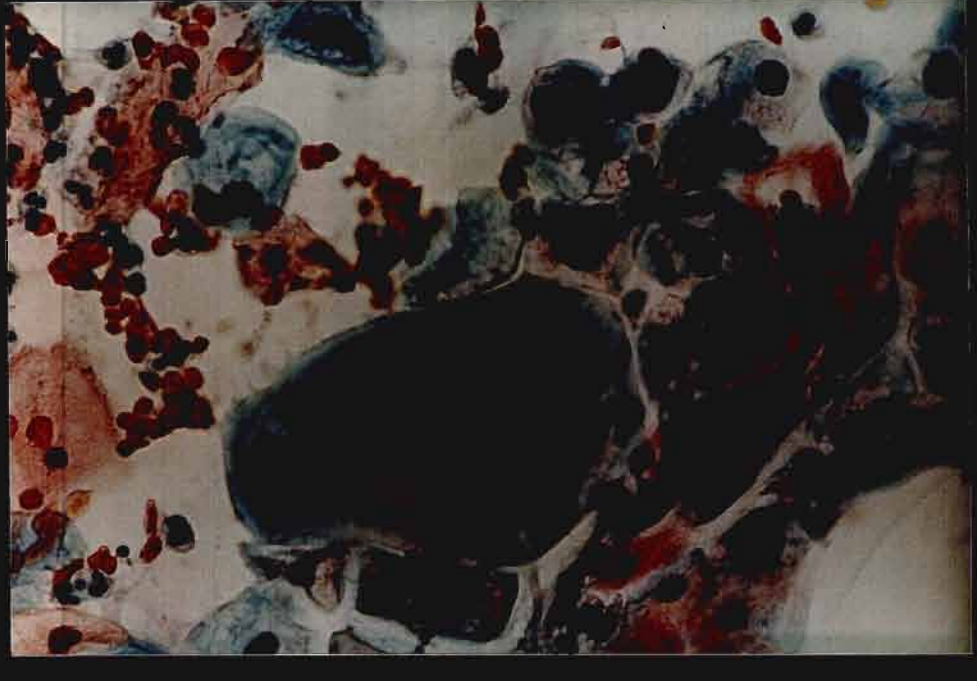
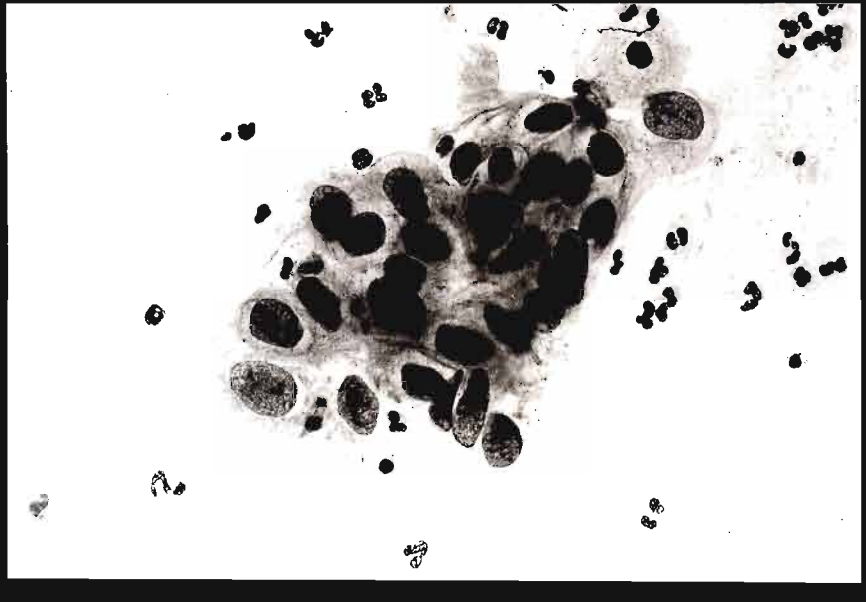


PLATE 63

CIN 3 with associated HPV infection in the presence of a high progesterone level. Cells show nuclear irregularity, anisocytosis, nuclear grooving and a moderate amount of cytoplasm (x100).



