AGE RELATED CHANGES IN THE HUMAN CERVIX UTERI WITH REFERENCE TO THE DEVELOPMENT OF NEOPLASIA

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M.D. THESIS

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FIGURES

SECTION ONE

The colposcopic study of the cervix at, and around, the menopause. The evaluation of the colposcope as an aid to standardisation of stigmata of hormone deficiency. The incidence and importance of the atypical transformation zone in the postmenopausal woman, and the limitations of available screening methods.

SECTION TWO

The microbiological environment of the cervix in CIN, human papilloma virus infections, and health. The variation in isolation of organisms with advancing age. The relationship of previous infection with certain organisms, in patients developing cervical cancer and matched controls. The concept of two forms of cervical cancer.

SECTION THREE

Structural study of the factors related to the apparent movement of the squamo-columnar junction. Biochemical investigation of the structure of the cervix at different ages, and the influence of exogenous oestrogens. Histological study of varying collagen presence, with advancing age.

INTRODUCTION AND ACKNOWLEDGEMENTS

The concept of this thesis, emerged when I was working as a registrar in Gynaecology and Obstetrics, at the Western Infirmary, and Queen Mothers Hospital, Glasgow. I had a concurrant commitment to colposcopy, gynaecological oncology, and menopausal clinics . A colposcopic study of postmenopausal women, who attended the menopausal clinic, was devised. The aim was to ascertain both the prevalence of the atypical transformation zone in the older woman, and the effect of hormone replacement therapy, on the colposcopic appearances of the cervix.

This study, however, raised further questions, and it was apparent that further work would have to be done. The surprise finding, of occult pre-invasive disease in an older population, has prompted questions, both on aetiology in the older patient, and on the screening aids available at present, for detection of neoplasia. General interest in a specific agent, as the initiator of cervical neoplasia, has never been greater, and the evidence is becoming very strong in the younger woman. The older woman, on the other hand, appeared to be so remote from the sexually transmissible agents, that I considered there may possibly be more than pathway for cervical cancer to develop. Age may be a co-factor, rather than a sexually transmissable agent. Working in a gynaecological oncology clinic, afforded an ideal opportunity to study putative aetiological agents, at a wide range of ages, in patients with cervical carcinoma.

Subsequent studies were devised, to attempt to answer the

questions raised by the colposcopic study on postmenopausal women. What were the differences in the cervix colposcopically in these older women, and what was the relevance to neoplasia? What were the microbiological differences, in the genital tract of older women, compared to the younger woman? In particular, was there any evidence of considerable prevalence of human papillomavirus, and what relevance did this have to neoplasia? Did the older woman with cervical cancer have the same genital pathogenic precursors as younger women, and has there been a change in prevalence of these possible precursors to neoplasia? Lastly, are there structural changes in the cervix, with advancing age, which are responsible for the problem of failure of diagnosis in the postmenopausal woman?

Such questions do not have easy answers, but there were interesting findings in each phase of this work. As the work lends itself into division into three parts, so the thesis is dealt with in three sections, each with two main studies. An introductory chapter, gives a historical overview of the background to each phase, and each study is described and discussed. A short summary of each phase is given after the papers.

All the studies were devised by myself, but the study on genital microbiological flora of patients at the colposcopy clinic, was devised jointly with Dr. Stephen Walkinshaw. Considerable help was given to me in the execution of the studies, and these are acknowledged forthwith. All work, (1983-4), and authorship, (1985-7), was carried out when I was working within the National Health Service. No grant was

employed for this work, and where laboratory services have been used,this was by virtue of the kindness of individual clinicians and scientists, to whom I am grateful.

ACKNOWLEDGEMENTS

I should like to thank Dr. James W. Cordiner, lately Senior Lecturer, Dept. Of Midwifery, University of Glasgow, and Consultant Obstetrician and Gynaecologist, for all the help and advice he has given, at all levels of this work.

CHAPTER 2

I recruited, councilled, and managed all the patients in this study, but I gratefully acknowledge the help of The Dept. of Cytology, Western Infirmary, Glasgow, who performed the cytology.

CHAPTER 3

I carried out all management of the patients, but am indebted to Dr. Rod Denholm, Lecturer, Dept. of Pathology, Western Infirmary, Glasgow, who performed histopathology and cytological review of all specimens. I am also grateful to Dr R.A. Burnett, Consultant in Administrative Charge, Dept of Pathology, Western Infirmary, and Dr. Clive Bouch, Consultant Pathologist, Leicester Royal Infirmary, for reviewing and advising on the histopathology. Histology was independently reviewed by Dr. Malcolm Anderson, Consultant in Gynaecological Pathology, Samaritan Hospital for Women, London, and I am most grateful to him for his help. I carried out all cytological review, for endocervical cells in all specimens, before and during therapy.

CHAPTER 4

This study was jointly devised as indicated above. Drs.

Stephen Walkinshaw and James Cordiner, recruited about 50% of the patients from the study group, and 13% of control patients, and I recruited the remainder. The cytology and pathology was performed by the depts. of Cytology and Pathology, Western Infirmary, Glasgow. The HSV isolation was performed by Dr. Joan Macnab and staff, at the Institute of Virology, Glasgow. The Chlamydia isolation, was by Dr. R. Sommerville and staff, at the Dept. of Virology, Belvidere Hospital, Glasgow. Bacterial isolation, was by Dr. Alastair Simmonds and staff, at the Dept. of Bacteriology, Western Infirmary, Glasgow. I am very grateful to all the above who gave help and advice so willingly.

CHAPTER 5

I obtained all specimens for study and control patients from this study. Drs. Macnab and Sommerville kindly performed antibody estimations.

CHAPTER 7

I obtained punch biopsies from the patients in this study, after written informed consent was obtained. Hysterectomy biopsies were obtained by myself, from patients undergoing this operation. I froze the specimens, and transported them to Dr. Ian Leggatt, Dept. of Biochemistry, Stobhill Hospital, Glasgow. He and his staff, performed the weighing and Hydroxyproline assay, and Dr. Leggatt's help and encouragement, were essential to this study.

CHAPTER 8

Dr. Rod Denholm and I, jointly selected the histological specimens for this study, and performed the study with the image analyser. Prof. W. Lee, advised on the use of this instrument

and I am indebted to both for help in this work.

Professor C.R. Whitfield and Dr. D.M. Hart have given considerable help and advice during the course of this thesis, and I am very grateful to them. I am also grateful to Professor J. MacVicar for his help and advice.

All data analysis, statistics and authorship have been performed by myself.

PUBLICATIONS AND PRESENTATIONS

The following parts of this thesis have been published, or are accepted for publication.

CERVICAL INTRAEPITHELIAL NEOPLASIA IN POSTMENOPAUSAL WOMEN WITH NEGATIVE CERVICAL CYTOLOGY. Roberts,A.D.G., Denholm,R.B., and Cordiner,J.W., Br. Med. J. (1985); 290:281.

THE VALUE OF CERVICAL COLPOSCOPIC ASSESSMENT IN THE MANAGEMENT OF GENITAL HORMONE DEFICIENCY. Roberts,A.D.G., Cordiner,J.W., and Hart,D.M., Colposcopy and Gynaecologic Laser Surgery (1986); 2: 141-6.

HUMAN PAPILLOMAVIRUS INFECTION OF THE UTERINE CERVIX OF WOMEN WITHOUT CYTOLOGICAL SIGNS OF NEOPLASIA. Walkinshaw,S.A., Roberts,A.D.G., and Cordiner,J.W., Br. Med. J. (1987); 294: 117-8.

AGE RELATED ISOLATION AND ANTIBODY STATUS TO CHLAMYDIA TRACHOMATIS. Roberts,A.D.G., Walkinshaw,S.A., and Cordiner,J.W., Proceedings of the Fifth World Congress on Human Reproduction, Athens, 1985. D. Aravantinos and G. Creatsas eds (1987) NOW PUBLISHED.

EVIDENCE FOR POSSIBLE INTERACTION BETWEEN VIRUS AND ENVIRONMENTAL RISK FACTORS IN WOMEN WITH CERVICAL INTRAEPITHELIAL NEOPLASIA. Walkinshaw,S.A., Roberts,A.D.G., and Cordiner,J.W., Colposcopy and Gynaecologic Laser Surgery. IN PRESS.

CHANGES IN CERVICAL COLLAGEN WITH AGE WITH RELEVANCE TO CERVICAL NEOPLASIA. Roberts,A.D.G., and Denholm,R.B., Contemporary Obstetrics and Gynaecology. G. Chamberlain ed. BY INVITATION

The author has presented various aspects of this work to the following learned societies:

to The American Society for Colposcopy and Cervical Pathology, Orlando, Florida 1984.

to The Fifth World Congress on Human Reproduction, Athens, 1985.

to The British Society for Colposcopy and Cervical Pathology, 1984 and 1987.

to The Nuffield Obstetrical and Gynaecological Society, 1985

to The Birmingham and Midland Obstetrical and Gynaecological Society, 1985.

to The Blair Bell research Society, 1986.

SUMMARY

This thesis,has attempted to indicate, that the cervix after the menopause,is a considerably different organ from that of the younger woman. A paradox now exists with carcinoma of the cervix, namely high incidences of pre-invasive disease in the younger woman, and low incidence of carcinoma, which contrasts with the situation in the older patient, who has high incidence of invasive disease, and low incidences of pre-invasive disease. This has led to the suspicion of two different aetiologies. The older patient may not progress through a detectable pre-invasive phase, as low rates of carcinoma in situ in the older woman would suggest (209). Thus, age itself, may be a co-variable in the genesis of disease (92,93). This questions whether the agents that are putative causes for cervical cancer, in the younger woman, are also aetiological factors in the older woman.

The work in chapter two, defined the colposcopic appearances of fifty peri- and postmenopausal women,who had symptoms of the climacteric, and were to be prescribed hormone replacement therapy (HRT). Colposcopic and cytologic variables were compared and related to serum analyses of oestradiol. A scoring system was devised, to enable the effect of HRT to be quantitated. HRT improved the colposcopic appearance of the cervix, and enabled the SCJ to be seen, when previously it was not visible.

Other work,carried out on the same patients, indicated that twelve of the fifty patients (24%),had a colposcopic abnormality or atypical transformation zone. Of greater alarm, was that four of these eleven patients,had histologically proven CIN, without

any cytological abnormality, despite repeated smearing. Doubt has been cast on the ability of these CIN lesions, in the postmenopausal patient ,to exfoliate abnormal cells, at least in the early grades of CIN found in this study. This has not been encouraging to the concept that cytology may be discontinued at the age of 60 years, if it has been negative up to that age (21).

The second section of the work of this thesis, considered the factors that have been proposed as causal to carcinoma of the cervix. A population of women with carcinoma of the cervix, were matched with a population of women of like age, parity, and socio-economic group, who had no previous evidence of cervical neoplasia. Three putative agents, which lend evidence of past infection were studied. No conclusive evidence could be found, to implicate any agent or combinations of agents. A trend, however, appeared with C. trachomatis and tumour differentiation, and it is possible that this agent, or combinations involving this agent, are implicated in more aggressive tumours.

Data on socio-economic grouping,proved crucial to the study. Seropositivity appears intimately related to S.E. group, and indicates the severe limitations of studies which do not have this information. Perhaps the greatest value of this work, lies in the illustration of seropositivity with age. The patient under the age of thirtyfive years, with carcinoma of the cervix, had much higher rates of seropositivity to all three agents, but especially to C. trachomatis and CMV, than the control patient. The older patient who had had carcinoma of the cervix, had

almost identical seropositivity rates to the control patients. This is further evidence that carcinoma in the older patient, may be of different origins to the carcinoma in the younger patient. One problem with these data, is the dominance of carcinoma among the older woman, thus burying any significant differences evident only in the younger woman, who have developed carcinoma.

The next section of the work, was to identify agents associated with CIN, and to compare these to a population of normal women, and healthy postmenopausal women. HPV was confirmed as an agent commonly associated with CIN, but no other agent was more commonly associated, than in the control group. The identification of a group with abnormal cytology and negative colposcopy, who had significantly greater microbiological isolation, gave support to cervical culture before referral to colposcopy, if such a service was limited. The postmenopausal women had higher isolations of vaginal pathogens, and an inexplicably high isolation rate of group B Streptococci. Surprisingly HRT did not reduce this reservoir of vaginal pathogens.

Having data available on both isolation and serology for the same patients, allowed closer examination of trends in Chlamydial infection with age. The conclusion from this evidence, is either that C. trachomatis is not detectable by the microimmunofluoresence technique in advancing age, or this organism is considerably more prevalent now, than in the past. This may still be a co-factor to HPV or smoking, in the genesis of carcinoma, in the younger woman with the aggressive tumour.

The final section of the work, has attempted to provide some explanation, for the difficulty in visualisation of the squamo-columnar junction (SCJ), that often accompanies age. Decreased cervical water, at the area around the SCJ was found. Hydroxyproline estimation suggests, that collagen is present in greater density, near the SCJ, in the older woman. The collagen content in the cervical stromal core, however, appears similar at all ages. A histological study, confirms increasing collagen density near the SCJ, with advancing age. HRT may increase cervical water, which may render the SCJ visible, by allowing greater eversion of the endocervix. This study found no effect on collagen by HRT.

It has been the aim of this work, to show the cervix in the older woman is different from that in the younger woman. The differences are in appearance, structure, microbiology, and prior vaginal pathogens. The differences may affect the ability to detect cervical neoplasia, and question whether cervical cancer in such older women, is truly the same disease as that in women of childbearing years.

CHAPTER 1

HISTORICAL REVIEW

A) SECTION 1 - CARCINOMA OF THE CERVIX IN THE POSTMENOPAUSAL PATIENT- A CAUSE FOR CONCERN?

B) SECTION 2 - FEATURES ASSOCIATED WITH AND POSSIBLY CAUSAL TO CARCINOMA OF THE CERVIX

C) SECTION 3 - VARIATION IN THE STRUCTURE OF THE HUMAN CERVIX WITH AGE

CARCINOMA OF THE CERVIX IN THE POSTMENOPAUSAL PATIENT- A CAUSE FOR CONCERN?

"With the exception of stopping the population from smoking, cervical cytological screening offers the only major proved public health measure for significantly reducing the burden of cancer today."

ICRF CO-ORDINATING COMMITTEE
ON CERVICAL SCREENING
(Br. Med. J. 1984;289:894-5)

Cytological screening of the cervix, has been practiced for almost twenty five years in Europe and America, and although initial promise of the eradication of cervical squamous carcinoma has not occurred, there has been an appreciable decrease in the incidences and the death rates from the disease (1,2,3,).

Those who have benefited to the greatest extent, are the women in the 35-54 year age group; the very patients, in whom the maximum effort has been expended to prevent the disease. Unfortunately, the women outwith this age range have not fared as well (4).

The increase in number of cervical smears, taken in England and Wales between 1965 and 1980, rose from 700,000 to 2,900,000, and yet there was only an overall fall in the death rate of 16% (5). Much of the explanation for this, lies in the women

younger, and older than the 35-54 year group.

Concern is now being expressed, at the alarming increase in the incidence of carcinoma of the cervix, in the patient under 35 years of age. British data indicate a three fold increase in the death rate for these women from 1965-1980 (5). The total numbers of cases in this young group is however, small, and greatly outweighed by the large improvement for the middle aged patient. It is a matter of speculation, what the incidence of carcinoma might have been, without screening in these younger patients.

There is now some evidence, to suggest cervical screening may not be so effective at preventing the deaths, in these young women. It is suggested that the transition time from normal squamous tissue to frank invasion, may be shorter than the recommended smear interval of 5, or even 2 years (6,7). It is possible that these tumours do not go through a prolonged pre-invasive phase, that is commoner in the older woman. The cervical cytology histories, of these young women who develop invasive carcinoma, frequently reveal negative cytology in the recent past (8,9,10,11). Series of such patients are usually very small; one however, reported 45 patients with invasive carcinoma under the age of 35 years. Almost all these patients had poorly differentiated lesions, and 26% had pelvic node involvement. Eighteen of these patients had negative cytology within the 6 years prior to diagnosis of carcinoma. The prognosis in this group was poor, and only 2 of the 9 patients with anaplastic tumours, were alive (8).

A similar German study, reported 15 of 87 patients with

invasive carcinoma,as having a negative smear and negative gynaecological examination, within one year of diagnosis. These patients had the highest incidence of pelvic node metastases (10).

Figures from cancer registrations, on the other hand, suggest an improved five year survival, for women under thirty five years. These data are based on cancer registrations of all stages, and include many with microinvasive carcinoma. The most recent figures available, indicate 5 year survival from cases diagnosed in 1978 (12). The situation for the younger woman is, however, dynamic; The number of cases of carcinoma of the cervix, registered in 1976, in women aged 25-34 years, was 348. Four years later, this number had increased to 492. There was also a 25% increase in the number of cases in women aged 35-44 years over this period, but women of other ages had similar rates in both years. There is also an extremely marked fall in the three year survival, of very young women under 25 years. It is apparent that the poor prognosis, recognised by individual gynaecologists, is now being reflected in the registry figures.

The rapidly progressive pattern of the disease in the young, has led to speculation that this may be a different form of tumour, but Anderson believes that these represent the malignant end of a continuous spectrum of neoplasia (13).

The concept, that there may be more than one form of invasive squamous carcinoma, with a different malignant potential, is not new; Ashley reintroduced the hypothesis in 1966 (14,15). He based his postulation on the bimodality of the death rate curve of the disease . A later report from Scandinavia, agreed in

principle with this theory (16), but others have not held the same view (17).

The precedent for considering more than one type of squamous tumour, depending on age, lies with tumours arising from other sites. Brain, eye, kidney, ovary and uterus, among others, are likely to harbour different tumours in the young, than in the old (16). Ashley's hypothesis, suggested tumour in old women, did not progress through the usual premalignant stages. Can the same thinking be applied to the tumours in the very young?

Anderson has suggested that some of these tumours on the cervix, may have arisen in the native squamous epithelium rather than in the transformation zone; the origin of carcinoma according to dogma (18).

Whatever the true progenitor cell or cells of carcinoma in the young, thought must be given to the possibility, that there has been a change in the natural history of cervical carcinoma. The increasing appreciation of the importance, of human papillomavirus in carcinogenesis, may reflect a true increase in the prevalence of this agent. Its greatest effect may have yet to be manifested.

One further explanation for this alarming increase in disease in the young, is the cohort effect (19,20); this is well known to be associated with high rates of carcinoma in the generations sexually active in times of social deprivation. There is some evidence, that women under 35 years in the mid nineteen seventies, will continue to have a high incidence of disease, but those born in later decades may have lower rates.

Although mortality trends in the young are worrying (12), the

numbers are very small when compared to the situation in the patient over 55 years. Here, there appears to have been a reduced impact of cervical screening (1,4). The effectiveness of screening in the 35-54 year age groups, has ensured that the mean age at diagnosis has risen, with women over 60 years now having the highest death rates from the disease (2).

There are several reasons why the death rate is higher in the older patient; Cervical screening is often not performed in the postmenopausal woman, in the belief that it is no longer necessary. This has the effect of delaying diagnosis until the disease is symptomatic. The re-convened Canadian Task Force on cervical screening, reaffirmed that screening was not necessary after the age of 60 years, if it had been repeatedly negative until that age (21). This is not synonymous with screening not being necessary in this age group; Macgregor and Teper, in presenting results from their screening campaign, concede that better results could be obtained, by the screening of more women over 55 years (1).

Lack of screening activity, is also due to a reduction in the belief in the test, in the older patient; Often the entire transformation zone cannot be smeared, as the squamo-columnar junction is in the endocervical canal, and any material obtained, may be too scanty for valuable comment. Failure to obtain appropriate cellular material, renders a smear inadequate. Some laboratories report the lack of endocervical cells, as an indication of limitation of the smear (7,22). Husain gives an excellent account of the difficulties encountered by the cytologist, in assessing whether a smear is

worthy of being classed as negative (23). Scandinavian data however, fully supports the value of the cervical smear, in the older patient.

The screening records of the Scandinavian countries, are to be envied; by means of population registers, stable populations, and efficient central computerisation of results, figures are obtained which are not available in other countries. Programmes were often aimed at all women up to 70 years of age, and one report suggested, that over 80% of women between 60 and 70 years of age, were screened (24). For cytological screening to be effective, the frequency of invasive disease, should fall as much in the older screened patient, as the younger woman. Although the incidence fell dramatically, even in such a well screened population, there was still a frequency of 30 cases of cervical cancer per 100,000 women. Such frequencies are attributed to carcinoma arising in the women who did not take part in the screening programme.

Recently a Swedish report cited an incidence of 16 per 100,000 women between 60 and 69 years, in whom there was evidence of at least one negative smear in the preceding 10 years (25).This rate was considerably higher, than rates for younger women who had been screened. This evidence indicates, although cytological screening reduces incidences of cervical carcinoma, it may be less effective in the older patient.

Negative cervical cytology, shortly preceding the development of invasive squamous carcinoma, is well documented (7,10,26,27). Often the explanation is obvious; Smear review accounts for many cases where positive smears are falsely interpreted (7,10).

Others are explained by patient or clinician, not acting on a positive report (6). Many, however, cannot be explained and postulations about rapidly growing tumours, are made (6). There is another explanation for this situation, which may be applicable to the postmenopausal patient with low oestrogen stimulus; Often in established cases of cancer, malignant cells are not exfoliated. In the older patient with dysplasia, possibly pre-invasive dysplastic cells are also not shed, and the smear may be reported as negative, in the presence of dysplasia. The insistance on seeing endocervical cells, to call a smear adequate, is a council of perfection, not often attainable in the postmenopausal woman (22).

Early work by Coppleson and Reid, suggests that a colposcopic examination, may increase diagnostic accuracy in cervical screening by 5%, in patients with lesions missed by cytology alone (28).

Older patients, often present with more advanced disease, due to awaiting symptoms, rather than availing themselves of screening (29). Routine cytology in the older patient, may reveal an invasive lesion, which has been caught at an earlier stage, than if symptoms were awaited. Screening the older patient, may thus decrease the chance of invasion, but also improve the death rate, which at present is considerably worse in the postmenopausal patient, than the younger woman (12). In terms of numbers, the major hurdle to be overcome by cervical screening, is now the reduction of cervical neoplasia in the older woman.

The foregoing discussion, has been based on the premise, that

cervical cancer is similar, whether in young or old. To study the converse, namely that there is a difference in the tumour at different ages, the aetiological factors, and their prevalence at different ages, need to be considered. The next section will discuss these agents.

FEATURES ASSOCIATED WITH AND POSSIBLY CAUSAL TO THE DEVELOPMENT CARCINOMA OF THE CERVIX UTERI.

No argument suggesting more than one type of squamous carcinoma of the cervix, would be tenable, without some investigation and discussion into the putative causal factors of the disease. In the past two decades, many agents have been postulated as having a role in the aetiology of neoplasia of the cervix. Many of the studies implicating such agents, have drawn data from younger patients. Data on possible precursors in older women are more scarce.

Whether infective agents are responsible or not, squamous cell carcinoma of the cervix, stands out as an example of a behaviour related disease. Although the rarity of the disease in nuns had been known for over 100 years (30), interest in the association with sexual behaviour, was stimulated by a report of rarity in virgins (31). Reports that exist, suggest that risk of the disease will increase in proportion to the number of sexual partners (32), and with earlier age at first coitus (33).

This latter risk factor, has led to the hypothesis, that it is age of the patient, that plays a factor in the genesis of cervical neoplasia. The exposure of immature epithelium to a mutagen, may render it unstable, and more likely to become neoplastic (34). A further view, suggests that an infective agent, with oncogenic potential, is more likely encountered with multiple sexual partners. Evidence of transmission of a factor involved in neoplasia, be it infective or chemical, is found in

unfortunate cases of sequential wives of the same man, developing carcinoma of the cervix (35). Such observations on the "high risk male", and also those indicating that male promiscuity will increase the risk to a monogymous partner (36), strengthen the case for a transmissable agent being an aetiological factor.

The search for an infective causal factor, while not apparently difficult, has been hampered by the numerous associations between sexually transmissable agents and cervical carcinoma. All such agents, will be more prevalent in a population with cervical carcinoma, than a control population. To suggest causation rather than association, has proved very difficult. Many workers in this field, now feel that human papillomavirus (HPV) is a likely culprit, but in the past other agents have also been seriously considered.

Treponema pallidum and Neisseria gonorrhoeae, are now infrequently found in association with carcinoma of the cervix. Trichomonas vaginalis, is now rarely investigated as a causal factor, but as yet it cannot yet be fully vindicated (37).

The first agent to have serious and intensive study, was herpes simplex virus (HSV). The observation of cytological changes, due to HSV, in greater prevalence among women with cervical dysplasia (38), heralded a new era in this search. Rawls et al., augmented the body of knowledge, by finding higher prevalence of antibodies to HSV II in cervical cancer patients than controls (39), and other reports have continued to confirm this association (40,41). Virus specific particles, have been identified in cervical neoplastic lesions (42), and mRNA

specific to HSV, was found more often in cancer biopsies, than control biopsies (43). HSV II, also exhibits two important characteristics; latency and transformation. It may remain latent in the neural ganglia (44,45), and it may transform mamalian cells, when incubated by ultraviolet light (46).

There has, however, been a great paucity of work, confirming HSV DNA in cervical cancer cell lines (47), but some work has advised vaccination against HSV, in an attempt to prevent cervical carcinoma. (48). The inability to conclusively prove HSV as a true causal factor, has led to the suggestion that HSV may be a co-factor in cervical cancer causation, rather than the sole agent (49,50).

Coincident with the realisation that HSV was not the sole agent in carcinogenesis, was the search for other factors. The cytomegalovirus (CMV) supplied an attractive model for carcinogenesis. This agent is sexually transmissible, able to transform cells in vitro (51), and has been isolated in an infectious form from cervical carcinoma (52). Serological studies draw conflicting conclusions; a study by Fucillo et.al., involving limited numbers, found no difference between the seropositivity in cervical cancer patients and matched controls (53). However, in another study around the same time, Vestergaard and colleagues did show significantly greater seropositivity among cancer patients (54). Such confusion, probably results from the natural prevalence of CMV antibodies in the normal population. Of young women under 16 years, only 40% will have antibodies to CMV, but this percentage rises steadily, such that 80% of women over 41 years, will have

evidence of previous infection (55).

Age is thus critical to any serological study involving CMV. Cytomegalovirus, being a herpesvirus, may yet play a part in cervical carcinogenesis. The interest in this agent in immunosuppression, and the observations of high rates of both CIN and CMV infections in renal transplant patients, raises suspicion; current work on this agent shows CMV DNA in tumour by restriction analysis (J.W. Cordiner personal communication).

All agents, at present accorded serious consideration as aetiological factors, are viruses, with the exception of Chlamydia trachomatis (56). Other genital bacteria, such as Mycoplasma hominis, and Ureaplasma urealyticum, have been studied, but no greater prevalences were found in cancer patients, compared to controls (57). Chlamydia is a Gram negative bacterial organism, which is intracellular during replication (58). It is responsible for blindness, with an estimated 500 million cases of trachoma worldwide. C trachomatis is also a genital pathogen, causing Lympho Granuloma Venereum, "non specific" lower genital tract infection (59), salpingitis (60) and cervicitis (61). The presence of the organism is related to sexual behaviour (60), and prevalence is high among promiscuous women, such as those with gonorrhea (62). The attraction of this organism as a causal agent, lies in its infectious sexual transmission (63), intracellular replication, and cervical reservoir of infection. The organism has been isolated in up to 16% of patients in early pregnancy (59).

The main body of data implicating C. trachomatis, comes from seroepidemiolical studies. Higher prevalences of antibodies to

C. trachomatis, have been found in patients with cervical dysplasia than controls (64,65,66,). There are however, several problems; C. trachomatis, being a sexually transmissable agent, is more likely to be prevalent in the population also at risk of cervical neoplasia, namely the more sexually active woman. A second less obvious factor, is whether the dysplasia associated with C. trachomatis, is truly neoplastic, or a form of inflammatory reaction. Hare et al., suggested that infection is associated with a follicular cervicitis (61), and such intense reactions may mimic CIN. Carr reports that mild changes thought to be CIN, may be due to C.trachomatis, and could be reversed with tetracycline therapy (67). This explanation may hold for the reported cases of regression of CIN, after application of local tetracycline (68,69). Genotypic studies, can now give more information; aneuploid lesions are neoplastic, and therefore less likely to regress. With these limitations in mind, the presence of current or past infection with C. trachomatis, may act as a marker indicating women at higher risk for cervical neoplasia.

The foregoing sexually transmissable agents have been overshadowed, for the role of the sole putative causal agent of carcinoma of the cervix; HPV has now the largest body of evidence to support its claim. No matter how strong the evidence incriminating this organism, however, there will be the potential for the role of other agents as co-factors.

Of the infective models for causation of cervical cancer, HPV fulfils many criteria. The virus is sexually transmitted (70). It has specific genital strains (71), which are genetically

different, from material from warts from other sites. Some of the subtypes of HPV have differing malignant potential. In particular, types 5 and 8 are found in epidermodysplasia verruciformis; a condition with a 30% chance of progression to malignancy (72). Some subtypes are associated with benign cervical infection (73,74), and others are associated with CIN (75,76,77).

The cytological changes associated with HPV, were described by Ayre as long ago as 1949 (78). But it was not until Meisels and Fortin categorised these as being due to HPV, that interest in the virus as an aetiological factor, was stimulated (79). The association with CIN, has now been confirmed by many authors (72,80).

HPV antigens have also been observed in CIN lesions. The frequency of observation, however, decreases with increasing severity of the CIN (81,82). Serological evidence does not answer the main questions surrounding the relationship between HPV infection, and CIN. This is mainly due to the inability to culture HPV, and hence raise an antibody that is specific enough to indicate previous genital wart, rather than plantar or common wart infection (72).

It has been mainly from genetic analysis, that the incriminating evidence has accumulated. In parallel with the decrease in the microscopic and immunohistochemical changes of HPV infection, with increasing grades of CIN, there has been increasing evidence of aneuploid lesions. Those lesions which appear warty, are likely to be diploid, with a transition through polyploidy in warty atypia, to aneuploidy in CIN (83).

DNA-DNA hybridisation studies, have revealed an association between HPV and both CIN and cervical carcinoma. This is very suggestive of a causal role. Hybridisation studies, suggest there are two varieties of HPV, that display homology with the DNA of the atypical condyloma. These are named types 6 and 11 (71). Although these may rarely be associated with cervical carcinoma, usually they are associated with lesser degrees of neoplasia. A report by McCance and colleagues, suggested the HPV DNA in cases of CIN, was present in a free form, and not integrated into the host DNA (84). A report in 1983, suggested that another HPV, type 16, was present in 61% of cervical cancers, but in only a small percentage of CIN lesions (85). A further subtype, type 18, has also been found in cervical cancers. Type 16 and type 18 HPV DNA may be integrated into the host genome, but there is also evidence that HPV DNA to type 16 and type 18, may be episomal in cases of CIN (86).

In addition to the evidence that the virus involves neoplastic tissue, there is exciting work to suggest that HPV may infect histologically normal tissue. Macnab and colleagues, have found DNA sequences which hybridise to HPV 16, from histologically normal tissue in patients with cervical cancer. The HPV 16 was also found, in histologically normal cervix from 11% of normal women (87). Like HSV (44,45), this work indicates that HPV may remain latent, or at least hidden.

Thus, there appears to be a logical progression, from cervical condyloma without atypia, through warty change with CIN, to carcinoma in situ. At this stage there is often no gross evidence of HPV infection, but the lesion may contain HPV 6 or

11 DNA episomally. The final progression, may be to invasive carcinoma, with HPV 16 or 18 DNA integrated into the host DNA. Whether HPV 16 and 18, are the same virus as HPV 6 or 11, but altered by the process of integration, is not yet known. With the information available at present, it seems that HPV gives a good model for cervical carcinogenesis, but not all women with genital HPV develop cervical neoplasia, and other factors must be important.

Recently, there has been interest in the role of cigarette smoking in cervical cancer (88). It appears that risk of neoplasia increases with heaviness of smoking, and duration of smoking (89). Nicotine is now considered a co-factor in carcinogenesis, and both this, and some of the other agents previously mentioned, may also act with HPV in a synergistic process which results in carcinoma. The strong evidence implicating HSV type 2, and the paucity of isolation of DNA homologous to HSV in cervical tumours (47), has led to the postulation of HSV as a "hit and run" agent ; HSV transforms the epithelium, but does not remain in the tissue (50). zur Hausen has proposed a synergistic process, in which a mutagen, whether it be HSV or nicotine, or some other agent, transforms the epithelium and renders it susceptible to malignant change, when subsequently infected with HPV (49).

This is as yet a hypothesis, but there are animal models supporting it; interaction between bovine papilloma virus type 4, and a substance quercetin, in cattle grazing on bracken fern, induces intestinal cancer (90). Also, chemical carcinogens are synergistic, in the conversion of Shope papilloma virus

papillomas into carcinoma, in rabbits (91).

Cancer, by virtue of the increased prevalence in the old, must have age as a co-factor. It has been suggested that the inflection with age, in the bimodality noted in the death rate from cervical cancer, by Ashley (14,15), is related to the process of ageing (92,93). Thus age itself, may be as important a co-factor as others previously mentioned. Where age fits into the above hypothesis, is not yet clear. Evidence of HPV infection in the older woman is unusual, but the true prevalence of infection decade by decade is not known. It is commoner in the 20-30 year decade than the 30-40 year decade, but does it confer risk if, after the acute infection, the epithelium is still normal?

If cancer (or pre-cancer), of the cervix, is dependent on a cascade of events, how does this apply to the postmenopausal woman? She may not have had intercourse for many years, or contact with these risk agents since her youth, and yet she maintains a high incidence of the disease. Do these agents remain dormant? HSV may remain latent in the dorsal root ganglion of spinal nerves (45), but this property in HPV has still to be fully proven. Generally, older women smoke less, have fewer sexual pathogens, and yet a higher rate of cervical cancer. Has cervical cancer, in the older patient, a different or modified series of initiators and promotors?

The following introduction, approaches the problem of high mortality in the older woman, from a different standpoint; If the aetiological factors for carcinoma of the cervix, are the same in the older patient, then the failure to reduce mortality,

may be due to a structural change in the cervix. This may alter the efficacy of the available screening, and diagnostic aids, used in the detection of cervical carcinoma.

VARIATION IN THE STRUCTURE OF THE HUMAN CERVIX WITH AGE

It is only now, in the latter part of the twentieth century, that most women live the majority of their lives, without functional reproductive organs. Life expectancy in the past, usually ensured most women died before reaching the menopause. The reproductive system, is the only bodily system that can cease functioning without death. Consequently, postmenopausally, the internal genital organs have no function, and are frequently the site of pathology.

Changes in ovarian follicular and endometrial activity, are paramount to a woman who becomes aware of the climacteric. There are, however, also changes in the cervix. The endocervical glands are under hormonal control, and during the menstrual cycle do have a cyclical activity, with increase in the height of the columnar epithelium, under the influence of oestradiol (94). Similarly, gross hyperplasia of the endocervical glands, may occur during pregnancy, resulting in an endocervical prolapse (95). This activity, is greatly reduced after the menopause, and mucoid discharge may disappear altogether. The epithelium of the cervix will become thinner, and a less adequate barrier to infection. Cervical bulk will diminish, resulting in the typical cervix of the postmenopausal woman, small,dry and fragile (96).

The cervix uteri has been recognised as a separate entity to the uterine corpus, since the first century A.D. (97). It consists of a vaginal and a supravaginal portion, and for the

purposes of study, can be considered to consist of stroma, an epithelium and endocervical glands. It is in the epithelium that neoplasia arises, but stromal changes may be responsibile for the difficulty in identifying these changes in the postmenopausal patient. The stroma consists predominately of fibrous tissue, with 10-15% smooth muscle (98). The muscle content is variable, depending on the site in the cervix; 28% in the upper third, 18% in the middle third, and 6% in the lower third (99).

In the immediate subepithelial layer, there is a capillary plexus, and this region has more vascular channels, than deeper in the cervical stroma. Lymphatic vessels and neural elements, are found in the subepithelial layer. Histological analysis, confirms greater neural tissue in the cervical isthmus, but fibres can still be seen in the distal cervix, where there is limited smooth muscle. These fibres are known to be lacking in the older woman, and probably regress with age (100). There is also evidence for elastin in the human cervix (101), but the main constituents of cervical stroma are collagen, ground substance, and water. These will be discussed in greater depth later.

A major problem in the older woman, is the failure to visualise colposcopically, or cytologically sample the entire transformation zone . The vaginal portion of the cervix, is covered mainly by squamous epithelium, and the transition to columnar epithelium, may either be abrupt or gradual (102). All epithelium that is metaplastic squamous in type, i.e. not native squamous or native columnar, constitutes the transformation

zone, and may be the site of neoplasia. The landmark of the squamo-columnar junction, is often not visible in the older woman (103), but the reasons for this are not easily explained. The process of squamous metaplasia, physiologically transforms all vaginally exposed columnar epithelium to squamous epithelium, due to the effect of the increased acidity of the vagina. The atrophy that accompanies the menopause, ensures this junction recedes into the cervical canal. The influence of the cervical stroma on this retraction, is not well documented. Cartier suggests that the stroma is more dense, with a reduced blood supply. He indicates the cervix is fibrosed rather than oedematous, when compared to the younger woman (104). Novak, illustrates a diminution in volume of the cervix, as inducing the squamo-columnar junction to recede into the endocervix (105). It would thus appear, that the diminution in bulk of the cervix, due to stromal volume changes, may be partly responsible.

The size of the cervix is determined by stromal volume, but deep in the stroma of the menstruating woman, are endocervical glands (106). In times of high steroid levels such as pregnancy, these may hypertrophy, and add to cervical bulk (107). Consequently, being under hormonal influence, they will diminish after the menopause, and this will contribute to the decrease in cervical bulk.

The cervical stroma itself, is also under hormonal control. The cervix is more rigid in the luteal phase (108). There are short term changes induced by prostaglandins (109), and the cervical softening of late pregnancy (110,111,112), all indicate

the cervical stroma is hormonally sensitive. Oestrogen binding sites, have been found not only in cervical glands, but also in cervical stroma (113). However, the changes in the cervical stroma that accompany the hormonally depleted state after the menopause, are not well documented.

There is no doubt that there is an alteration in the volume of the cervix in the postmenopausal woman, and this is due to a change in the composition of the cervix. Most likely, there are decreases in cervical water content, ground substances, cellular elements, collagen, and smooth muscle.

Hughesdon (114) compiled meticulous records on the histology of the human cervix, under certain conditions, and at certain ages. He believed, like many previous authors, that the cervix contained an outer layer of smooth muscle, which comprised about 25% of the cervix. This was believed to have a physiological action, in the 'taking up' of the cervix in pre-labour. This layer enveloped a much more dense structure, composed largely of collagen. Danforth (98,115) took a different view, believing smooth muscle to be an ineffectual and unimportant structure, in comparison to collagen, as far as cervical dilatation was concerned. Hughesdon (114) however,believed that the difficulty in recognising smooth muscle amid a dense collagenous matrix, in addition to tangential cutting, served to reduce the apparent muscle presence, as indicated by histological examination. Ferroni (116) and others (117), in earlier histological studies, describe an inner layer of muscle sweeping round under the portio, to join the vagina.

It was believed that contraction of this submucous layer of

muscle, in the primigravid, would 'take up' the cervix without dilating the internal os. In the multigravid patient, Hughesdon thought, as the muscle had been disrupted by previous labour, contraction of the upper cervical muscle, would cause dilatation before full effacement.

Danforth (118), did not agree with this hypothesis on cervical effacement. He proposed that changes in collagen, and ground substance, would produce effacement and dilatation. He had shown that collagen is broken down and excreted in pre-labour and labour. He also found an increase in cervical water content, and this further reduced the density of collagen. In addition to these changes, Danforth and Buckingham (119), have identified amino acids appearing in late pregnancy and labour, thus suggesting, the formation of a new protein in the cervical stroma. The role of smooth muscle when the cervix is already changed by the diminution of collagen, is not known.

These obstetric observations, have relevance to the apparent movement of the squamo-columnar junction. The cervical stroma is under hormonal control in labour, and the location of the junction may be related to hormonal effects on the stroma. In the primigravid patient undergoing colposcopy, the squamo-columnar junction is easily visible at the external os. The multigravid patient, on the other hand, may have a gaping ectocervix but closed internal os. The effect of squamous metaplasia on this exposed columnar epithelium, would tend to site the junction high in the cervical canal, and when postnatal, this would regress into the canal.

The failure to visualise the entire transformation zone, in

the older patient, is certainly a problem for the colposcopist. Considerations of the reasons for the ease of visibility in the younger patient, yields clues to this occurrance. Pregnancy certainly allows adequate exposure of the squamo-columnar junction, for the reasons stated above, but also because the strong progesterone influence, induces hyperplasia of the endocervical columnar cells, which secrete mucus. This causes an effective endocervical prolapse, and easy visualisation of the junction (120).

Cervical water may also be a factor. In hormonal environments low in ovarian steroids, with their water retention effect, a low percentage of water, will make the cervix more rigid and less liable to manipulation and eversion by a speculum.

Postmenopausally, the cervix may also have a firm consistancy, due to a higher density of collagen. In pregnancy, administration of oestradiol directly to the cervix, will induce cervical softening, in part at least, due to collagen resorption (121). The postmenopausal patient, with oestrogen deficiency, has a gradual demineralisation of the skeleton and increase in collagen breakdown, a process that is reversed by exogenous oestrogen (122,123). It is unknown whether this also occurs on the cervix, resulting in a cervix with a lessened total collagen content. The effect, however, of oestrogen on the amount of subcutaneous collagen in abdominal skin, indicates an increase with therapy, (124). The cervical stroma has oestrogen receptors (113), and the response of the cervical collagen to oestrogen is unknown in the postmenopausal woman.