CHAPTER NINE

CONCLUSIONS

This study represents the first comprehensive research undertaken to investigate, identify and explain the effect of DMPA and similar contraceptives on the epithelium of the female genital tract. There were two major objectives. The first was to establish a normal range of cellular change related to the use of long term injectable progesterone-only contraceptives, particularly DMPA. The second was to define such change in a range of abnormal conditions commonly occurring in the female genital tract.

As a result of the findings, new clinically significant diagnostic criteria have been established. Although these will be primarily of value in populations of women using progesterone contraceptives they will also be applicable in other low oestrogen states such as pregnancy and late menopause. Considerable benefit is anticipated from their implementation.

The most important effect of this study will be to improve the accuracy of the cytological assessment of cervical smears by making it possible to identify progesterone related change, both on its own and in the presence of disease processes. The new criteria will provide the potential for distinguishing progesterone change from intraepithelial neoplasia and will facilitate the separate identification of co-existing conditions in the presence of progesterone.

These findings also provide a counter argument to occasional widely publicised reports of dysplastic change associated with the use of DMPA. It is highly likely that such reports may have been due to the misinterpretation of the changes encountered as a result of the use of this contraceptive, either with or without the complicating factor of co-existing pathology.

The contraceptive may be expected to produce many of the effects of pregnancy and since one of these is a diminished immunologic defence system, an increased susceptibility to sexually transmitted and other diseases may occur (Meisels 1992). It is a well known fact that certain types of HPV have been credited with carcinogenic properties. The

identification of all such cases is therefore clinically important. The polymerase chain reaction and related techniques (de Villers et al. 1987) have demonstrated this virus in a high percentage of cervices in women of child bearing age but visual identication has lagged behind. With the demonstration of the minimal cellular changes which may be found in immature squamous epithelium, reporting of this condition may be expected to increase in frequency. Still to be established is what effect DMPA might have on the replication and progression of the virus.

There were two particularly interesting and unexpected findings in the study. The first was the apparent differential multipotentiality displayed by endocervical columnar cells. The demonstrated potential for columnar endocervical cells to undergo squamous differentiation supports the work of Fluman (1961) in this regard. The second finding was the establishment of the origin and content of the nipple-like protrusions found in these cells. A source of worldwide controversy for many years, this problem was resolved through observations made possible by the action of progesterone which slowed the normal cell replication process to the extent where relevant structures in a transformation stage became clearly visible. Previously thought to be associated with either a degenerative or high oestrogen state this phenomenon has now been shown to be due to the increase in progesterone level and as such may prove to be useful in the future in determining the occurrence of ovulation.

An in depth examination of the biochemical action and influence of progesterone undertaken in the course of this investigation, established the point of its maximum concentration during the menstrual cycle as well as the effect of the hormone on the utilisation and absorption of folate, the latter having important implications for patient treatment. A potential area of research is the further investigation of the action of the contraceptive in interfering with other biochemical processes and pathways. It is known for instance that the action of progesterone leads to competition for occupation of the globin locus (Flavell and Martini 1982) and a connection may exist here with the development of porphyria in some users.

Further aspects worth investigating would be the DNA ploidy analysis of the enlarged nuclei found in hypertrophic cells displaying delayed maturation. Appropriate techniques would be flow cytometry or image

analysis using grey densitometry. Hormone receptor studies on cytological and histological material would also be of interest in determining whether fluctuations occur during the course of therapy.

Clarification of the molecular biology of equivocal differentiation could be obtained by flow cytometry and fluorescence microscopy using monoclonal antibodies as markers. The most likely of these to make a contribution towards resolving this problem would be antibodies against involucrin, a marker for normal suprabasal squamous differentiation and filaggrin, a protein only found in well differentiated (keratinised) epithelial cells. A further potential marker, bromodeoxyuridine (anti-BrdU), a thymidine analogue, is of particular interest through its ability to distinguish cells undergoing DNA synthesis and therefore the proportion of cells in the S phase of the cell cycle. This would permit evaluation of the extent to which progesterone prolongs the duration of the mitotic cycle through its inhibition of folate absorption.