The cervix may be more firm postmenopausally, due to

decreased water content, decreased collagen breakdown, or a true replacement of collagen with fibrous tissue (107). Study of the collagen density and water content near the squamo-columnar junction, at a variety of ages, and under exogenous hormonal influence, may answer some of these points.

CHAPTER 2

THE VALUE OF CERVICAL COLPOSCOPIC ASSESSMENT IN THE MANAGEMENT OF GENITAL HORMONE DEFICIENCY

INTRODUCTION

The natural point, from which to start any investigation is observation. The problems associated with the cervix in the postmenopausal woman, are no exception. Observation and description of cervix and cervical abnormalities at this age, will lead to further avenues of exploration.

In countries with active cervical screening campaigns, the peak incidence of cervical carcinoma, now occurs in the postmenopausal years . Screening has reduced the incidence of the disease in younger women, but little impact has been made on the death rate in older women (1,4). After the menopause, screening is less likely to be performed, and interpretation of cytological findings becomes more difficult. In addition, colposcopy has limited value, due to the failure to visualise the entire transformation zone (103).

Hormone deficiency is thought to be responsible for the altered colposcopic appearance of the cervix, after the menopause. Hormone replacement therapy (HRT), has been reported to rejuvenate the colposcopic appearance of the cervix, and enable a more complete examination of the transformation zone (107,125). The degree of hormone deficiency, when assessed colposcopically, has hitherto been subjective. Quantitation and standardisation of colposcopically visible stigmata of hormonal deficiency, would allow comparison before and after therapy. Such assessments would also act as aids in the management of the hormone deficient states.

This study, attempts to define the colposcopic findings in

peri- and postmenopausal women; To confirm the colposcopically visible changes due to HRT, and to quantify these changes allowing a reproducible assessment of hormonal status. The value of such a system, will be compared to vaginal cytology and serum oestradiol levels.

METHODS AND PATIENTS

Women presenting to a menopausal clinic, with symptoms of the climacteric, had an objective assessment of their hormonal status made by three methods. Lateral vaginal wall cytology, and serum oestradiol estimation, were performed as a routine aid to management, and these were compared to a colposcopic assessment of genital hormone status. Patients with a past history of hysterectomy, or cervical neoplasia, were excluded. Initially, fifty women were examined colposcopically, and the appearances of the transformation zone are recorded in the next chapter. A variety of hormone replacement therapy (HRT), was commenced. Forty two women returned after at least 2 months HRT, and they were re-examined. This group of women, received a variety of oestrogens, and a homogeneous group of 29 women was selected, and subjected to further study. Full details of all 50 women, are shown in Appendix 1.

There were 34 women who were commenced on conjugated equine oestrogens and norgestrel, and the patients were invited to return after at least two months therapy. At the return visit, colposcopic and cytologic examinations were repeated.

The serum oestrogen, was expressed as the oestradiol in picomoles per litre, and the vaginal smear was graded on a three point scale, corresponding to the three point maturation value

of superficial, intermediate, and parabasal cells (126,127).

At colposcopic examination,four indicators of hormonal status were systematically scored.

The first colposcopic assessment, was of the thickness of the epithelium by a subjective consideration of the prominence of the capillary vascular pattern; 0 was scored if the epithelium was very thin, and 1 to 3 were scored depending on the thickness of the epithelium.

Secondly, the presence of subepithelial haemorrhages was assessed on a three point scale; 0 for severe , 1 for present and 2 for absent. Thirdly, the reaction to Lugol's iodine, was scored from 0,indicating poor uptake,to 3 with good uptake. The last indicator, cervical mucus, was assessed on a four point scale; 0 none, 1 slight, 2 moderate and 3 copious.

To give each indicator equal weighting, these scores were converted to a denominator of 6, and thus a maximum colposcopic score of 24, was possible. A young woman would have a score approaching 24.

A fifth colposcopic feature was the location of the squamo-columnar junction (SCJ). This was also recorded for the study patients. These data are valid, however, for all 50 patients, and are recorded in Appendix 1

The initial colposcopic score, the score during HRT and the change in score, were evaluated for each patient and correlated to duration of menopause. The colposcopic score was correlated to serum oestradiol, and in some patients to gonadotrophin estimations. The colposcopic assessment was compared to vaginal cytology, as both an indicator of hormonal status, and a method

of monitoring response to HRT. The colposcopic score and the serum estradiol were related to the visibility of the SCJ.

RESULTS

Fifty patients of mean age 50.38 years (S.D.4.51), and mean postmenopausal time of 31 months (S.D.33.87), were examined colposcopically before therapy. The SCJ was only visible in 12 patients (24%). Further data is available in Appendix 1, but as this group received several preparations, these results are not commented on. The conclusions are drawn from the following patients.

Thirty four patients were enrolled in the study, and twenty nine patients returned after at least two months therapy, all of whom were symptomatically improved. The mean age of these patients was 49.66 yrs. (S.D. 5.22) and the mean postmenopausal time was 34.41 months (S.D. 39.1). Twenty three patients were postmenopausal, and six were perimenopausal, but all had significant symptoms. Of the five patients who did not return, four discontinued therapy as a preference, and multiple myeloma was diagnosed in one patient. The ages and postmenopausal times were not different from those who completed the study.

Analyses are confined to the twenty nine patients who were assessed before, and during therapy.

COLPOSCOPIC SCORE

Twenty six patients, had a colposcopically visible improvement in the hormone status of the cervix, after the administration of HRT. The colposcopic features and the response to therapy are shown in table 1.

The mean initial score was 12.90 (S.D. 4.64) and the mean

final score was 19.62 (S.D. 2.52). This difference is significant (paired students t test-($t = 8.74; p<0.001$)). Some patients, however, had a very small increase in score with the administration of HRT, while others had a much larger improvement.

The initial colposcopic assessment correlated closely with postmenopausal time. The distribution of postmenopausal time, was not normal, with 12 patients within 1 year of menopause, and the rest up to 12 years postmenopausal. The correlation coefficient of logarithm of postmenopausal time, to initial score was significant ($r = -.515$, $t=3.12$; $p<0.01$). There was a deteriorating colposcopic picture of hormonal status, with the elapse of time from menopause.

Of the three patients who showed no colposcopic change, initial scores were high at 18, 22 and 22, two had serum oestradiols before therapy, of 793 and 540 picomol/L, and all three had some menstruation within three months prior to therapy. All three had high colposcopic scores, indicating endogenous oestrogen, but vaginal cytology was mainly intermediates in all three.

Three further patients, had a minimal change in score of 2, with therapy. Initial scores were 20, 18 and 18, and two of these had oestradiols of 237 and 542 picomol/L. Two were menstruating irregularly, and one was 5 years postmenopausal. These six patients are illustrated in table 2.

Thus, of patients with a minimal colposcopic response to therapy, all had an oestrogenic initial colposcopic picture, four had high oestrogen levels, and five had menstruated within

three months previously. The colposcopic score, clearly indicated those women who, despite being symptomatic, had not yet developed genital hormone deficiency.

Women who showed the greatest change in colposcopic score were, surprisingly, not the oldest women, but a group whose menopause was fairly recent. Of 5 patients with a change in score of 12 or more, 3 had a menopause within 6 months, and 2 were 3 years postmenopausal. The six patients, whose menopause was more than 6 years previously, had low initial scores (mean 10.67) but the change in score was less (mean 8) than younger women, with an initially hormone deficient picture.

OESTROGEN AND GONADOTROPHIN LEVELS

Oestradiol(E2) estimations were available for 26 of the 29 patients. Five patients had initial E2 levels of greater than 150 picomoles per litre. The mean initial score in these patients was 19 (S.D. 1.79). In the 21 patients with E2 levels less than 150 picomoles per litre, the mean initial score was 11.76 (S.D. 4.07) (unpaired students t test;$t=3.73$;$p<0.01$). Three of these 5 patients with E2 levels greater than 150 picomoles per litre, were still menstruating irregularly, but two were post menopausal. Colposcopy was able to identify those patients in whom the serum oestrogen was not yet in the postmenopausal range, and the two methods were in accord. These data are shown in table 3

The data on gonadotrophin status were not so full, with only 18 results being available. Two patients had premenopausal values (E2 540 and 542pmol/L, both irregular menstruation) and their initial scores were 22 and 18. The patients with

perimenopausal gonadotrophin values, had slightly higher scores than those with postmenopausal values, but the difference was not significant.

VAGINAL CYTOLOGY

Using a three point maturation value, rendered the vaginal cytology less sensitive, when compared with the colposcopic score. Nineteen patients had intermediates, and only seven had basals or parabasals. The mean colposcopic score for those with parabasals was 10.14 (S.D. 2.74) and for those with intermediates 13.89 (S.D. 5.05),(t test, $t=1.79$; n.s.). There was agreement in one of the three patients with oestrogenic cytology before therapy. She was having irregular menstruation, E2 was 542 pmol/L and initial colposcopic score was 18. The other two, had low colposcopic scores and low serum E2, and one was 12 years postmenopausal. The repeat assessments on therapy showed better agreement; 16 patients intermediate cytology, mean score 18.69 (S.D.2.51), and 13 patients superficial cytology, mean score 20.77 (S.D.2.15), (t test, $t=2.28$; $p<0.05$), (table 4). A more critical assessment of cytology, may have made these differences more dramatic, and increased the accord.

VISIBILITY OF THE SQUAMO-COLUMNAR JUNCTION

Of the 29 patients analysed here, seven patients had a visible SCJ before therapy (Table 5). Their mean initial score was 17.14 (S.D. 2.64) The other 22 patients had a mean initial score of 11.55 (S.D. 4.45), significantly lower than those in whom the SCJ was visible ($t= 3.04$;$p<0.01$). While taking HRT, the SCJ became visible in a further 12 patients. Significantly more squamo-columnarjunctions were seen after commencement of HRT ($\chi^2=$

10.04;chi sq.test; $p<0.01$)

DISCUSSION

Oestrogen deficiency which occurs peri- and postmenopausally, causes gross morphological and cytological changes. Colposcopically visible stigmata of hormone deficiency of the cervical epithelium, have been documented (128,129), and vaginal cytology has been used for many years as an indicator for the need for HRT (130).

Exogenous oestrogen, increases proliferation of basal epithelial cells, resulting in a thicker epithelium. Cartier has proposed that exogenous oestrogen will increase the thickness of the cervical epithelium, within ten days, and this therapy may improve the diagnostic accuracy in the postmenopausal patient (131). This process, in vivo, is evident within 11 days (132), and certainly should be complete by the 2 months for which therapy was used in this study. Such an effect would improve vaginal cytology, increase epithelial thickness, decrease likelihood of haemorrhages, and improve the uptake of Lugols iodine.

The cervix, however, is not a good source of cells for cytological assessment of hormonal status. Local trauma, infections, and sexual intercourse all act to reduce the value of exfoliated cervical cells, as indiators of end organ response to oestrogen (133). Consequently, cytological assessments have largely been confined to the lateral vaginal wall. Perhaps, for similar reasons, colposcopic examination of the cervix has not been widely used as an aid in the management of hormone deficiency. This study, however, confirms that hormone

replacement therapy, alters the colposcopic appearance of the cervix. The change in appearance is greatest in those who have the most marked stigmata of hormone deficiency, before therapy. A significant inverse correlation was found between the post menopausal time and the colposcopic score.

There is a strong relationship between cytological estimation of hormonal deficiency, and low serum oestradiol (134). This study, indicates that the colposcopically visible changes due to hormone deficiency, are quantifiable, and comparable to serum hormonal estimations. This work also suggests, that those who menstruate, or have higher oestradiol levels, are likely to have a higher colposcopic score. These data, augment the cytological evidence of the end organ response to HRT. Colposcopy also examines the end organ response. It may have a role as a true indicator of hormone deficiency, and possibly of greater value than the protein bound serum oestradiol, especially as a dynamic test, useful in assessing response to therapy. This is an area where serum estimations are less valuable, and reliance has been on vaginal cytology.

The observations on the squamo-columnar junction, have importance to the colposcopic and cytologic exclusion of pre-invasive and invasive neoplasia of the cervix. The SCJ is less frequently seen in the postmenopausal patient, than the younger woman (103,135), and this renders colposcopic evaluation less valuable. Paterson et.al. reviewed the effect of HRT on the visibility of the SCJ, and found that the squamo-columnar junction became visible in three of six patients, with the administration of HRT (125). There has been a recent report,

involving the use of ethinyl oestradiol in women with abnormal smears, in whom the whole transformation zone was not visible. It has shown that complete examination was possible in 16 of 25 such women, after oestrogen (136). All of these patients, however, were under 50 years, and the majority were fairly young, but there is no comment as to whether any were postmenopausal.

Other authors, have failed to find cervical eversion in older women treated with oestrogen (137). HRT, however, can enable the entire transformation zone to be seen in the older woman, when previously this was not possible. Mildly atypical or atrophic cytology, in the older woman, may be managed by oestrogen therapy, both as a theraputic measure and to enable more serious disease to be excluded by colposcopy. More suspicious or positively dyskaryotic cytology in the older patient, demands cone biopsy, if the entire transformation zone is not visible.

Twenty four percent of patients in this study, in whom the SCJ was visible before therapy, had a higher colposcopic score than those with a colposcopically invisible SCJ. This finding, implies that retraction of the SCJ into the endocervical canal, is a hormone dependent event, and is probably due to a deficiency of endogenous oestrogen. Administration of HRT appears to allow visualisation of the SCJ.

Some comment, on the role of progestogens on this observation should now be made. While oestrogen allows greater eversion of the cervix, and has an effect on the thickness of the epithelium, natural progesterone and synthetic progestogens, also play a part in allowing the SCJ to remain visible.

Oestrogens stimulate the columnar cells (138), but the degree of hyperplasia and hypersecretion of the columnar glands, depends on the progestogen influence (139). This effect is similar to the endocervical prolapse seen in pregnancy, where columnar cells and thus the SCJ, are pushed from above by hyperplasia, and thus are more easily visible. This property of progestogens in oestrogen primed columnar epithelium, contributed to the ease of visibility of the SCJ in this study, and in one patient in particular, was very apparent.

TABLES FROM CHAPTER 2

TABLE 1 — COLPOSCOPIC SCORES

n=29	BEFORE HRT				DURING HRT			
PARAMETER	0	1	2	3	0	1	2	3
epithelial thickness	1	3	14	11	0	1	3	25
haemorrhages	6	6	17		1	1	27	
iodine reaction	6	11	11	1	0	7	17	5
mucus production	13	7	7	2	0	7	9	13

TABLE 2
n=6 — Patient characteristics of those with minimal change in colposcopic score

age	meno-pause	E2 pmol/L	initial cytology	initial score	final score	change in score
48yrs	3mts	793	2	18	18	0
46yrs	1mt	540	2	22	22	0
48yrs	1mt	<150	2	22	22	0
50yrs	1mt	542	3	18	20	2
45yrs	1mt	<150	2	18	20	2
41yrs	60mts	237	2	20	22	2

TABLE 3
n=5 — Patient characteristics of those with E2 levels greater than 150 picomoles per L

age	meno-pause	E2 pmol/L	initial cytology	initial score	final score	change in score
48yrs	3mts	793	2	18	18	0
50yrs	1mt	542	3	18	20	2
46yrs	1mt	540	2	22	22	0
45yrs	12mts	409	2	17	22	5
41yrs	3mts	237	2	20	22	2

TABLES FROM CHAPTER 2

TABLE 4
n=29 Colposcopic scores related to vaginal cytology

pre-treatment smear score	n	mean colp score(SD)	during treatment smear score	n	mean colp score(SD)
1	7	10.14(2.74)	1	-	-
2	19	13.89(5.05)	2	16	18.69(2.51)*
3	3	13 (3.74)	3	13	20.77(2.15)*

* t=2.28; p<0.05 the students t test

TABLE 5
n=29 Visibility of the squamo-columnar junction

visible before therapy	mean initial score(SD)	invisible before therapy	mean initial score
7	17.14(2.64)*	22	11.55(4.45)*

easily with Koggans forceps
1 6

visible during therapy	invisible during therapy
19	10

easily with Koggans forceps
5 14

* t=3.04; p<0.01 the students t test

COLPOPHOTOGRAPHS OF STUDY PATIENTS

It was intended that all study patients had colpophotographs performed, both before and during HRT. In all, there were 162 colpophotographs taken from the fifty women, but this has been one of the less successful aspects of this work. Colposcopy in the postmenopausal woman is not usually easy, and a good view is not always obtained. The quality of the photographs, was less than desired, and half way through the study, the flash unit broke, and the remaining photographs had to be taken with available light. This considerably reduced their quality.

A series of slides has been assembled from the patients in this study, and they illustrate the points made in this Chapter. Annotation to the slides is given in Appendix 2.

CHAPTER 3

THE ATYPICAL TRANSFORMATION ZONE AND UNEXPECTED CERVICAL INTRAEPITHELIAL NEOPLASIA, IN POSTMENOPAUSAL WOMEN WITH NEGATIVE CERVICAL CYTOLOGY

INTRODUCTION

There is again concern,that mortality from carcinoma of the cervix in the woman past childbearing years, is not falling. (140). The experience from British Columbia, indicates that although the incidence of cervical cancer is highest in women over fifty years of age, such women are less and less likely to attend for screening as age advances (141). Among this older population there is therefore, proportionally a greater incidence of invasive cancer, in unscreened women.

In the older woman, the diagnosis of preinvasive neoplasia, is made less frequently as age advances, but the incidence of invasive cancer rises (142). The explanation for this aparent paradox may lie either in a failure to detect preinvasive lesions in the older woman, or indeed, carcinoma in the old is less likely to be preceded by intraepithelial neoplasia. The failure to detect existing preinvasive lesions, may be due to lack of screening, or a failure of the scereening test in this age group.

Certain questions need to be addressed in this context: Firstly, is there a reservoir of cervical neoplasia in this population, which is undetected by screening methods? Secondly is screening being performed? and Thirdly, is there a preinvasive element to the neoplasia? The second and third questions, can be answered by reference to the work, of those who extensively screen all ages, for cervical neoplasia. The Scandinavian countries, have comprehensive screening programmes, which have shown a decrease in incidence of cervical cancer for

all ages (2,24,25,27). This implies preinvasive neoplasia can be detected, and invasion prevented. This first question may be partly answered by these studies, but in order for this concept to be totally refuted, the incidence of cervical cancer, in the older screened woman, should be the same as that in the younger screened woman. This is not the case according to the work of Stenkvist et al., who found an incidence five times greater in women 60-69 years, who were sreened, compared to women 30-39 years, who were screened (25).

There may therefore be a reduction in efficacy, of the cervical smear in the postmenopausal years. This may be due to the abnormal areas not being available to the cervical spatula, i.e. endocervical, or it may be due a biological failure of the neoplastic tissue to exfoliate. The following study will attempt to address this subject.

Colposcopic study of the cervix in the peri- and postmenopausal woman, will identify the incidence of the atypical transformation zone (ATZ), at these ages. Cytological examination of the cervix, will be correlated to the colposcopic findings,and the presence of endocervical cells as an indicator of adequacy of the smear,will be assessed.

PATIENTS AND METHODS

Cervical cytology and colposcopic examination of the cervix, were performed in the fifty women mentioned in the previous chapter. These were 50 consecutive patients with intact uteri, seeking aid for symptoms of the climacteric. No patient was known to have had a past history of cervical dysplasia, a

previous abnormal smear, or clinically suspicious cervix, before examination. The only indication to perform colposcopy and cytology, was to document the incidence of visible abnormalities and the effect of HRT.

Repeat colposcopy and cytology were also performed in forty two patients, after at least two months therapy. If a colposcopic abnormality was seen, then a punch biopsy was taken with Leech-Williamson biopsy forceps for histopathological study. If histology was neoplastic, the patient was managed according to the nature of the abnormality, i.e. cone biopsy if the entire transformation zone was not visible, otherwise local ablation or follow up. All cervical smears, before and during therapy, were studied for unequivocal sheets of endocervical cells; a cytological indicator that the upper limit of the transformation zone has been smeared.

RESULTS

The mean age of the fifty patients was 50.38 years(S.D.4.51) and the mean postmenopausal time interval was 31 months (S.D.33.87). One hundred and one colposcopic examinations were performed on these patients, and cervical cytology was obtained at each examination.

a)CYTOLOGICAL FINDINGS

Initial cytology was negative in all patients. Cytology from one patient was documented as suspicious at review, colposcopy revealing a diffuse aceto-white lesion. Biopsy confirmed a subclinical papilloma virus infection, without CIN. None of the remaining 49 patients had abnormal cytology during the course of

the study.

Review of the smears for sheets of endocervical cells, revealed only two patients with definite evidence of these cells. They were present in both patients before and during treatment, but HRT did not render endocervical cells visible when previously not seen, in the other patients.

b)COLPOSCOPIC FINDINGS

Colposcopic examination of the cervix, in the remaining 49 patients, disclosed 11 patients with an ATZ. Aceto-white epithelium (AWE),was the predominent feature in all eleven patients, seven of whom did not display a vascular pattern. A mosaic pattern was seen in three patients and one had a punctate vascular pattern. In ten of these patients,the abnormality was noted at the first examination. In the eleventh patient, an inflamed cervix was noted at the first visit, and a McDonald suture ,in situ for 19 years was removed. Two months later when taking HRT, an aceto-white lesion was seen.