The information so obtained would be of value in establishing the type and origin of dysplastic processes and might add significantly to the understanding of tumour morphogenesis. It has been demonstrated in this study that progesterone administration has a marked effect on restricting keratinisation. This has implications when assessing the histological grade of squamous carcinomas, in which keratinisation is a marker of differentiation. Retarded keratinisation may also lead to the lack of recognition of a squamous component in an adenosquamous carcinoma, leading to the erroneous classification of the tumour as an adenocarcinoma.

A question frequently raised regarding the use of DMPA is the reversibility of its action and effect. Although it was noted that some changes decreased in intensity after 9 months, the study was not long enough to demonstrate persistence or reversal of the cellular changes observed. In routine screening however, persistence of cellular effect has been noted up to 2 years after discontinuation of use with a reduced effect indicating a probable return to normal hormonal function. No evidence was found in the course of this investigation to support a permanent change and in fact the most marked effects were found within the first 6 months. A parallel study comparing progesterone blood levels with the incidence and prominence of cellular changes could supply prognostic i ndicators in this regard to establish the

significance of persistent change and its correlation with restored fertility.

The study highlighted certain aspects having implications for quality control and quality assurance with regard to the correlation of the results of routine cervical smear evaluation with other investigative methods. The evidence of the existence of hormonally modified differentiation at the transition zone of the uterine cervix explains some of the variability found in colposcopic, histologic and cytologic investigations which often take place at significant intervals of time. The absence of equivalent histological criteria is also seen as a contributory factor to the occurrence of discrepancies in reporting and it is recommended that this area be investigated. Specialised techniques such as flow cytometry may facilitate the definition of the newly established cytological criteria in histological terms.

Since the effect of DMPA varies from woman to woman according to menstrual and recent pregnancy status, it is evident that access to a comprehensive clinical history is vital for accurate cytological assessment. It is therefore recommended that all request forms for gynaecological cytology should make adequate provision for the insertion of relevant history including contraceptive use and any other type of therapy likely to produce cellular alteration.

Education of cytologically trained staff in the recognition of progesterone related cellular change is very necessary. It is strongly recommended that these criteria be introduced into the training curriculum of all those engaged in, or associated with, the practice of cytological diagnosis. Incorporation of the newly established criteria into the training system of a cytology unit processing approximately 60 000 cervical smears per annum has resulted in more accurate and efficient reporting. The service provided is consequently better directed to the needs of its immediate community of women, a large proportion of whom are on injectable progesterone contraceptives.

The aim of research must ultimately be to further knowledge along specific lines and preferably to make a contribution which will improve an existing situation. The results of this study are immediately applicable and have the potential to improve cytological reporting by increasing specificity and sensitivity. This has important implications

for the patient in that correct therapy and management may be selected and instituted. A related reduction in clinic visits, hospitalisation and repeat cytological screening can be expected to have long term benefits for the use and deployment of scarce material resources, equipment and trained personnel.

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REQUEST FORM:

NATAL PROVINCIAL ADMINISTRATION
REGIONAL PATHOLOGY LABORATORY

CYTOPATHOLOGY

USE BALL POINT, PRESS FIRMLY

SMEARS MUST NOT DRY BEFORE FIXATION

PATIENT PARTICULARS (Block letters please)

PREVIOUS LAB. No.	THERAPY	Hospital:	Hospital No.:	Ward/Dept.
	Oestrogen			
First specimen	Progesterone	Surname:		Age:
Repeat specimen	Androgen			
Vaginal	Digitalis	First name:		Sex: (Mark with X)
Cervical	Radiation			Male / Female
Endocervical	Other			
Sputum	Comment on radiologic and other clinical findings:			
Pleural fluid				
Ascitic fluid				
C.S.F.				
Urine				
Gastric				
F.N.A.B. SITE:				
Other (Specify)				

Signature of M.O. .....

Date specimen taken	Name of M.O.

FOR LABORATORY USE ONLY:	Screener	Checked	Date Received	Laboratory No.

Cervix	Normal	
Cervix	Abnormal	
Regular	menses	
Irregular	menses	
P.M.P.		
Parity		
L.M.P. commenced		
Biopsy taken		

REPORT

Signed .....