All aceto-white epithelia were biopsied. In seven patients, histology revealed a benign epithelium. In the remaining four patients, histological evidence of cervical intraepithelial neoplasia (CIN) was found. This was thought to be CIN 1 in two cases and CIN 2 in one case. In the fourth case, the patient did not take HRT. The biopsy displayed epithelial atypia bordering on CIN 1.

The histopathology was reviewed by several local histopathologists, and all agreed the epithelia in these patients, were abnormal. Therefore, for independent confirmation

of this finding, histological sections from these four patients were sent to Dr. Malcolm Anderson, Senior lecturer in Histopathology, Samaritan Hospital, London, for his opinion. He agreed that all displayed changes due to CIN. In one case he considered the CIN may even be grade 3, (see Appendix 3). Cytology from these four patients were reviewed by cytologists and cytopathologists for evidence of false reporting. All these smears were considered negative. A colposcopic diagram, and photocopies of cytology and pathology reports from the four patients, are illustrated in Appendix 3. Photomicrographs of histology and cytology are shown in the figures.

A summary of the cytological, colposcopic and histological findings, in all patients with an ATZ, is shown in table 6.

Three additional patients (nos.12,13 and 14) presented during the course of the study, and are included as they appear to confirm the findings of the study. Their evidence is, however, anecdotal. Patient no.12 had a clinically benign cervix, but a colposcopic examination was performed, due to a complaint of post-menopausal bleeding. An aceto-white lesion was noted and biopsy confirmed CIN 2. Concurrent cervical cytology was negative.

Patient no. 13 presented at the colposcopy clinic,due to the presence of cervical polyps. Cytology in the past was negative. Colposcopy confirmed the polyps, but in addition there was aceto-white epithelium on the cervix, and biopsy confirmed CIN 1. Concurrant cytology was inflammatory but negative, with no dyskaryotic or atypical cells noted. Cone biopsy confirmed CIN 1.

Patient no. 14,had a clinically benign cervix and negative cervical cytology, prior to Manchester repair, for utero-vaginal prolapse. Histopathological examination revealed CIN 1 on the cervix.

It is of interest, that in addition to having CIN with negative cytology,all seven patients in table 6 with CIN, had a comparatively early menopause. The average age of these patients is 49.5 years and the average time since the menopause is 8.1 years.

DISCUSSION

The disappointing situation for the older woman with cervical neoplasia, results from a combination of factors. Postmenopausal women, are less likely to present for cervical screening, than women of childbearing age. There is less enthusiasm, for performing cervical smears in the elderly, whether due to the wish to spare embarrassment, or due to lack of faith in screening. Gynaecologists see less of a place for colposcopy in the older women, as the transformation zone is frequently not totally visible (103,125,135).

There is, however, a pressing need for action to reduce the incidence and mortality of this preventable condition. Yule has shown,that although there is a substantial improvement in mortality in the 35-54 year age group, the mortality has been static in women aged 55 years and over (4). This has occurred despite screening greater numbers of women. The explanation

usually given, is those needing screening, are those who do not attend, and this seems to be the case in the majority of England and Wales (142). In Scotland too, Elizabeth Macgregor has found, that ninety percent of women developing cervical cancer, have never had a smear (143). Sadly, experience from America and elsewhere is very similar, (144,145). Scandinavian data, on the other hand, shows the reduction in mortality that good screening can bring to the older woman (24, 25). Day has found, from these Scandinavian data, that the incidence of invasive cervical cancer, may be reduced by 80%, by screening every 2-5 years, but there is little advantage in screening more frequently (2).

High penetration of screening programmes into the population, such as the 80 or 90 % found in the Scandinavian series (24,25), can be emulated in the United Kingdom, though usually on a much smaller scale. Standing and Mercer, achieved a remarkable 96% uptake rate among their albeit small female population (146). To achieve this degree of penetration required home visits for non attenders.

Frequently, if neoplasia of the cervix is diagnosed in the older woman, it has already passed the pre-invasive stage. However even in such a case, there is a substantial benefit in the earlier diagnosis of the tumour in the older woman (147,148). The feeling that the prognosis is worse in the elderly woman with cancer of the cervix, due to late presentation and poorer general health, is reinforced by cancer registry statistics (12). The five year survival figures are certainly reduced, however, there is some work to show that if cancer of the cervix, is diagnosed at an early stage in the

older patient, then the prognosis may be better,than that in the younger patient, whether the treatment is surgery (147), or radiotherapy (148).

There is no doubt, that the mainstay of cervical screening is cytology, and this is indubitably effective. Work indicating that it may be less effective in the old, than the young (25), supports the findings of Coppleson and Reid (28). Some years ago, they suggested that if colposcopy and cytology are used in collaboration, then the diagnostic accuracy of cervical screening, is greatly improved.

This present study, suggests that there are lesions in the cervix, in this age group, which have not been detected by cytology alone. Colposcopy is a very sensitive aid to the diagnosis of pre-invasive neoplasia of the cervix. It is less specific, however, and an ATZ does not always indicate CIN. A recent series of sexually active patients,found an ATZ in 65% (149). Older patients are also known to exhibit an ATZ, and Coppleson and Reid found atypical epithelium in a series of 40-65 year old women (28). This present study, has documented atypical features in 12,of 50 peri and postmenopausal women. Only one patient had evidence of HPV and although postmenopausal, this patient was aged 37 years. This comparatively low incidence of HPV infection in menopausal women,is probably due to the reduced sexual promiscuity in this age group.

Twentyfour percent of postmenopausal patients with a colposcopic abnormality, would render this form of examination less suitable for screening,as it is both time consuming and

demands expertise. The presence of AWE in itself, has not the same importance, provided this is not due to CIN. The diagnosis of CIN, however, means the pathologist considers the epithelium may have neoplastic potential. The colposcopic distinction between an ATZ due to CIN, and one not due to CIN, may be very difficult in a postmenopausal woman, without recourse to cervical biopsy. Other workers, reviewing the role of colposcopy in postmenopausal women, found colposcopy to be unsatisfactory in 53% of such patients (150). The explanation for this finding, is the lack of visibility of the entire transformation zone. The clinical application of the use of oestrogen, to improve the visibility of the squamo-columnar junction, may be of value, and Prendiville and colleagues reduced their cone biopsy rate by such a treatment (136). All their patients were under 50 years of age, and the use of oestrogen, for this purpose, in older women is not widespread.

The Schiller test, useful in outlining areas of abnormal epithelium on the cervix, has less value in the older woman. Schiller positive (iodine negative) areas,are not uncommon in the postmenopausal woman's cervix (135), and atrophic changes with a very thin epithelium may render the ATZ rather difficult to recognise. The finding, however, of four patients with epithelial abnormalities with neoplastic potential, from an unselected population of 50 women with symptoms of hormone deficiency, has raised concern. This concern is heightened, by the repeated failure of cytology to indicate these lesions.

Traditionally, cervical cytology is regarded as a successful method of screening. Macgregor reports a 2% false negative rate,

(151), although many other centres have higher rates. There are many reviews of cervical cytology in women who subsequently develop invasive cervical cancer. Some, such as the study by Martin are depressing,in that they show errors in the patient, the physician, and the laboratory (6). Sadly, over 50% of cases,demonstrated physician error. In addition, failure of the patient to present for further treatment, lessened the effectiveness of cervical screening in his study. He therefore concluded, that cytologic screening was only 50% protective against cervical carcinoma. Similar audit by British reseachers in Nottingham, has also indicated adequate follow-up of abnormal cytology, in only 59% (152). In this study, despite exhaustive efforts, 8% of patients could not be traced.

Adopting a different line of thought, other American reviewers, have vindicated the failure of cytology to detect cervical tumours, which presented soon after negative cytology, by the tumour not having a prolonged pre-invasive phase (3). Macgregor, however, advises caution, and the need for closer study of the so called 'rapidly advancing cases' before there is a change in screening policy (143). The importance and need for centralised cytological services, to improve the quality of follow-up, has been stressed by others (153).

Once again, the most complete data on the cytological failure to detect invasive cancer, come from Scandinavia. Bjerre and Johansson found, that of 131 women developing carcinoma after screening, 90 had abnormal cytology which did not have appropriate treatment (24). Ryelander, reported a 60% laboratory

misdiagnosis, among patients who subsequently developed invasive neoplasia (7). In another work, she reported that 3 of the women who developed cancer after screening, had abnormal colposcopy, but the reassurance of normal cytology prevented further action (27). Most works on this subject, do not mention the ages of the patients, who develop the cancer after screening, but a recent British study found that seven of fifteen such women, were over fifty years of age (26).

The cervical smear, is only as good as the people obtaining and reporting it. It is further limited in the older woman, as the relevant area may not be smeared, as the SCJ has receded into the endocervix.

One cause for the normal smear shortly before cervical neoplasia, has not had the same attention as these others. This is the concept that the cervical intraepithelial neoplasia, may not be actively exfoliating. Husain terms this 'biological error', when no other identifiable cause for negative cytology can be found in women with neoplastic lesions on the cervix (23). The diagnosis of this has, however, been by exclusion and this concept can only be tested in women who are known to have CIN, and concurrent cytology is examined for exfoliated dysplastic cells. This condition is almost certainly unusual in the woman of childbearing years, however, it may be less unusual in the older woman.

In this present study, four women who were hormone deficient, had a histologically proven intraepithelial neoplasia or atypia, with repeatedly negative cytology. In total, nine normal smears were obtained from these four women. Sadly, the expected

exfoliation of abnormal cells by the administration of oestrogen, to three of these women, (See previous chapter),did not occur. The possibility that the lesions were endocervical, and thus not smeared, would be a cogent argument if colposcopy had not been performed. All lesions were ectocervical and colposcopically visible, and were thus adequately scraped. Two of the four patients with CIN in the study, had cone biopsy as the SCJ was not adequately visible, and the other two were followed up with review colposcopy.

The addition of three similar anecdotal cases, with CIN and negative cytology, heightens concern about this finding and may indicate limitations of cytology in this age group. The finding of a comparatively early menopause, in these women, may be spurious. There is the possibility, however, that continued sexual activity, such as most women in the early fifth decade may have, may make the epithelium unstable if there is an early menopause. A similar argument has been used in the incrimination of mutagens,on the epithelium of very young women having sexual intercourse. Such exposure, may render an immature epithelium unstable (34). This is speculation and further work on this hypothesis would be necessary for any definitive comment to be made.

The review of the smears for endocervical cells, proved disappointing. Gondos and co-workers,found that although 92.5% of women under 45 years had endocervical or metaplastic cells, this figure fell to under 64% in women over 45 years (22). The finding in this present study of only four percent,is explained by the strict, and possibly too strict, criteria of sheets of

endocervical cells. Metaplastic cells only indicate that the transformation zone is being smeared, endocervical cells indicate the SCJ is being smeared. Looking for endocervical cells, yields few positives in this age group. If all smears in which they did not appear were classified as inadequate, then cervical cytology by Ayres spatula would have to be seriously questioned. There are now newer forms of spatula, which are shaped to allow endocervical cell sampling, and these may be more appropriate to the older woman. Weid showed that an endocervical swab could yield more abnormal cells than a cervical smear (154). Certainly, if the SCJ is not easily visible,in the older woman having cervical cytology, consideration should be given to performing an endocervical sampling with a swab moistened with saline.

With approximately 4000 cases of cervical cancer registered in the U.K. in 1980 (12), extrapolation, gives a woman a 1% chance of developing cervical cancer, throughout her lifetime. Clearly, the finding in this study, of eight percent of a 'normal' post-menopausal population, harbouring a mild cervical abnormality, does not indicate that all would progress to invasion. It does, however, raise concern that there is an undiagnosed reservoir of cervical abnormality which may have neoplastic potential. In their paper on rates of progression of cervical dysplasia, Richart and Barron found that only 6 of 462 women with very mild dysplasia, regressed by the first follow-up (155). The median rate of progression from mild dysplasia to carcinoma in situ was 86 months. Such progression, from a mild abnormality to a lesion with serious invasive potential, would

be expected to be slow, and there would be the opportunity to detect the lesions at a later cytological examination. The recently re-convened Canadian Task Force on cervical cytology (21), has reaffirmed that if regular cytology is negative until the age of 60 years, then it may be discontinued. This study appears to indicate that such a policy will not allow these mild intraepithelial lesions to be detected at a later date.

TABLE FROM CHAPTER THREE

TABLE 6

Patients with negative cervical cytology and an atypical transformation zone

PATIENT	AGE	MENOPAUSE	CYTOLOGY	COLPOSCOPY	HISTOLOGY
1	54YRS	4YRS	NEGATIVEx3	AWE	CIN 2/3
2	53YRS	4YRS	NEGATIVEx3	AWE	NORMAL EPITH
3	52YRS	3MTS	NEGATIVEx2*	AWE+MOSAIC	METAPLASIA
4	51YRS	5YRS	NEGATIVEx2	AWE	CIN 1/ATYPIA
5**	50YRS	9YRS	NEGATIVEx2*	AWE+MOSAIC	CIN 1
6	50YRS	10YRS	NEGATIVEx3	AWE	NORMAL EPITH
7	49YRS	MENST	NEGATIVEx3	AWE	NORMAL EPITH
8	49YRS	3YRS	NEGATIVEx2	AWE+PUNCT	METAPLASIA
9	49YRS	9MTS	NEGATIVEx3	AWE	ACANTHOSIS
10	45YRS	MENST	NEGATIVEx2*	AWE+MOSAIC	INFLAM EPITH
11	37YRS	6YRS	NEGATIVEx2*	AWE	CIN 1
12	50YRS	12YRS	NEGATIVEx1	AWE+PUNCT	CIN 2
13	53YRS	11YRS	NEGATIVEx1*	AWE	CIN 1
14	52YRS	10YRS	NEGATIVEx1	-	CIN 1

* Negative with inflamatory exudate on one occasion

** AWE seen at second visit

YRS-YEARS MTS-MONTHS AWE-ACETO-WHITE EPITHELIUM

CIN-CERVICAL INTRAEPITHELIAL NEOPLASIA PUNCT-PUNCTATION

EPITH-EPITHELIUM INFLAM-INFLAMATORY MENST-STILL MENSTRUATING

SUMMARY TO SECTION 1

Research into descriptive studies of cervical colposcopic appearance, is limited in the age group here studied. A common, and reasonable view, is that colposcopy has a limited place in such women. If cytology is abnormal, the fear of missing invasive carcinoma, when fertility is not a concern, often ensures cone biopsy is the first diagnostic procedure. Cartier, sees a use for exogenous oestrogen in improving the colposcopic picture, in older women with abnormal cytology (131). As regards visibility of the SCJ, this study has confirmed his experience, and that of others (125,136), In the study group of 50 women, four had intraepithelial neoplasia diagnosed, and three of these had HRT for at least 2 months. One of the three women had CIN 2/3, and although the SCJ was visible, it was high in the endocervix, and she required cone biopsy. One other patient with atypia/CIN 1 in the study group, did not take HRT. The SCJ was, and remained invisible, and despite only having a very mild abnormality, she also required cone biopsy. The two others had CIN 1, and the SCJs were visible before HRT. They were, however, high in the endocervix, but administration of HRT, rendered them easily visible and continued surveillance, rather than cone biopsy was possible.

In this clinical trial, the presence of unsuspected CIN was discovered. The administration of HRT, prevented the need for cone biopsy in two patients, in whom it would otherwise have been necessary.

The other aspects of this investigation, indicate a use for

colposcopy in the management of the hormone deficient woman. A logical scoring system,and a standardised technique, allow a reproducible quantitative appraisal of genital hormone status. This assessment, may be superior to a three point maturation value assessment by cytology, although comparison to more sensitive and critical cytological assessments, was not made. Agreement with serum estimations of oestradiol was frequent, but colposcopy had advantages over hormonal assays. Degrees of hormone deficiency were diagnosed by colposcopy, but oestradiol estimations were of little value, if in the menopausal range, below 150 picomoles per litre. Additionally, colposcopy could be used to monitor response to therapy, and was an indicator of end organ response.

Utilisation of the scoring system, and association with the visibility of the SCJ,suggests that retraction of the junction into the endocervical canal, is a hormonally dependent event.

CHAPTER 4

THE MICROBIOLOGICAL FLORA OF PATIENTS WITH CERVICAL INTRAEPITHELIAL NEOPLASIA AND NORMAL WOMEN, AT DIFFERENT AGES

INTRODUCTION

There is little doubt that the human papilloma virus (HPV), is closely associated with CIN, and it may have an aetiological role in the genesis of cervical carcinoma (72,75,76,77,80). If HPV is a cause of CIN, and CIN progresses to invasive cancer, then HPV has importance as a cause of cervical cancer. This, however, is perceived as the importance of cervical HPV. CIN is not a disease, being asymptomatic and unharmful, provided it does not progress to invasion. A slightly different approach, is to perceive HPV as a genital tract pathogen, which occurs with, and without CIN. Consideration can be given to what other effects it may have locally, other than its probable carcinogenic action.

Research, attempting to prove causation of cervical cancer, is necessarily sophisticated. Associations, however, may still be observed between putative causal agents, and valuable comment can be made. HPV, frequently yields cytological evidence of its presence, in the pre-invasive stage, but histological evidence of the virus, is rarely found in invasive cancer. The classical cytological changes, are due to death of the cells, and these have no part in cervical carcinogenesis. The cells, which have the potential to become malignant, are those which have their DNA altered by HPV, without cell death, and these changes are not histologically visible when invasion occurs. Until HPV can be cultured, and a specific antibody to each subtype can be raised for serological study (72), cytological, histological and microbiological study, must be confined to patients with

pre-invasive disease, for any associations with HPV to be made.

The influence of HPV, on age at diagnosis of CIN, may be valuable. If CIN was occuring at an earlier age, due to HPV, then possibly the age of onset of invasive cancer would fall. The parallel increase in the number of cases of cervical HPV infection, and the number of very young women developing cervical cancer, may be related. A study relating HPV presence to both age at diagnosis of cervical abnormality, and other organisms predominant in the lower genital tract, may be of value. HPV may be a marker not only for CIN, but also for changes in microbiological flora,which may also have importance.

A study was designed, involving all patients presenting with abnormal cervical cytology, to a colposcopy clinic. Evidence of viral and bacterial infection was sought, and related to CIN. The influence of age on the recovery of these organisms, was also correlated, and these findings were compared to a group of normal women. A further group of women at the climacteric, were also examined for these organisms, both before and during the administration of HRT.

PATIENTS AND METHODS

Two hundred and eighty-seven (study) patients with abnormal cervical cytology, were referred to the colposcopy clinic at the Western Infirmary, Glasgow, between June 1983 and June 1984. These patients were matched with 100 control patients, with normal cytology, and no past history of cervical neoplasia, vaginal infection, or pelvic inflammatory disease. These patients were recruited from women seeking gynaecological treatment, for conditions other than the above. Patients and

controls were matched for age, parity, contraception and socio-economic status. An attempt was made to match for smoking, but an excess in the study group was found, and this precluded matching. There was no attempt to match for sexual behaviour, both because this information was felt to be unsound, and also any attempt to do this may dissuade patients, with abnormal smears, to return for treatment. All study patients had cytology, colposcopy and biopsy, for confirmation of diagnosis. All controls had current negative cytology and 24% had negative colposcopy.

Identification of HPV, was by typical cytological and histological changes (156,157). Other putative aetiological agents were sought by isolation techniques, in particular herpes simplex virus (HSV) and C. trachomatis, to assess their prevalence in the local population. Endocervical swabs were obtained for both these agents. Swabs for HSV were transported in BHK 21 (Baby Hamster Kidney) medium (Gibco(Europe)Ltd.), and cultured in BHK c13 cells in transport medium, supplemented by 10% v/v fetal calf serum (Gibco). The swab for C. trachomatis was collected into 2SP transport medium, and innoculated into monolayer culture of McCoys cells, without prior freezing. The innoculation method used, was that of Harper et.al.(158), except that Lugol's iodine was used to stain the culture, three days after innoculation.

Triple bacteriology swabs from endocervix, high vagina and urethra, were also obtained from all patients, to determine the predominant aerobic and anaerobic organisms in the lower genital tract. These swabs were transported to the laboratory, in

Stuart's Medium (Oxoid), and plated onto blood agar (Aerobic and anaerobic), sensitivity blood agar, Macconkey agar, Thayer and Martin medium, and Robertsons meat broth fluid medium, with subculturing if necessary. Trichomonas vaginalis was identified on the cervical smear, but was not cultured.

No attempt was made to culture each organism present in the vagina. Such exhaustive descriptive studies, have been carried out previously (159,160). Only the predominant organism, found at culture, was correlated to the histological group from which it was isolated.

The same isolation techiques for C. trachomatis, HSV, and aerobic and anaerobic bacteria, were also performed on a group of twenty women patients with symptoms of the climacteric. These patients were taken from the population of women undergoing colposcopy, before and during HRT (Chapters 2,3), and isolation was performed at both these times. The culture results from these patients, are compared to the control group of younger women for evidence of changes in bacterial flora, and presence of HPV.

RESULTS

Two hundred of the two hundred and eightyseven patients had CIN, eightyfour of which also had evidence of HPV. Thirty-six patients had HPV alone, and fifty-one patients had a benign abnormality, without CIN or HPV. Patient details are shown in tables 7 and 8. There were no significant differences between study and control groups for age, parity, or distribution of socio-economic group. There were slightly more oral contraceptive users, and slightly fewer barrier contraceptive

users in the study group, compared to controls. Of 120 patients with HPV infection, only 3 (2.5%) used barrier contraception, compared to 15% of controls. This is indirect evidence, of the protective effect of barrier contraception, to HPV infection.

HPV and AGE

The influence of the finding of HPV associated with CIN on age at diagnosis, is shown in table 9. For CIN 1 and CIN 3, the presence of HPV, reduced the age at diagnosis by two years, but in CIN 2, those with HPV were slightly older. The differences were not significant.

C.TRACHOMATIS and AGE

Fewer patients were infected with C. trachomatis than HPV in the study group, but an almost identical proportion of controls, were isolation positive. When age was related to C. trachomatis infection, the patients were significantly younger than those who were not infected, irrespective of which group was studied (table 10).

HSV AND AGE

Among the 287 study and 100 control patients, no evidence of HSV was found on culture.

OTHER ORGANISMS

The three common vaginal pathogens, yeasts, T.vaginalis, and Gardnerella vaginalis, were found in similar proportions in study and control groups, but yeasts were apparently more prevalent in the control group. The group with HPV alone, had low rates for T. vaginalis, and none had any yeasts.

If an organism other than these noted above was identified as a significant growth, this was correlated to the histological

group. The five most commonly isolated organisms, were related to histology, and are shown with these other vaginal pathogens in table 11.

Clearly the group with a benign abnormality, which was mainly 'cervicitis', has a significantly greater isolation of these organisms, than the control group. Coliforms and Staph. albus, were more frequently found in the CIN alone, and HPV alone, groups but not in the CIN and HPV group.

POSTMENOPAUSAL PATIENTS

Twenty patients had microbiological isolation performed before the commencement of HRT. None had a specific complaint of vaginal discharge, although vaginal dryness was common. Eleven of these patients had no growth, or any significant growth at any site. Three patients had C.albicans or yeasts, one patient each had T. vaginalis, Gardnerella vaginalis, enterococci, and group B Streptococci. Two patients had coliforms, and one had non haemolytic Streptococci (Table 12).

Sixteen patients returned after at least two months treatment with HRT. Four did not, three because they did not like HRT, and the fourth, the patient in whom enterococci was isolated, had a multiple myeloma diagnosed, and did not receive therapy. During therapy, there appeared to be greater isolation of vaginal pathogens. Only seven out of sixteen had no growth, or no significant growth. Four patients had a group B Streptococcus isolated, one of which also had C. trachomatis. Two patients had yeasts, two had T. vaginalis and one had Gardnerella vaginalis. Excluding the GBS, there were still six of sixteen patients with vaginal pathogens.

DISCUSSION

It is clearly outwith the scope of a study of this size, to accurately determine all the micro-organisms present in the lower genital tract. The large proportion of patients, with HPV associated with CIN, adds further weight to the large body of information implicating this agent as an associated factor in the aetiology of CIN (75,80,81). It does not make any causal role for HPV any clearer. Were each grade of CIN to be diagnosed at an earlier age, if there is the presence of HPV, then several explanations are possible. Firstly, the HPV may be hastening the progression of the CIN, resulting in more advanced CIN at a younger age. Secondly, the HPV may be a marker for a more active sexual practice, which may render epithelium neoplastic, at an earlier age. Lastly, it is possible that the presence of HPV may be the factor that renders the smear abnormal, rather than the dyskaryosis due to the CIN. Although the cytological features of HPV infection have been documented for many years (78), the features of dyskaryosis are unlikely to be missed, if attention has already been attracted by koilocytosis. No firm conclusion as to whether the HPV hastens CIN, can be drawn from these data on age incidence, but there is a trend to be found.

Similar thinking, can be applied to C. trachomatis, an organism known for its sexual transmission. The same prevalence was found in study and control groups. Although groups were not matched for sexual behaviour, as it was felt this line of questioning may jeopardise the women with CIN returning for treatment, this information suggests, that study and control groups had similar sexual practices. Little comment, can be made

for C. trachomatis, as a causal factor in CIN, from these isolation studies. The work in the following chapter, will consider past infection, in this role more closely. This present study, however, shows that a suitably controlled group of normal women, have almost identical prevalence rates, to women with CIN for the isolation of C. trachomatis. Group for group, women with positive isolation for C. trachomatis, were younger than isolation negative women, almost certainly a manifestation of increased sexual activity in the younger, than older woman.

Although, perhaps the least sophisticated investigation performed, triple bacteriology swabs from endocervix, high vagina and urethra, gave very interesting results. The organisms that can be isolated from the vagina are legion, even in perfect health (161,162). Bacteroides species alone, can be isolated from the vagina, in over 50% of healthy women (162). Descriptive studies, employing meticulous and exhaustive culturing techniques are necessary to describe all vaginal micro-organisms. Such studies are usually of small numbers (160,163). Studies on microbiology of the vagina, may either concentrate on detection of all possible organisms, detection of a specific organism to establish the prevalence, or an attempt at quantitation of the organism (164). The first and third of these objectives, were beyond the scope of this study, in view of the numbers, and thus the predominant growth on plating was considered, and associated to the histological groups.

The culture techniques used, would show the pathogenic organisms and other bacterial growths, which may be significant. Larsen and Galask point out, that results are likely to be of

greater value if all isolations within the study populations are performed in the same laboratory, rather than culling information from more than one source (164). All isolations in this study, for specific agents, were performed in the same laboratory, from matched patients. Thus, any differences in isolation can be explained, by differences in the lower genital tract microbiological environment.

It was felt that perhaps the presence of CIN or HPV, may alter the predominant organisms, or balance of organisms, and that this may have been detected. CIN is found in association with numerous commensal and pathogenic organisms, and several studies have attempted to detail these. A large study of patients from India, found a markedly high infestation rate of T. vaginalis in dysplasia patients, compared to controls, but little other comment could be made regarding associations (165). This work, supported the views of others, that T. vaginalis may still have a part to play in the genesis of neoplasia (37,166). A similar study to the present one reported, but carried out in North America, sought evidence of HPV and C. trachomatis, in addition to other bacteria. This group found similar incidences of HPV and C. trachomatis as found here, but higher incidences of Gardnerella and HSV (167). A smaller, but more intensive investigation on patients with dysplasia, and invasive carcinoma, found little to comment on the alteration of flora in dysplasia, but found much lower incidences of Gram negative anaerobic baccilli, in patients with invasive carcinoma (168).

Other workers have reported on flora, associated with invasive neoplasia. One series, reported that among 21 cases of

cervical cancer, there was an increased frequency of E. coli and Bacteroides species (169). Another series, however, found no increase in bacterial growth among necrotic cancerous cervices (170). The data found in this study, are suggestive of a shift in predominant bacteria to the enterobacteria, and enterococci, among the CIN group. A similarly high incidence of enterococci, was found by Thadepalli and co-workers, among patients with CIN (168). A conclusion from this work, and that of others, on the organisms isolated at the time of diagnosis of CIN or HPV, must be that there are no strong correlations of any one agent, to CIN. HPV appears to be a strong risk factor in its own right, and possibly its presence may inhibit other agents. The role of prior infections and the genesis of CIN, is discussed in the next chapter.

The main value of bacteriological screening of women with abnormal cervical cytology, may be illustrated by the findings in the group with no subsequent evidence of CIN or HPV, after colposcopy and biopsy. By routine lower genital tract microbiological screening, these patients were significantly more likely to have a positive isolate than controls, for the most commonly isolated groups of organisms. Significant cervical infection, can cause abnormalities on cervical cytology, which may be misinterpreted as CIN (67). This may account for reports for cases of CIN being controlled, by local application of antibiotics to the cervix (68). If a gynaecologist, or general practitioner has limited access to colposcopic facilities and is faced with a mildly abnormal smear, he may perform these routinely available microbiological tests, and treat any growth.

Subsequent cervical cytology may prove normal and cone biopsy may be avoided.

No herpes simplex was isolated from the cervix, during the study. This is a prevalent organism in the United States, but it does not appear so in the population from which these patients were recruited. No positive isolate among 387 cases was unexpected. The isolation technique was validated during the course of the study, by women with known genital herpes being screened by the same technique, and proving positive. The virus may remain latent in the sacral ganglia (45)and be found on the cervix, only during acute infections, but it seems likely that herpes simplex is not a significant problem in this area.

Early work, on the change in microbiological flora that accompanies the menopause, concentrated on the decrease in the lactobacillus (171). Since then, there has been further work implicating other changes. Blum and Elian suggested, that the flora in postmenopausal women, was more akin to that found in children, or in pregnancy (172).Osborne and colleagues, could find no significant differences in culture rate, between postmenopausal women receiving HRT and those who did not (173). Some have suggested, there may be an increased isolation rate of anaerobic bacteria in women not treated with HRT, and certainly there will be a lower isolation of the lactobacillus (164).

The work of this present study, indicates that the group of women at the climacteric, had a considerably higher rate of isolation of vaginal pathogens, such as yeasts, T. vaginalis or Gardnerella vaginalis. Five of twenty patients (25%) isolated one of these pathogens before treatment, and the situation was

even worse during therapy, with six of sixteen patients having a pathogen isolated. The rate of isolation of these organisms, is higher in comparison to the rates in younger women (Table 11). HRT does not appear to improve the situation. C trachomatis, T. vaginalis, and G. vaginalis all have a positive correlation to cervical mucopus (174), but this was not found in these patients. The finding of four of sixteen hormone deficient patients, having a group B Streptococcus during HRT, was surprising. The significance of this finding is not clear.

The conclusion from these data, must be that the study population observed in this older age group, have a reservoir of pathogens, at least as large as that in the younger woman. HRT may not improve the genital microbiological environment.

TABLES FROM CHAPTER 4

TABLE 7 Patient details

A) AGE AND PARITY

	NUMBER n	MEAN AGE yrs.(SD)	MEAN PARITY (SD)
CONTROLS	100	29.61(8.05)	1.93(1.59)
CIN	116	29.81(6.43)	1.71(1.56)
CIN+HPV	84	28.82(6.05)	1.86(1.57)
HPV	36	28.80(9.18)	1.08(1.61)
BENIGN	51	30.69(9.82)	1.43(1.37)
TOTAL STUDY	287	29.54(7.41)	1.63(1.55)

B) CONTRACEPTIVE PRACTICE

	n	STER/ NO CONT	O.C. PILL	IUCD	BARRIER
CONTROLS	100	44(44%)	31(31%)	10(10%)	15(15%)
CIN	116	37(32%)	53(46%)	17(15%)	9(7%)
CIN+HPV	84	25(30%)	45(54%)	12(14%)	2(2%)
HPV	36	17(47%)	16(44%)	2(6%)	1(3%)
BENIGN	51	15(29%)	27(53%)	4(8%)	5(10%)
TOTAL STUDY	287	94(33%)	141(50%)	35(12%)	17(6%)

C) SMOKING PRACTICE

		CPD				
	n	0	-10	-20	-30	>30	UNKNOWN
CONTROLS	100	41(41%)	9(9%)	22(22%)	2(2%)	2(2%)	24(24%
CIN	116	31(27%)	7(6%)	38(33%)	11(9%)	3(3%)	26(22%
CIN+HPV	84	19(23%)	12(14%)	26(31%)	6(7%)	1(1%)	20(24%
HPV	36	9(25%)	10(28%)	8(22%)	2(6%)	0(0)	7(19%
BENIGN	51	17(33%)	7(14%)	11(22%)	0(0)	0(0)	16(31%
TOTAL STUDY	287	76(26%)	36(13%)	83(29%)	19(7%)	4(1%)	69(24%

TABLES FROM CHAPTER 4

TABLE 8 Patient details

SOCIO-ECONOMIC STATUS

S.E. GROUP	1	2	3	4	5	6	UNKNOWN
CONTROLS	2(2%)	17(17%)	27(27%)	10(10%)	8(8%)	12(12%)	24(24%
CIN	3(3%)	19(16%)	32(28%)	20(17%)	7(6%)	14(12%)	21(18%
CIN+HPV	0(0)	14(17%)	21(25%)	9(11%)	3(4%)	18(21%)	19(22%
HPV	0(0)	4(11%)	8(22%)	10(28%)	1(3%)	6(17%)	7(19%
BENIGN	3(6%)	9(18%)	10(20%)	10(20%)	1(2%)	3(6%)	15(29%
TOTAL STUDY	6(2%)	46(16%)	71(25%)	49(17%)	12(4%)	41(14%)	62(22%

TABLE 9 Age, CIN and HPV

GROUP	n	AGE(SD)yrs.	GROUP	n	AGE(SD)yrs.
CIN	116	29.83(6.43)	CIN+HPV	84	28.82(6.05)
CIN 1	26	29.11(7.40)	CIN1+HPV	18	27.06(6.41)
CIN 2	51	28.54(5.22)	CIN2+HPV	47	29.00(5.91)
CIN 3	39	31.97(6.74)	CIN3+HPV	19	30.05(5.99)

TABLES FROM CHAPTER 4

TABLE 10 Age and C.trachomatis culture

GROUP	+veCULTURE (%)	AGE(SD)	-veCULTURE (%)	AGE(SD)
CONTROLS	10(10)	26.60(5.18)	90(90)	30.02(8.23)
CIN	11(10)	23.25(7.96)	105(90)	30.31(6.50)
CIN+HPV	12(14)	25.83(4.40)	72(86)	29.32(6.16)
HPV	4(11)	26.50(14.47)	32(89)	29.09(8.61)

TABLE 11 Prevalence of positive cultures in each group

	CONTROL (%)	CIN (%)	CIN+HPV (%)	HPV (%)	BENIGN (%)	TOTAL STUDY (%)	*MENOPAUSAL bef	dur
enterococci	6(6)	14(12)	8(9)	1(3)	10(20)	33(11)	1	-
coliforms	2(2)	11(9)	5(6)	5(14)	8(16)	29(10)	2	-
Staph.albus	2(2)	10(9)	5(6)	3(8)	4(8)	22(8)	-	-
Gp. B Strep.	2(2)	2(2)	2(2)	0(0)	5(10)	9(3)	1	4
BacteroidesSp	1(1)	2(2)	3(4)	0(0)	3(6)	8(3)	-	-
C.trachomatis	10(10)	11(10)	12(14)	4(11)	6(12)	33(11)	-	1
Yeasts	15(15)	8(7)	5(6)	0(0)	6(12)	19(7)	3	2
T.vaginalis	12(12)	10(9)	4(5)	1(3)	7(14)	22(8)	1	2
G.vaginalis	2(2)	4(3)	1(1)	1(3)	1(2)	7(2)	1	1

* n=20 for before(bef) HRT menopausal group and n=16 for during (dur) HRT group

TABLES FROM CHAPTER 4

TABLE 12 Microbiology before and during HRT in perimenopausal and postmenopausal women

		BEFORE HRT	DURING HRT
Patient 1	HVS	all group B	all group B
	Cx	Streptococcus	Streptococcus
	U		
Patient 2	HVS	no growth	no signif growth
	Cx	no growth	no signif growth
	U	no signif growth	no signif growth
Patient 3	HVS	C. albicans	no signif growth
	Cx	no growth	no signif growth
	U	coliforms	no signif growth
Patient 4	HVS	all non	gp B Streptococcus
	Cx	haemolytic	no signif growth
	U	Streptococci	no signif growth
Patient 5	HVS	no signif growth	Trichomonas but no
	Cx	no signif growth	sigificant growth
	U	no signif growth	no signif growth
Patient 6	HVS	no signif growth	Trichomonas
	Cx	no growth	no growth
	U	no signif growth	no signif growth
Patient 7	HVS	no signif growth	gp B Streptococcus
	Cx	no signif growth	gp B Streptococcus
	U	no signif growth	no growth
Patient 8	HVS	Gardnerella	mixed anaerobes
	Cx	Gardnerella	C. trachomatis
	U	Gardnerella	gp B streptococcus
Patient 9	HVS	Streptococcus	no signif growth
	Cx	C. albicans	no signif growth
	U	no growth	no growth
Patient 10	HVS	Trichomonas but	yeasts
	Cx	no signif growth	yeasts
	U	no signif growth	no growth
Patient 11	HVS	no signif growth	Gardnerella
	Cx	no signif growth	Gardnerella
	U	no signif growth	Gardnerella
Patient 12	HVS	yeasts	yeasts
	Cx	yeasts	yeasts
	U	yeasts	yeasts
Patient 13	HVS	no signif growth	no signif growth
	Cx	no signif growth	no signif growth
	U	no signif growth	no signif growth
Patient 14	HVS	coliforms	no signif growth
	Cx	coliforms	no signif growth
	U	coliforms	no signif growth
Patient 15	HVS	no signif growth	no growth
	Cx	no signif growth	no growth
	U	no signif growth	no growth
Patient 16	HVS	no signif growth	normal flora
	Cx	no signif growth	normal flora
	U	no signif growth	normal flora

TABLE 12 cont.

Patient 17	HVS	no signif growth	
	Cx	no signif growth	NO RETURN
	U	no signif growth	
Patient 18	HVS	enterococci	
	Cx	enterococci	NO RETURN
	U	enterococci	
Patient 19	HVS	no growth	
	Cx	no growth	NO RETURN
	U	no growth	
Patient 20	HVS	no growth	
	Cx	no signif growth	NO RETURN
	U	no signif growth	

CHAPTER 5

AGE RELATED ANTIBODY STATUS OF PATIENTS WITH CERVICAL CANCER AND MATCHED CONTROLS

INTRODUCTION

In the United Kingdom, cancer registry figures indicate that carcinoma of the cervix is a disease, affecting more postmenopausal, than premenopausal women (12). There are more cases, affecting women over 65 years than women under 45 years. Despite these figures, the main thrust of cervical cancer protection schemes, has been to screen younger women, so they do not develop the disease later in life. This principle is sound, provided that firstly, the disease has a long preinvasive phase, and secondly all the population can be protected. The second, has not been achieved, and the length of the preinvasive phase, has been questioned by Ashley. He postulates that the disease may be different in the older woman (14,15). A reduction in the preinvasive phase, or a reduction in the efficacy of screening methods in the older woman, imply the need for renewed vigour in screening as age advances, rather than a reduction.

Evidence is mounting, that much of the intraepithelial neoplasia in the younger woman, has an association with human papilloma virus (HPV) (71,72,74,75,76,81,82,83,84). HPV is a sexually related organism (70), and thus is understandably more common, in the younger woman. The question of how HPV may be involved in cervical neoplasia in these older women, many celibate for years, has not yet been addressed. The property of latency, evident in herpes simplex virus infections, is only now being questioned for HPV (87). Does HPV alter the genotype of squamous cells, but the effect remains dormant for many years, before leading to carcinoma in old age? Or does carcinoma in the

older woman have a different series of initiators, with age a co-factor in its own right?

The following sero-epidemiological study investigates three putative causal agents for carcinoma of the cervix. Rates of sero-positivity in a study and control group are compared, and prevalence of the evidence of prior infection with these factors, is related to age. This attempts to identify, whether the older woman with carcinoma of the cervix, has been exposed to the same sexual or other pathogens as the younger woman. HPV may not yet be investigated in this way, as culture and specific antibody production have not yet been possible (72).

PATIENTS AND METHODS

One hundred patients with invasive carcinoma of the cervix (study), were matched with one hundred control patients (control), for age, parity, and socio-economic status. A serum sample was obtained from all patients, and analysed for antibody titres to three genital pathogens which have been implicated in the causation of carcinoma of the cervix; Herpes simplex virus, cytomegalovirus and Chlamydia trachomatis.

Control patients had never had cervical neoplasia, or previous atypical Pap smear, and many were postmenopausal, seeking aid for symptoms of the climacteric. The serum sample, was obtained from the study patients, before therapy in 27%, and after therapy, usually radiotherapy, in the remaining 73 patients. The matching for age with control patients, was based on the age at diagnosis of the carcinoma, and not age at sampling. It was felt that genital infection after therapy for carcinoma was unlikely, and this matching criteria, would avoid

a skew in the distribution toward the older woman.

Serology for Chlamydia trachomatis was performed by the standard micro-immunofloresence method,using as substrate McCoys cells, which had been previously treated with cycloheximide, pre-infected with C. trachomatis type i-cal. The reciprocal of the greatest serum dilution, showing typical inclusion body fluorescence, was recorded as the titration endpoint. Titrations were then read on a Leitz microscope, fitted with Ploemopak illumination for fluorescein isothiocyanate.

The antibody titres to herpes simplex virus (HSV), and cytomegalovirus (CMV), were obtained by a standard complement fixation technique.

The serological status of study and control patients, was compared for difference in infection rates, or synergism between agents possibly causing the cancer. The serological status, was also linked to stage of disease, histological differentiation of the tumour, and socio-economic status.