Stores Cat. No. 23-31950/F-589650/HJPDBN./08.92

APPENDIX B

PAPANICOLAOU METHOD

STAINING SCHEDULE FOR CERVICAL SMEARS

STATION	CONTENTS	TIME
1.	70% alcohol	2 minutes
2.	Water	30 seconds
3.	Water	30 seconds
4.	Gill's haematoxylin	4 minutes
5.	Water	30 seconds
6.	Water	30 seconds
7.	Scott's tap water	1 minute
8.	Water	30 seconds
9.	Water	30 seconds
10.	SVR	30 seconds
11.	SVR	30 seconds
12.	OG6	10 seconds
13.	SVR	30 seconds
14.	SVR	30 seconds
15.	EA 65	3 minutes
16.	SVR	30 seconds
17.	SVR	30 seconds
18.	Absolute alcohol	1 minute
19.	Absolute alcohol	30 seconds
20.	½ absolute & ½ xylol	30 seconds
21.	Xylol	30 seconds
22.	Xylol	30 seconds
23.	Xylol	30 seconds
24.	Xylol	30 seconds

APPENDIX C

GW 1/6.4.1

Departement van Gesondheid en Welsyn
Department of Health and Welfare

Streekdirekteur
Regional Director
Commercial City
Commercialweg/Road
Privaatsak X54318
Private Bag
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4000

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Dr V. Chrystal
Senior Specialist
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Pathology
Faculty of Medicine
University of Natal
King George V Avenue
DURBAN
4000

Navrae/Inquiries: Dr J. McDonald

Verwysing/Reference:

1986 -08- 28

Dear Dr Chrystal

REQUEST FOR INVESTIGATION OF PROGESTERONE RELATED CELLULAR CHANGE IN THE UTERINE CERVIX WITH PARTICULAR REFERENCE TO PROGESTERONE-ONLY CONTRACEPTIVES

STUDY BY MRS S. McCALLUM M.Sc (Med. Sci.)

I am pleased to inform you that the re-written protocol for the above study has been approved by Dr J.H.O. Pretorius. A copy of this protocol has been forwarded to Head Office, Department of Health and Population Development, Pretoria.

Enclosed are written details of patient selection and practical methodology to be conveyed to all clinic staff involved in taking the Pap smears for this study. These details are based on Criteria agreed upon by ourselves and Mrs McCullum at our last meeting and I trust will meet with your approval. A copy of the new protocol, and details of patient selection will be forwarded to Dr M. Richter City Health enabling local authority clinics to assist in the collection of Pap Smears for the study.

Yours sincerely

for REGIONAL DIRECTOR
NATIONAL HEALTH AND
POPULATION DEVELOPMENT : DURBAN

PRACTICAL DETAILS OF PATIENT SELECTION AND METHODOLOGY TO BE CONVEYED TO CLINIC STAFF

Aim of the study is to differentiate between changes that have previously been grouped together as dysplastic or atypical changed from a specificant, reversable progesterone induced cytological change.

PATIENT SELECTION

1. Only patients who have never had Depot/Nuristerate/Micronovum or Microval previously i.e. no previous progesterone agents.

2. Exclude patients who have and are being treated for megaloblastic anaemia.

3. Exclude patients on Epinutan (for epilepsy).

FORMS

The routine GW7/11 green cytopathology form alone is required plus a Green sticker and notation below the sticker saying "study". A green sticker to be placed temporarily on patients card.
All details on the form are to be completed with particular care in these patients. Additionally details of treatment also to be included on GW7/11 form eg. Septran, Flagyl, Ovral stating dose, for how long, and dates, medication was taken.

WHAT IS REQUIRED

First time acceptors of Depot Provera are to have 2 smears taken prior to their initial injection. One smear is from the lateral vaginal wall, the other from the cervix. These smears may still be taken if the patient is menstruating.

The patient should be asked to return to us every 3 months (when each injection is due) for repeat pap smears "to ensure the Depot is working correctly". Should she have one of her injections at another clinic it would be greatly appreciated if she could still return to us for her pap smear.

2 smears are to be taken at the initial visit, and after 3 months, 6 months, 9 months and 12 months. The vaginal smear marked "V" and the cervical one "C"

Should a patient have a florid vaginal infection eg. Trichomonas or purulant cervicitis she should be treated appropriately and reviewed one week later to ensure the discharge has resolved and take the pap smears.