RESULTS

The mean age of the study patients, at the time of sampling, was 58.7 years (S.D.12.31), and the mean age at diagnosis of cervical carcinoma was 53.4 (S.D.11.85), very similar to the mean age of the control patients. Age, parity and socio-economic matching, are shown in table 13.

Serological titres, were available for all study and control patients for HSV and CMV, however the results from five samples of the study group, and four samples of the control group, were not available for Chlamydia. These samples were broken, or lost in transit to the laboratory. The denominator for analyses

involving chlamydia titres, or combinations involving Chlamydia titres, is therefore 95 for the study group, and 96 for the control group, but it is 100 for controls and study for HSV and CMV. Stage of tumour was known in 96 study patients, and tumour differentiation was available in 80 study patients (table 14).

a) SEROLOGY

The number of patients, with evidence of previous infection, is shown for each agent, and combination of agents in control and study patients (table 15). There is no significant difference in the numbers with evidence of previous infection to HSV ,CMV or C. trachomatis, between study and control groups. An apparent difference between the groups for previous Chlamydia infection (8 cf. 17, chi sq. 3.83 N.S), did not reach significance. Similarly, differences in combinations of previous infection, involving Chlamydia, were not significant.

Consideration of the absolute values for the titres to each agent, did not reveal any differences between the study and the control groups (table 16).

b) STAGE AND TUMOUR DIFFERENTIATION

The serological status, was linked to the above features of the disease in the study patients; Neither HSV or CMV antibodies, were found to be associated with more advanced disease, or poor tumour differentiation. The tumour type, was known in fifteen of the seventeen patients, who had antibodies to C. trachomatis; nine of these patients' tumours were poorly differentiated. Thus 24% of patients with poorly differentiated tumours, had antibodies to C. trachomatis, compared to 14% in patients with well or moderately differentiated tumours (n.s.).

Only 8% of the control group patients had these antibodies. There were seven patients, under the age of fifty years, with poorly differentiated tumours, five of these had antibodies to C. trachomatis. There was no correlation between tumour stage and serological status for any agent (table 17).

c) SOCIO-ECONOMIC STATUS (SE) AND ANTIBODY PREVALENCE

The data for the control patients, indicate that the prevalence of antibody to HSV is usually between 65 and 75 % of S.E. groups. There is no increase in proportion affected with decreasing status, until group 5 which has a higher rate. The study group behave in the same manner.

The CMV status of the control patients, was slightly different, and there appeared to be an increasing prevalence of antibody, with decreasing status. In the control group, 37 of 48 patients (77%), in S.E. groups 4,5 and 6, had antibodies to CMV, compared to only 26 of 48 patients (54%) in groups 1,2 and 3. This trend was was also evident in the study group. The numbers in the C.trachomatis group are so small, that meaningful comment cannot be made (Table 18).

d) SYNERGY

Synergy between agents such as these, has been suggested as a model for cervical squamous neoplasia (49), but there has been no evidence from these data that synergy exists between these agents. There is no more prevalent seropositivity for combinations of agents in the study group.

e) AGE AND ANTIBODY STATUS

A database of prevalence of seropositivity rates at different ages, was constructed among the control group. To expand the

numbers of premenopausal patients, data from the group of control patients, from the CIN study patients in the previous chapter, were added. These patients also had serology to HSV, CMV, and C. trachomatis evaluated. This gave a group of patients, of varied socio-economic status, all of whom had no past history of cervical neoplasia. It is considered valid to add this group to the control patients of the present study. This enables reasonable cohorts in each age range to be compared to the patients with carcinoma. There will be very few patients with carcinoma, in the young age group, and their infection profile can now be compared to a larger group of normal women. This avoids bias against the findings in the young women, by the overwhelming number of older women. A one to one matching study will not give useful information on the young woman who are few in number. This exercise will allow population comparison, but will not aid in the ascertainment of aetiology. Data were available for 200 patients on HSV,CMV status, and 194 patients on C. trachomatis status (table 19).

Study and control patients were analysed by proportion, having antibodies at each decade of reproductive and post-menopausal life. As stated, age at tumour diagnosis, rather than age at sampling, was chosen for the study patients, but figures by both methods are shown; (table 20a study patients age at diagnosis, table 20b study patients age at sampling). Among the control patients, the percentage with positive serology to HSV, did not vary significantly, from one decade to the next. Thus, if a patient was not seropositive by age 35, then new primary infection was unlikely. The antibody profile of CMV was

different; The percentage seropositivity continued to increase with advancing age, indicating primary infection occuring throughout the reproductive years. The numbers with titres to C. trachomatis are commented on in the next chapter.

Comparison of these figures, (table 19 to table 20a,20b), reveals that for patients over the age of 45 years, each agent follows the same age/infection pattern. The situation in the younger woman appears different. The very young patient, developing carcinoma, is more likely to have antibodies to one, two, or all three agents, than the young woman in the control group. The trend persisted in the 35-44 year age group, but was less marked. The large number of older patients with carcinoma, tends to obscure this observation. It appears the younger patient with carcinoma of the cervix, has a different prior infection profile, than control patients who were obtained from similar socio-economic circumstances.

DISCUSSION

A study of this nature, will not identify a causal agent, or agents, for cervical carcinoma. The control patients form a database, on which the limitations of data from study patients, are evident. The three putative causal agents for cervical neoplasia, are viewed against variations in the normal patient, with age and socio-economic status. The data from control patients, indicates increasing seropositivity with age, and decreasing SE status; Both risk factors in the development of cervical carcinoma. If this is the natural progression of these infections in population terms, seroepidemiological trials of this size will not produce an answer, as to whether these agents

are implicated in carcinogenesis.

Many previous, similar sero-epidemiological studies, into associations with cervical carcinoma, conclude that further more specific research is required, to implicate any organism in carcinogenesis. The choice of which antibody test to use to indicate previous infection, can greatly influence prevalence estimates. Generally, microimmunofluorescence techniques will give considerably higher numbers with evidence of previous infection to C. trachomatis, than complement fixation (C.F.) tests (64). In one study, 56% of patients had antichlamydial C.F. antibodies, but 81% of the same patients, had positive immunoflorescent titres (65). It is most important, that comments and conclusions are confined to the group of patients under study, all who have the same tests and are comparable.

Comparison of study and control groups, does reveal that previous infections with all three agents, are more prevalent in the carcinoma group, but statistical significance is not reached. Similarly, those with more than one previous infection, although more common in the study group, do not constitute an identifiable high risk group. Much of the difference, is due to the greater prevalence of C. trachomatis in the study group. The concept of synergy between two or more agents to initiate and promote carcinoma, is attractive (49,50). There is little evidence from this work, to suggest these agents act in this way. Definitive comments on synergy between organisms, cannot be made on data such as these. Patients who have had cervical cancer, also appear to have had exposure to more sexual pathogens, than other patients. No extremely high risk group has

emerged. To exclude HPV from consideration of synergy, is to omit a powerful aetiological contender. At present,there is no reliable way of indicating prior HPV infection, by serological means (72). Similar work with cervical intraepithelial neoplasia, does not indicate evidence of synergism between HPV and any of these other agents (Walkinshaw, Roberts and Cordiner, unpublished observation).

The influence of socio-economic status, may be overlooked in studies such as this. When dealing with genital pathogens, social attitudes to sexual promiscuity, are very important in the rate of spread of the disease. Spread of Chlamydia, whether genital or extragenital, will be more rapid in conditions of poor hygiene, overcrowding and sexual promiscuity. While sexual behaviour may not equate with socio-economic status, the other aspects of relative or absolute poverty, may considerably affect seropositivity rates.

Socio-economic groups 2 through to 5, are well represented in the control data. Although all seropositivity is more common, in SE group 4, there are differences in the profile of infections. HSV seropositivity, appears less linked to SE status, than CMV. Among the control patients, seventyseven percent of SE 4,5,6 had antibodies to CMV, whereas only 54% SE 1,2,3, had antibodies to CMV. Others, however, have found that CMV,is not correlated to social class,and only HSV 2 is more prevalent in lower SE groups (54). Such large variations in the normal population,with change in SE group, render studies such as that by Fucillo et.al. (53) which utilise case control matching, of greater value. This study confirmed an association between cervical carcinoma and

CMV. Other population studies, which may appear to confirm an association (54), have less value unless they are very large.

Age is another extremely important variable, in the rate of seroconversion to CMV. Griffiths and Baboonian, have shown a progressive increase in seropositivity, during childbearing years. They found only 40% of a large population of women seropositive under 16 years, but this percentage increased to over 80% seropositive over 41 years (55). Others have confirmed this finding (54). Alexander has suggested this organism is sexually transmitted (175), but eighty percent of normal women, are unlikely to be infected solely by a venereal vector of transmission. These data confirm this relationship of CMV seropositivity to age, but as a womans sexual behaviour, is usually determined by her midtwenties, it is unlikely that CMV is transmitted solely by venereal contact.

The relationship of increasing seropositivity to age, does not apply to HSV. Vestergaard et.al. have shown higher proportions of cervical cancer patients, than controls have antibodies to HSV type 1 and type 2; With a more impressive relationship to type 2 antibody (54). The study reported here, has involved complement fixating antibody to HSV, and this is not type specific. Recent data, however, implicates both type 1 and type 2 HSV in genital herpes (176), and perhaps it is now valid to consider both as oncogenic. The majority of people with HSV CF antibody, will have produced, this due to oral herpes and have had no previous genital herpetic lesion. But to consider only type 2 HSV, will ignore the increasing group who have genital herpetic lesions due to HSV type 1.

It is because of the high prevalence of HSV antibodies, that these data are not helpful in identification of differences between controls, and carcinoma patients.

Chlamydia trachomatis, on the other hand, is an organism with a considerably lower seropositive prevalence. The micro-immunoflorescent test, however, is more specific for the organism than the CF test. Because of the lower prevalence, this organism is more noticeable in the study group, than the control group and it appears that both alone, and in combinations with the other organisms, this agent is more frequently encountered in the cervical cancer group. This finding, illustrates the difficulty that studies such as this encounter. Although the agent is more frequently found associated with cervical cancer, is this a causal role, or a casual association due to both conditions arising in the same high risk patient? A more full discussion on the rates of isolation and seropositivity, is given on these data in the next chapter.

Two interesting observations have arisen from this study. Firstly, the presence of evidence of prior infection with C. trachomatis, is more prevalent in patients with advanced and poorly differentiated tumours. This is especially true in the young woman who develops a poorly differentiated tumour. Possibly C. trachomatis is a co-factor in tumours in the young woman, but is less important in the older woman. The large numbers of older women, however, have rendered this association less apparent. C. trachomatis infection, related to tumour differentiation, may be an indication that advanced disease accompanies the increased sexual promiscuity, that chlamydial

infection implies. The alternative is that Chlamydia does modify the natural progress of the disease.

Secondly, the concept of different aetiological factors in older women developing cervical carcinoma, finds support in this work. In the control group, the prevalence of antibodies to all three agents, is greater in women over the age of 55 years, when compared to the study group. This contrasts with the younger woman, in whom the highest prevalences of prior infection are found, in the study group. The older woman may have a less sexually related disease.

TABLES FROM CHAPTER 5

TABLE 13

Patient details

	n	mean age at sampling(SD)	mean age at diagnosis(SD)	mean parity (SD)
STUDY	100	58.7yrs.(12.3)	53.4yrs.(11.85)	3.44(2.40)
CONTROLS	100	53.5yrs.(9.16)	-	2.93(1.96)

	n	SE1	SE2	SE3	SE4	SE5	SE6	SEunknown
STUDY	100	2	12	23	39	18	3	3
CONTROLS	100	3	14	31	31	13	4	4

TABLE 14

Tumour stage and differentiation

n=100

STAGE 1	44	WELL DIFFERENTIATED	18
STAGE 2	31	MOD DIFFERENTIATED	24
STAGE 3	17	POOR DIFFERENTIATED	38
STAGE 4	4	UNKNOWN	20
STAGE UNK.	4		

TABLES FROM CHAPTER 5

TABLE 15

Evidence ofprevious infection

	n	HSV+	CMV+	HSV+CMV+
STUDY	100	80	77	63
CONTROL	100	74	67	55

	n	Ch.+	Ch.+HSV+	Ch.+CMV+	Ch.+HSV+CMV+
STUDY	95	17	14	12	11
CONTROL	96	8	6	6	5

TABLE 16

Absolute values of titres

n=100 for HSV/CMV

n=95 for Ch. study and n=96 for Ch. controls

	8			16			32			64			>64		
	HSV	CMV	Ch.	HSV	CMV	Ch.	HSV	CMV	Ch.	HSV	CMV	Ch.	HSV	CMV	C
STUDY	46	23	4	23	21	6	10	26	4	1	5	2	0	2	1
CONTROL	25	27	2	35	24	4	13	11	2	1	4	0	0	1	0

TABLES FROM CHAPTER 5

TABLE 17

Antibody status related to stage and tumour differentiation

	n	HSV+	CMV+	Ch.+	HSV+CMV+	HSV+CMV+Ch.+
WELL DIFF	18	11	11	4	6	2
MOD DIFF	24	24	20	2	20	2
POOR DIFF	38	29	29	9*	22	6
STAGE 1	44	37	33	7	28	5
STAGE 2	31	23	25	6	19	4
STAGE 3	17	13	15	3	11	3
STAGE 4	4	4	3	0	3	0

* 5/7 patients under 50 years with poorly differentiated tumours had antibodies to C. trachomatis

TABLE 18

Socio-economic status and antibody status

	cont(c)	study(s)	HSV+		CMV+		Ch.+	
	n	n	(c)	(s)	(c)	(s)	(c)	(s)
SE 1	3	2	2	1	1	1	0	0
SE 2	14	12	10	9	8	6	1	2
SE 3	31	23	21	16	17	17	3	3
SE 4	31	39	25	33	25	33	2	9
SE 5	13	18	11	16	8	14	0	0
SE 6	4	3	2	2	4	3	1	1
SE unk.	4	3	3	3	4	3	1	2

TABLES FROM CHAPTER 5

TABLE 19

Prevalence of antibody at different ages

CONTROL GROUPS

1 n=200

age(yrs.)		HSV+(%)	CMV+(%)	HSV+CMV+(%)
<35	76	50(67)	20(26)	16(21)
35-44	24	15(63)	11(46)	8 (33)
45-54	74	52(70)	46(62)	38(51)
55-64	14	12(86)	13(93)	11(79)
65+	12	8 (67)	9 (75)	6 (50)

2 n=194

age(yrs.)		Ch.+(%)	HSV+CMV+Ch.+(%)
<35	76	11(14)	3 (4)
35-44	23	3 (12)	1 (4)
45-54	72	4 (6)	3 (4)
55-64	13	2 (15)	2 (15)
65+	10	1 (10)	1 (10)

TABLES FROM CHAPTER 5

TABLE 20
Prevalence of antibody at different ages
a) STUDY GROUP - AGE AT DIAGNOSIS OF CARCINOMA
1 n=100

age(yrs.)	n	HSV+(%)	CMV+(%)	HSV+CMV+(%)
<35	8	6 (75)	6 (75)	5 (62)
35-44	14	13(93)	9 (64)	8 (57)
45-54	24	20(83)	17(71)	15(62)
55-64	34	26(76)	30(82)	24(71)
65+	20	15(75)	15(75)	11(55)

2 n=95

age(yrs.)	n	Ch.+(%)	HSV+CMV+Ch.+(%)
<35	7	3 (43)	1 (14)
35-44	14	3 (21)	2 (14)
45-54	23	3 (13)	3 (13)
55-64	32	5 (16)	3 (9)
65+	19	3 (16)	2 (11)

b) STUDY GROUP - AGE AT SAMPLING
1 n=100

age(yrs.)	n	HSV+(%)	CMV+(%)	HSV+CMV+(%)
<35	4	2 (50)	2 (50)	1 (25)
35-44	13	13(100)	8 (62)	8 (62)
45-54	10	9 (90)	7 (70)	6 (60)
55-64	39	29(74)	35(90)	28(72)
65+	34	27(79)	25(74)	16(47)

2 n=95

age(yrs.)	n	Ch.+(%)	HSV+CMV+Ch.+(%)
<35	3	2 (50)	0 (0)
35-44	13	3 (23)	2 (15)
45-54	10	3 (30)	3 (30)
55-64	37	3 (8)	2 (5)
65+	32	6 (19)	4 (12)

CHAPTER 6

AGE RELATED ISOLATION AND ANTIBODY STATUS TO CHLAMYDIA TRACHOMATIS

INTRODUCTION

Chlamydia trachomatis, is an extremely prevalent pathogen in the 20th. century. It is the cause of trachoma, the commonest preventable blindness, with around 500 million cases worldwide (177). Some serotypes cause lymphogranuloma venereum, and genital carriage of the organism can lead to inclusion conjunctivitis and non gonococcal urethritis. Asymptomatic carriage, however, can affect the greatest number of women, with rates of isolation of the organism of up to 16% of pregnant women (178). Most studies, however, indicate an isolation rate of around 7% of sexually active females (59,179,180).

The role of C. trachomatis, in cervical carcinogenesis has already been discussed, and this at present, is unproven. It is however, an agent, that is isolated in the woman who is also at risk of developing CIN. As HPV is being increasingly recognised as a factor, associated with, and possibly causal to CIN, so too may C. trachomatis be important. The data shown in these present studies, indicate that this organism is prevalent in the population studied, and this concurs with the work of others (59,64,65,66,67,178,179,180). It has been shown by Griffiths and Baboonian that the prevalence of seropositivity to CMV, was a factor of age (55). Similar work on C. trachomatis is not available, and although the infection is known to be prevalent in the younger more sexually active woman, the rate in older and postmenopausal women is not clearly established (64,181).

The two previous studies, have supplied data on both isolation and serology to C. trachomatis, on a large body of

normal women. By pooling these data, it is possible to observe the female population, for rates of isolation and seroconversion, at different ages. It may be that there is a change in the prevalence of the organism, and thus a possible co-variable for cervical neoplasia.

PATIENTS AND METHODS

The study population consisted of 214 women, with no evidence of pathological vaginal discharge, cervical infection, pelvic inflammatory disease or CIN. One hundred patients had both isolation and serology for C. trachomatis, 20 additional patients had isolation only, and 94 patients had serology alone. There were 120 patients who had isolation performed (age range was 16-56 years), and 194 serological patients (age range of 16-89 years).

The isolation techniques, and antibody titrations have been described in the previous chapters.

RESULTS

Twenty of the 194 patients (10.3%), were seropositive with titres>=16 and 10 of 120 (8.3%), were isolation positive. Patients were grouped into 5 year age bands, ranging from under 20 to over 80. There were few patients over the age of 60 years. The prevalence of seropositivity was rarely below 10% for any age group up to 60 years, but there was a peak of 26% (6/23) at age 30-34 years. Only one subject was seropositive over the age of 60 years. Data are displayed in table 21, and 10 year age bands are shown in fig. 1.

Isolation was confined to younger more sexually active women, with no positive cases over the age of 39 years. The peak age

range of isolation, was 20-24 years with 28% (5/18). Data are shown in table 22, and 10 year age bands in fig. 2. Isolation and serology rates are related to age in fig.3.

There was no predilection for any socio-economic group. The mean parity of the isolation positive patients was 1.22 (S.D. 1.09), considerably lower than the mean parity of the sero-positive patients; 2.62 (S.D. 1.74). This effect is likely to be due to the difference in the ages of the two groups, rather than any effect on fertility.

DISCUSSION

There is wide disparity, in the isolation rates of C. trachomatis in the literature, and overall this figure of 8% is in accord with many studies (59,64,179,180,181). It is surprising however, that the age range is so narrow, with such high percentages of young women affected; More than one in four women between 20 and 24 years affected. These women were all asymptomatic and as the organism is predominately sexually transmitted, the chance of exposure to the organism must be very high, among sexually promiscuous women. The prevalence at different ages in this study, is in accord with the findings of Khurana and co-workers (181), who found 18% of women up to 30 years, and 13% of women up to 40 years, isolation positive. No patient over 40 years was isolation positive. Chlamydia isolation from the genitalia has, however, also been found in the postmenopausal patient, who has had hysterectomy (182). Schachter and co-workers showed that antibodies to C. trachomatis are less prevalent with increasing age (64). Complement fixating antibody was found in 25.9% of women under

35 years of age, but in only 5.3% of women over 35 years of age. This difference was not seen in women tested with the microimmunoflorescent test, and the possibility of change in sexual behaviour of the population, was raised as an explanation for this observation.

We are probably witnessing a prevalence of C. trachomatis infection, not previously seen in less sexually relaxed times. Similar seropositive rates to the isolation rates were seen, but these occurred a decade later. Those patients who had both tests done, did not always have positive serology when isolation was positive. Isolation is likely to be the more sensitive test. One explanation for this observation, was suggested by Kalimo et al. (66), who felt that asymptomatic carriage only stimulated local antibody in the cervix. It may take a decade of asymptomatic carriage, for antibody to be detected in serum by this particular microimmunoflorescent test. Other workers have found a similar lack of detectible antibody in isolation positive patients (179).

The finding in this study, of such high colonisation rates in control women, who are healthy, suggests that this organism may either be commensal in a number of cases, or it has possibly a long latent phase as suggested by Kalimo et. al. (66). These figures are very much higher, than those studies which indicated higher isolation rates in CIN patients, than controls. In two such papers, the prevalence rate of C. trachomatis in control patients by isolation techniques, was .8% and 1% respectively (57,64). Such low prevalence rates may be a feature of matching of controls, especially for sexual behaviour. In another paper,

Hare and colleagues showed that Chlamydia was highly sexually transmissible with 18% of partners of men with NGU, harbouring Chlamydia on the cervix (183). The strongest evidence implicating C. trachomatis in aetiology of CIN, still lies with the observation of high rates of CIN, in C. trachomatis isolation positive patients (184). This, of course, may be association and not aetiology.

Such infection rates as have been found here, would add support to either pre-operative cervical culture for C. trachomatis, or prophylactic antibiotics for procedures such as termination of pregnancy, or laparoscopic hydrotubation. This would reduce the chance of a chlamydial pelvic infection following the procedure. There is little doubt, that Chlamydia causes tubal damage, and that this is a cause of infertility (185). Patients with tubal infertility, have the highest rates of positive chlamydial titres (186).

There appears to be a high 'infection' rate with C. trachomatis, on the cervix of young healthy women. There is a lag time to the development of systemic antibodies. These high rates in young women, and low rates for isolation and serology in the older woman, may suggest one of two things; Either the immunological memory for C. trachomatis, falls below the sensitivity of this test, with the passage of time, or indeed of much greater importance, C. trachomatis is a much more prevalent cervical organism than in the past. If this is so, an increase in Chlamydia related problems such as salpingitis, cervicitis, and possibly CIN, may be inevitable. If C. trachomatis is an

important co-factor in aetiology of cervical neoplasia, then it is likely to be considerably less important in the older patient, where it is more rarely detected.

TABLES FROM CHAPTER 6

TABLE 21

Seropositivity to C. trachomatis related to age

AGE GROUP	NUMBER	SEROPOSITIVE	%
<20 years	10	0	0
20-24 years	18	2	11
25-29 years	25	3	12
30-34 years	23	6	26
35-39 years	14	2	14
40-44 years	9	1	11
45-49 years	24	0	0
50-54 years	48	4	8
55-59 years	8	1	12
>60 years	15	1	7
TOTAL	194	20	10.3

TABLE 22

Isolation of C. trachomatis related to age

AGE GROUP	NUMBER	CULTURE POSITIVE	%
<20 years	10	0	0
20-24 years	18	5	28
25-29 years	25	2	8
30-34 years	22	2	9
35-39 years	14	1	7
40-44 years	6	0	0
45-49 years	10	0	0
50-54 years	13	0	0
55-59 years	2	0	0
>60 years	0	0	0
TOTAL	120	10	8.3

figure 1

Age specific frequency of positive titres to C. trachomatis

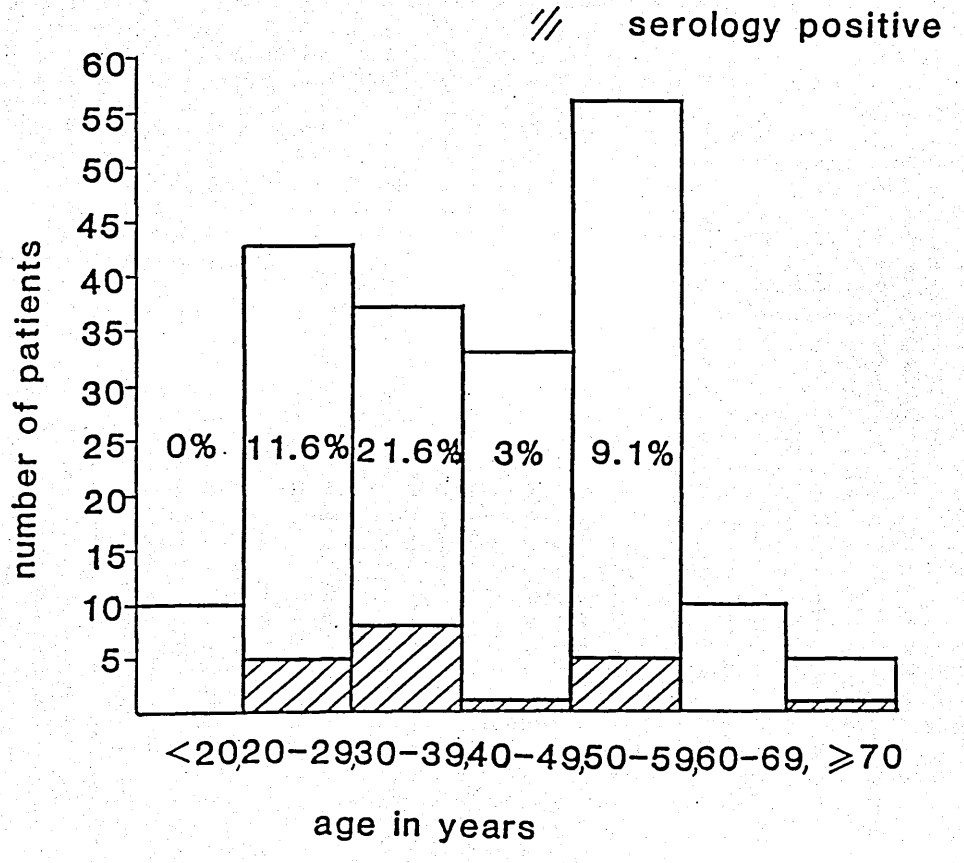


figure 2

Age specific frequency of isolation of C. trachomatis

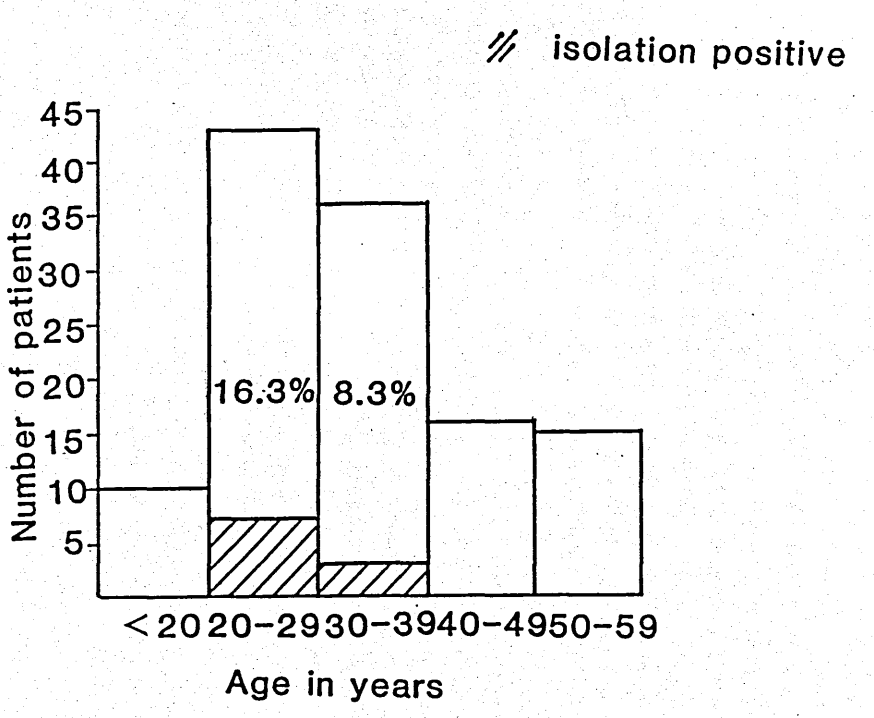
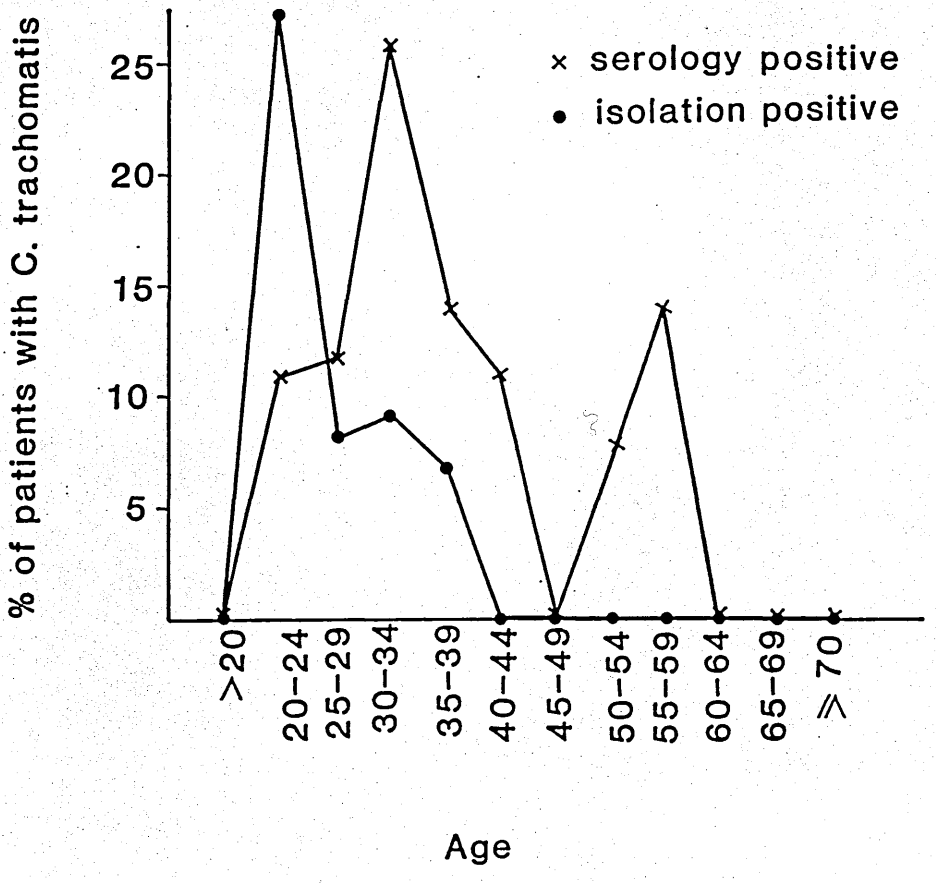


figure 3

Age specific groups of women with evidence of infection with C. trachomatis



SUMMARY TO SECTION 2

The purpose of the investigations in this section, has been to relate agents, which are associated with cervical neoplasia, to age of the patient. The microbiological insults, to the lower genital tract, are different as age progresses; Sexual intercourse, after the menopause, is less frequent. Cessation of menstruation, removes both the curettage effect, and the need for tampons which are an ideal culture medium (c.f. toxic shock syndrome). The barriers to infection are less effective, as hormone deficiency becomes established, after the menopause.

In comparison to the childbearing years, the cervix is in a different microbiological environment after the menopause. An environment in which carcinoma more frequently develops. The factors, however, that are strongly associated with cervical neoplasia, namely HPV, HSV type 2 and possibly C. trachomatis, are rarely found in the older woman. The postulate, is of alteration in genetic composition, many years before manifestation as carcinoma.

The effect of age on isolation, or evidence, of these agents, has been addressed by these studies in this section. HPV gives evidence of its presence by, histological or cytological techniques, only in pre-invasive disease. To determine that HPV is associated with a cervical carcinoma, requires DNA-DNA hybridisation. This indicates the presence of DNA homologous to the DNA found in certain types of HPV. Investigation of HPV, by techniques other than in situ hybridisation, necessitates use of patients with CIN, rather than carcinoma. Relating the presence of HPV changes, to age at detection of CIN, gives some

indication of the influence of HPV, on rate at which carcinogenesis develops. This agent may hasten the development of carcinoma, by its action as a co-factor (49). Similarly the carcinoma may develop later in life, with age as a co-factor. A small, and insignificant decrease in age at diagnosis of CIN, was seen in CIN 1 and 3. The population samples are probably not large enough for this point to be clearly made.

It is important that HPV was not found in any control patient, cytologically, clinically, (or colposcopically in 24%), and this confirms HPV as a risk factor for CIN. No HPV was found in the menopausal patients, who all underwent colposcopy and cytology (chapter 4), neither was it found in the control patients to chapter 5 who had cytology.

HPV is an infrequent finding in older women.

There is indirect evidence of the protective effect of the sheath, with very low rates of usage in the groups with HPV (chapter 4). Other workers have found that barrier contraception prevents an anaerobic shift in cervical flora (187), and a protective effect to cervical carcinoma is known.

There was confirmation that cervical inflammation may give rise to abnormal cytology, and the value of pre-colposcopic cervical culture and treatment was shown.

A more pronounced effect of age, on rates of isolation, than that due to HPV, was seen with C. trachomatis. The ages of isolation positive patients was younger than isolation negative women, for all groups (chapter 4, table 10). As this observation was equally evident in the control group, as the study groups, it appears likely that both C. trachomatis, and sexual activity,

are more closely related to youth, than any effect due to C. trachomatis hastening the development of CIN.

The work of chapter 5, indicates that, although overall, C. trachomatis previous infection, is no more prevalent in the study than control group, there may be a difference in a small subset of women. A small cohort of women who are under 50 years and had poorly differentiated tumours, had a greater prevalence of antibodies to C. trachomatis.

The compiled data in chapter 6, do indicate, this is an infection of the younger woman. Isolation was not found in women over 40 years, and antibodies were rarely detected in postmenopausal women. The sensitivity of the test, in detecting very low levels of antibody, in women who may have had infection many years previously, may be low. The alternative explanation is that C. trachomatis is more prevalent in the population, due to a change in sexual practices, and further population effects, on fertility, and possibly CIN may yet be manifest.

The data available for the postmenopausal women, although limited in numbers, prove interesting. There is no evidence, that postmenopausal women, are less prone to develop common vaginal infections, than younger women. HRT similarly, did not improve the situation, and indeed, more potential pathogens were isolated after HRT, than in the hormone deficient state.

CHAPTER 7

VARIATION IN CERVICAL WATER AND CERVICAL HYDROXYPROLINE WITH AGE

INTRODUCTION

The success of cervical screening programmes in the U.K., has reduced the numbers of women, dying from cervical cancer, in the fifth and sixth decades (1,4). There has been less benefit to women older than this. They are less likely to avail themselves of cervical screening, and the screening process itself may be less effective. If a cytological abnormality is found in the postmenopausal woman, often the first diagnostic procedure is a cone biopsy. It is felt that colposcopy has limited value in these older patients, as often there is a failure to visualise the entire transformation zone. This raises the question of why the squamo-columnar junction (SCJ), recedes into the endocervical canal as a woman ages.

The cervix of the postmenopausal woman, is smaller than that of the premenopausal woman, and the reduction in the cervical bulk tends to make the SCJ recede into the canal (135). Eversion, due to opening of the bivalve speculum, is less in the tight postmenopausal vagina, and may cause more distress or even trauma (188). Ostergard found the SCJ in the canal of 100% of patients over 60 years (103), and Paterson et.al. could only visualise the SCJ in half their postmenopausal patients (125). This study and the following study, investigate the cervical stromal structure in women of various ages, for factors relevant to the re-location of the SCJ with age. The first study investigates the water and collagen content near the SCJ, and deeper in the cervical stroma. It also assesses the influence of exogenous oestrogens on both these cervical components. The

second study, searches for differences in collagen distribution in the cervix, throughout adult life.

Each study utilises a different approach to collagen quantitation. The first study, investigates the hydroxyproline content both per unit weight, and as a proportion of total protein in the sample. Hydroxyproline is an amino acid, that is virtually specific to collagen, and as such is a reliable indicator of collagen content. It has been variously reported as 10% (111) and 12% (112) of collagen. Collagen represents 82% of the total protein in the non-pregnant cervix (118), and 85% of dry weight in the non-pregnant cervix (112). Acid soluble hydroxyproline, on the other hand, accounts for only 28% of the non-pregnant cervix (111), and thus the majority of collagen in the non-pregnant state, is old, with acid stable cross-links (112). Measurement of the hydroxyproline content, is an established investigation of collagen content (110,111,112, 118,119). The second study, quantitates collagen, by relying on its property of birefringency, and quantitating the transmitted light of histological preparations.

PATIENTS AND METHODS

Cervical biopsies, were obtained from 76 women (age range 22-85 years), who attended the menopausal, gynaecological and colposcopic clinics at the Western Infirmary, Glasgow. Written informed consent for cervical biopsy was obtained, and approval was granted by the Western Infirmary Ethical Committee. All biopsies, were obtained from healthy tissue; In 64 patients, punch biopsy by Leech-Williamson forceps was obtained, and the sample included a small piece of cervical epithelium. The biopsy

was taken on the ectocervix, several millimitres from the external os. In the remaining 12 patients, biopsy was obtained at hysterectomy from the cervical stroma, .5 cm. deep to the epithelium at the SCJ. No epithelium was present in these samples.

Biopsies were immediately frozen, stored at -20° C until analysed, (maximum storage period 3 weeks). The tissue was dried at 37°C for one week, and weighed to determine dry weight. Ten millilitres of 6N HCl was added, and incubated at 37°C for one week, to break down the tissue. One ml. of this solution in 6N HCl is hydrolysed, by heating at 105°C in an oil filled heating block for 16 hours, and the hydroxyproline in the hydrolysate, assayed by the method of Blumenkranz et. al. (189). The protein content of the tissue, was estimated by the method of Lowry et. al.(190).

The first analysis was performed on punch biopsies, from 30 patients of age range 25-85 years. In these patients, the hydroxyproline protein ratio, was correlated to age and menopause.

In the second analysis, performed on punch biopsy specimens from 27 further patients, water content was evaluated from wet and dry weights. Hydroxyproline was compared to dry weight, to exclude variation in cervical water with age. Hydroxyproline/dry weight ratio, and percentage water, were correlated to age and menopause. The biopsies in the second analysis, were more tightly controlled for weight, with an attempt to obtain larger biopsies. If the dry weight of biopsy was <8mg. in either analysis group, then results were excluded, as the epithelial

mass (with no hydroxyproline) was considered to be too great, relative to the stromal mass. The effect of too great an epithelial mass, would be to falsely lower the hydroxyproline/dry weight ratio, but by exclusion of small biopsies, this effect was minimal.

The same percentage water and hydroxyproline/dry weight ratio measurements, were made on 7 punch biopsies, from women taking hormone replacement therapy. They were also made on 12 deep biopsies of cervical stroma, obtained at hysterectomy for benign conditions, other than prolapse. Pre and postmenopausal samples were compared.

RESULTS

In the first group of 30 specimens, the dry weight range was from 3-31 mg.; Ten were excluded, as they had a dry weight less than 8 mg. The results on the remaining 20, are shown in table 23. The mean hydroxyproline total protein ratio, for the premenopausal women, was lower than the same ratio for postmenopausal women. The correlation of ratio to age, was not significant. As biopsies of comparable size were obtained from both pre and postmenopausal women, and the difference was still apparent, the effect of the slightly thicker epithelium in the premenopausal patient, was considered minimal.

The second group analysed, were more closely matched for size, but still the range was 5.4-29 mg. Three were excluded; 2 for being under 8 mg., and one because clinical data could not be traced. Twentyfour samples were thus included in the analysis. Comparison of cervical water, between the pre and postmenopausal samples, revealed a difference (premenopausal

mean 79.3% postmenopausal mean 75.9%, $p<0.05$; table 24). This difference, however, became apparent at the menopause, and was not a gradual decrease in cervical water with age. The hydroxyproline dry weight ratio, is not dependent on cervical water, so this ratio is not affected by these differences. This ratio is also shown in table 24, and it is not dependent on the other proteins present in the tissue. This ratio is simply a measurement of hydroxyproline in a given piece of tissue, and as such is perhaps a clearer reflection of collagen content, than the ratio comparing hydroxyproline to total protein.

In the second group, there were 13 premenopausal patients, and 11 postmenopausal patients. By summating the ratios for both groups, there were significant differences between mean hydroxyproline dry weight ratios; (pre .055, post .080; $t=4.12$, $p<0.001$). The correlation co-efficient of age to hydroxyproline content, was significant ($r=.742, t=5.20, p<0.001$). As the age increased, so also did the hydroxyproline content, indicating greater collagen density with advancing age.

The final group of samples analysed, included 12 biopsies taken from the deep cervical stroma, and 7 punch biopsies from women taking HRT. Seven of the deep biopsies were measured wet and dry, and the cervical water was found to be considerably lower in the deep biopsies, when compared to superficial biopsies. There was no decrease in water content with age. Similarly, there was little variation in the hydroxyproline content with age, in the deeper biopsies (table 25). In general, these biopsies had a hydroxyproline content, similar to that found in superficial biopsies, in postmenopausal women. Cervical

water near the epithelium therefore, appears variable, and is dependent on age. Deeper in the cervix, there is a collagenous core, with a water content that is not variable with age.

The samples from patients taking HRT, included three small biopsies (table 26). However, despite this, the hydroxyproline dry weight ratios remained high, and were in the range of the postmenopausal patient, and above the values found in premenopausal women (cf. table 24). The water content however, had risen almost to the levels seen in the premenopausal patients.

DISCUSSION

The aim of this study, is to account for the change in appearance of the cervix with age. From the clinical viewpoint, the most important change, is the inability to visualise colposcopically, the squamo-columnar junction in the older woman (103,125,135). Biopsies taken from this area, have shown differences between pre- and postmenopausal women. There is a change in the proportion of cervical water between these women, but this change appears to occur at the time of the menopause, and is not gradual. The diminution of ovarian steroids, with their water retaining effect, may be responsible for this. The effect of such a change, will be to make the cervix more dense, and less amenable to manipulation, during colposcopic examination of the endocervix.

The percentage cervical water content, found in this study, are slightly higher than the findings of other authors. Kleissl and colleagues, found 73% water in cervical biopsies, taken at hysterectomy in 5 women aged 29-41 years (111). Danforth et al.

found 74.4% cervical water in non-pregnant subjects (118), but both these studies used full thickness blocks, obtained at hysterectomy. Such figures are in agreement with the data obtained in the deep biopsy group, in this study. One study obtained cervical tissue, by a rotating needle biopsy of cervix, a technique yielding similar tissue to the present punch biopsy study. This found an 80.8% cervical water in premenopausal women (112). These figures, are closely in accord with the data in this study for the premenopausal woman. Comparison of the values for postmenopausal women, is not available, however animal models indicate, that there is an increased water loss from connective tissue with age (191).

These observations on cervical water, explain some of the clinical findings. The cervix in the premenopausal woman, is more oedematous (104), and in the subepithelial layer has an abundant blood supply. Both of these will reduce after the menopause. These changes, appear confined to the stroma near the surface epithelium, as the cervical stroma core appears similar irrespective of age.

As the water content, of the cervical stroma at the SCJ decreases with age, the hydroxyproline concentration rises. This is independent of the water content, as the ratio to dry weight confirms. This change is more a gradual one, and not as dependant on the event of the menopause. The young patient has a thicker epithelium which, with age, gradually thins, especially after the menopause (192,193). The biopsies included the epithelium, and this must have some effect. By considering only biopsies of a reasonable size, this effect is minimised.

The change in the hydroxyproline content is real, not merely apparent, but it only affects the stroma adjacent to the epithelium. Deeper biopsies (table 24), show no difference with ageing, and the hydroxyproline values are similar to those biopsies taken from the postmenopausal patients by punch biopsy.

Study of the data available on the anatomy of the cervix, do not readily explain these findings. Hughesdon, in a monograph, compiled from histological study, firmly believed in a peripheral layer of smooth muscle which was continuous with the myometrium, and the smooth muscle of the vagina (114). He believed this to be a functional layer, and relevant to the process of cervical dilatation. Danforth, although accepting the existance of a peripheral layer of smooth muscle (115), took a different view. He believed that dilatation was due to the breakdown of cervical collagen, with resulting urinary excretion of hydroxyproline (110,115,118,119).

The findings reported here, are compatible with both views. Collagen content per weight, is a reflection of collagen density. This will be increased, if there is a greater amount of collagen, or if there is a decrease in the other constituents of cervical stroma. The presence of smooth muscle will decrease collagen density. If this is found round the periphery of the cervix, it will have the effect of producing lower hydroxyproline values, in the superficial samples of young women. After the menopause, there are fewer nerves found in the cervix (100), and it seems reasonable to presume there will be reduced smooth muscle. This would increase the collagen density, without an increase in total collagen. Similarly, the

subepithelial vessels regress after the menopause, and this may further increase collagen density. The cervix could atrophy by loss of smooth muscle fibres and tissue fluid, without either an increase or loss in total collagen. This would account for the impression found by others, that the cervix becomes more fibrous after the menopause (104).

Danforth's views of hormonal control of destruction of collagen, also explain these findings. Oestrogen will soften the 'pregnant' cervix, resulting in the breakdown of collagen (121) and collagenolysis certainly occurs in labour (111,194). It is thus likely, that in the childbearing years, the cervical collagen is being created and broken down continuously. Outwith labour, collagen fibrils are much more regular and not disordered. However, with labour, the structure becomes disrupted, and amorphous material is deposited (195). It is the fibroblast which controls collagen and ground substance synthesis, and it is able to control collagen content, by decreasing collagen synthesis, and increasing ground substance, in addition to the reverse. Prostaglandins are able to influence this balance and so too may ovarian steroids (196).

After the menopause, when the oestrogen stimulus is considerably reduced, collagen is likely to be more stable, with more cross linkages, and consequently a greater proportion of insoluble collagen (193,197).

The collagen content in the deep cervical stroma, appears little influenced by advancing age. The percentage cervical water at this depth, is in accord with the findings of others on biopsies from similar sites (111,118). From this study, at the

depth of 0.5 cm. from the epithelium, the more dense collagen matrix does not affect the malleability of the cervix at colposcopic examination.

The findings on the patients receiving HRT, suggest the water content is similar to the premenopausal woman, probably indicating the fluid retaining properties of the ovarian steroids. The collagen content, is unaffected by HRT. The findings of chapter 2, indicate that HRT improves the visibility of the SCJ in menopausal women. It may be that tissue oedema, induced by ovarian steroids, aids manoeuverability and malleability of the cervix, without a significant contribution from a decrease in the collagen content. If collagen, in the cervix of the older woman, is stable with many cross linkages, it may not be amenable to degradation and resynthesis, as happens in the younger woman.

This hypothesis, is somewhat at variance with other data on skin collagen. Collagen is known to be responsible for skin elasticity, and is thought to decrease with age, both per dry weight and per skin area (198,199). Oestrogen has effects on the dermis of the skin, and in primates, will cause increase in water and glycosaminoglycans (200). This is similar to the findings in HRT treated women in this study. Further evidence suggests, that oestrogen may speed up the process of polymerisation of soluble to insoluble collagen (201), and oestrogen treatment, may increase dermal thickness (202). Recent work, has suggested that HRT will increase total skin thickness collagen content (124,203). This suggests, that oestrogen is responsible for synthesis of collagen, and possibly also the

decrease in collagenolysis in the skin. Indeed this is its effect in increasing mineralisation of the skeleton (122). The current study, has not investigated the total collagen content of the cervix, and certainly the postmenopausal patient, usually has a considerably smaller cervix than the premenopausal woman. The total collagen content of the cervix, may be lower in the postmenopausal patient, and consequently HRT may indeed increase the total collagen content in the cervix. This study has shown that it does not increase collagen density.

In addition to tissue water, the important point as regards visualisation of the SCJ, is not total collagen of the cervix, but collagen density near the epithelium.

One last factor which should be mentioned, is the possibility that biopsies may contain glandular tissue. Such a finding, would be more likely in the younger woman, and this would increase the water content, and reduce the hydroxyproline dry weight ratio. The following study, will refute this as a cause of reduced collagen ratios in the younger patient.

TABLES FROM CHAPTER 7

TABLE 23

Hydroxyproline protein ratio from cervical biopsies

PREMENOPAUSAL			POSTMENOPAUSAL		
AGE	WEIGHT MG.	OHP/PROTEIN	AGE	WEIGHT mg.	OHP/PROTEIN
25	11.3	.0216	47	10.0	.0937
26	20.0	.0343	49	13.5	.0710
30	17.0	.0560	50	10.3	.1560
31	18.0	.0739	52	10.4	.0402
31	10.4	.0270	52	11.6	.0759
32	12.5	.0342	53	10.0	.0413
38	29.0	.0271	60	20.4	.0689
41	24.6	.0560	66	26.6	.0579
			74	21.0	.1291
			76	14.2	.0795
			77	31.5	.0752
			85	21.2	.0750
MEAN OHP/PROTEIN RATIO			MEAN OHP/PROTEIN RATIO		
.0468(S.D..0277)			.0803(S.D..0333)		

$t=2.35; p<0.05$

TABLES FROM CHAPTER 7

TABLE 24

Hydroxyproline dry weight ratio in cervical biopsies

PREMENOPAUSAL					POSTMENOPAUSAL				
AGE	WET WT.mg.	DRY WT.mg.	%H2O	OHP/ DRYWT.	AGE	WET WT.mg.	DRY WT.mg.	%H2O	OHP/ DRYWT.
22	49.0	9.8	80	.031	43	50.8	10.5	79.3	.048
23	70.0	14.6	79.2	.034	49	85.8	18.9	78	.069
24	88.0	19.5	77.9	.056	50	90.6	21.2	76.6	.099
25	141.0	29.0	79.2	.068	53	108.2	27.6	74.5	.080
27	116.0	12.7	89	.039	56	98.6	25.1	74.5	.100
30	60.5	20.0	67	.055	60	73.1	17.8	75.6	.079
31	125.0	24.2	80.6	.050	61	64.1	15.6	76.6	.087
31	41.8	8.8	79	.068	62	89.5	22.5	74.9	.084
32	109.2	18.2	83.3	.055	67	100.0	24.2	75.8	.066
37	96.4	19.4	79.9	.067	69	62.8	16.1	74.4	.087
37	135.0	27.6	78.1	.058	79	47.5	11.8	75.2	.085
44	74.8	16.4	78.1	.049					
45	114.0	23.4	79.5	.085					
MEAN OHP/DRY WT. RATIO					MEAN OHP/DRY WT. RATIO				
.055(S.D..015)					.080(S.D..015)				
t=4.12;p<0.001									
CORRELATION COEFFICIENT AGE Vs. OHP/WT. r=.742;t=5.20;p<0.001									
MEAN %H2O 79.3(SD 4.7)					MEAN %H2O 75.9 (SD 2.2)				
t=2.2: p<0.05									

TABLES FROM CHAPTER 7

TABLE 25

Hydroxyproline dry weight ratio and cervical water in deep biopsies

AGE	WET WT. mg.	DRY WT. mg.	%H2O	OHP/DRY WT.
26	23.7	7.6	67.9	.092
27	-	14.4	-	.083
32	17.5	6.5	62.9	.092
40	-	19.5	-	.052
43	60.5	16.0	73.6	.075
46	-	37.8	-	.083
47	-	14.7	-	.097
59	64.0	18.3	71.7	.066
60	-	21.3	-	.076
64	94.7	27.6	70.9	.065
72	25.3	8.1	68.0	.086
76	46.1	10.3	77.7	.068

mean %H2O 70.4 (SD 4.7)

mean OHP/DRY WT. .078(S.D..013)

TABLES FROM CHAPTER 7

TABLE 26

Hydroxyproline dry weight ratios and cervical water in women taking HRT

AGE	WET WT. mg.	DRY WT. mg.	%H2O	OHP/DRY WT.
45	20.1*	4.4	78.1	.091
50	122.0	26.6	78.2	.083
51	66.1	15.2	77.0	.066
51	30.3*	6.2	79.5	.065
52	118.2	25.9	78.1	.069
53	19.6*	4.2	78.6	.071
63	42.5	11.8	72.2	.085

*-small biopsies

MEAN %H2O77.4 (SD 2.4)

MEAN OHP/DRY WT. 0.076 (SD 0.010)

CHAPTER 8

VARIATION IN THE COLLAGEN CONTENT OF THE CERVIX AT THE SQUAMOCOLUMNAR JUNCTION

INTRODUCTION

The previous study, inferred collagen presence by detection of hydroxyproline. Direct microscopic examination of sections of cervical stroma, does not clearly show collagen when viewed under scattered light. If, however, the section is illumuminated by polarised light, collagen is easily recognisable (fig.4). Due to being a coiled structure, collagen has the property of birefringency, and refracts light in three dimensions. As such, it is easily visible when illuminated by polarised light. The intensity of the light, refracted by collagen, is much greater than light transmitted by other structures. An image analyser can be used to measure the area of light of a certain density, seen under the microscope. As collagen appears much brighter than other structures, the image analyser can easily quantitate collagen. A quantitative analysis, comparable to quantity of collagen present, is obtained. This method may be used, to compare histological sections for collagen content, but it will not yield results of absolute collagen content. Provided histological sections are comparable, and examined under identical circumstances, then comparative estimations of collagen content, may be made. These estimations may be made on similar cervical sections, at a range of ages, and the variation in collagen, per area of microscopic field, can be ascertained. Such data will augment that found in the previous study.

METHODS

Histological sections of complete cervix, from women at a range of ages, were examined. In order to obtain a full spectrum of ages, sections were chosen from routine surgical

histopathology, obtained over the period 1972-83, at the Western Infirmary, Glasgow. The ICD (International Classification of Diseases) coded records, were searched for patients who underwent hysterectomy, or removal of entire cervix, such as at Manchester repair. The period of search was so large, in order to obtain a large enough population of older patients. Indications for hysterectomy, are generally limited to malignancy or prolapse in the elderly, and many of such specimens were unsuitable.

From the 12 years searched, 182 patients were selected as likely to yield sections, taken from an intact and healthy cervix, in which cervical collagen could be comparatively assessed. Cervical sections from these 182 patients, were examined microscopically, and criteria of acceptibility were applied. These criteria for acceptibility were as follows; 1) The tissue must be well prepared, and of equitable thickness. 2) The overlying epithelium must be healthy, and the SCJ must be visible histologically. 3) There must be no stromal disease or malignancy in the cervix, and there must be no large glands or Nabothian follicles, which obscure the cervical stroma.

The application of these criteria, excluded 84 patients, leaving 98, suitable for further analysis. The great majority, were from patients undergoing hysterectomy, although 18 patients had a procidentia.

The indications for surgery, were usually menstrual problems in the pre-menopausal patients, postmenopausal bleeding or benign ovarian enlargement in the immediately postmenopausal patients, and prolapse in the old patient. As the cervix uteri

was the area examined for collagen, no patient had any cervical disease, six patients had neoplasia of the endometrium; In situ in one, and invasive in five. There was no neoplasia at any other site.

The histological sections, were identified only by their histopathology number, and were examined without knowledge of data from the patient, from whom the section was obtained. Two areas in each slide were examined by an image analyser (Optomax Image Analyser, Micro Measurements Ltd. figs.5 and 6) The first field, known as the superficial field, was selected immediately deep to the basement membrane of the cervical epithelium, at the squamocolumnar junction. A field was selected which did not contain Nabothian follicles, or cervical glands, and which was representative of the collagen content of the cervix, beneath the epithelium. A second field, known as the deep field, was also examined. This was 3 mm. deep to the basement membrane in the cervical stroma. This also was representative of the collagen content in the cervix, at this depth from the epithelium. Both selected fields from each slide, were examined under polarised light, this rendered collagen distinct from other tissue.

The image of each field was recorded by a video camera, and relayed to an image analyser, which had the facility to record transmitted light as a digital readout. The analyser could be programmed to respond to a large range of light intensities. Several sections were examined to obtain the likely range of transmitted light, from highly collagenous tissue, to almost collagen free tissue. When the analyser was programmed to

respond to this range of refracted light, the sensitivity settings were not changed for the examinations of study sections. All the sections, both superficial and deep, were examined at one session, and all observations were made without altering video camera or image analyser.

The size of both fields was 1.3 x 1.5 mm., and each was examined at x 32 magnification by the analyser camera. To reveal error due to artefact,such as dust in the lenses, three background counts with no histological section were taken. These counts were 28, and the range of values for collagen in the sections examined in the study, was from 245 to 15467.

From each section, three recordings of the intensity of the refracted light were taken from each field. Although results for the recordings at superficial or deep field, showed little inter-observational variation, the mean was taken as the value for the field. The standard deviation of the three recordings from each field, was less than 1% of the value. The comparison of the superficial and the deep results, gives an indication of the homogeneity of the collagen in the cervix, and allows an impression of either increasing, decreasing, or similar collagen presence, with increasing depth below the epithelium.

Age, histology of the endometrium, and presence of procidentia, were correlated to these recordings. Only three patients received exogenous hormones; One receiving oestrogens, one norethisterone and one danazol. The effect of these was thought to be minimal. Data on parity, where available, was correlated.

RESULTS

The age range of the 98 patients from whom the sections were obtained, was 28 to 79 years. A mean count was obtained from 3 analyses, in each field, for each section. There was a very large variation in the quantity of collagen present, between different sections, and the distribution of the counts was not normal, being skewed to the right. Logarithmic transformation, rendered the distribution normal. Each count is transformed to the logarithm, to base e, and mean values to age ranges, histological groups,etc. are compared.

AGE

The values obtained for each field, were grouped according to age, into decades from under 30 years to over 70 years. Table 27, fig. 7) illustrates the variation according to age. There was little variation in the deeper value with advancing age. However, the superficial value increased until the menopause, and then fell somewhat. A paired students t test comparing collagen in the deep field, to collagen in the superficial field, showed significantly more collagen in the deeper field for each decade, but the level of confidence decreased with age. The collagen content, appeared to become more homogeneous with age. The mean superficial value for the 30-39 year olds, was significantly lower than the mean superficial value for the 50-59 year olds. A correlation coefficient, of age to superficial value was signifiant, $p<0.05$.

The same data can be viewed as a ratio of deep to superficial readings. The ratio was taken as deep value-28, for background count/ superficial value-28, and this was computed for each

patient. The mean ratio, for each 5 year age band, to age greater than 70 years, was evaluated and correlated to age (table 28, fig. 8). A high ratio indicated a cervix, which became more dense in collagen, with increasing distance below the basement membrane. A low ratio, implied a more homogeneous distribution of collagen. This ratio was derived, from numbers which were not normally distributed, so a non parametric test was used. Correlation of age to this ratio, using Kendalls coefficient of rank correlation, was significant, $\tau = .216$ $p < .0027$. A correlation of ratio to age, in patients without procidentia, was also significant.

These data, indicate that for women aged 30 to 54 years, there is a progressive decrease in this ratio with advancing age. This is not due to decreasing collagen in the deeper field, but due to increasing collagen, in the superficial field. It would appear, that collagen in the superficial field area, increases per unit area (in two dimensional sections), with advancing age until the menopause. The ratio remains fairly constant after the menopause.

HISTOLOGY

Endometrial histology was available for 83 of the 98 patients. Three premenopausal and twelve postmonopausal patients, had unknown histology, usually as a result of no curettings being available, at amputation of the cervix, during vaginal repair. Table 29 shows the ages, mean log values and comparisons between the groups. The premenopausal subjects were divided, by histology, into proliferative and secretory endometrium. The mean ages of the two groups, was similar as was

the superficial value. The deep value, however, was significantly different, with more collagen in the luteal phase. The thirty patients with atrophic histology, had similar values to the patients with hyperplastic histology, suggesting little effect on collagen of the endogenous oestrogen. Numbers were, however, small. Seven patients had endometritis, or neoplastic histology but meaningful conclusions cannot be drawn from this number.

PROCIDENTIA

There were 19 patients who had a clinical diagnosis of procidentia, and 18 of these were postmenopausal, (mean age 69 years). These patients, had significantly lower values for both the superficial, and deep fields, than the patients over 50 years, who did not have procidentia. They were, however, considerably older (table 30). Those postmenopausal patients with procidentia, had reduced collagen per unit area, and by inference, lower collagen density, than postmenopausal patients without procidentia. This is likely to be a reflection of tissue oedema that accompanies procidentia.

PARITY

Data on parity was only available for 26 patients, due to the long accumulation period, during which patients records were no longer obtainable. The only feature which was thought to possibly affect collagen content, was nulliparity. There were very few nulliparous patients, and their values were not different from the mean for their age group.

DISCUSSION

This method, for the histological estimation of collagen, by

examination under polarised light, was described by Danforth in 1960 (110), and the use of spectrophotometric methods has been validated by other authors to quantitate collagen (195,204). It has not been used to quantitate the collagen content, at different ages, nor have conclusions, about the variation in collagen with age, been drawn from this investigative line before.

Image analysers, are used to measure area of a given density, from electron photomicrographs (205,206). They are less suitable for examining histological sections, as the density of the structures is often too similar, to allow quantitation of any one structure. This, however, is not the case with collagen, which is very much brighter than other structures, when illuminated with polarised light.

It is crucial, to confidence in the study, that all recordings were made at one session, and taken without alteration of the image analyser, microscope, or camera. This avoids the pitfalls in the use of the image analyser, reported by Bradbury (207). Pre-selection of these slides was essential, but slides were examined without the knowledge of the age or other data.

These data have shown, that the values for collagen taken deeper in the cervical stroma, are very constant throughout a spectrum of ages (table 27). The main difference is in the superficial value, which varies considerably with age. The values in the younger women, are lower and show a greater difference to the respective deep values, than those of the older women. The ratio of deep to superficial value, is a

reflection of this change in superficial value with age. This ratio becomes lower with age, indicating that collagen presence, is distributed equally throughout the cervix. This has relevance to consideration of the visibility of the squamo-columnar junction. High superficial values, such as those found in the older patient, will represent a cervix which is not amenable to manipulation. This renders examination of the entire transformation zone difficult, or impossible. Lower superficial values, as found in the younger women, whether due to decreased collagen, increased water, or more smooth muscle, will allow greater cervical manoeuverability.

This information is in accord with that found in the previous study, and it affords numerical evidence, that the cervix has greater collagen density deeper in the stroma than superficially, irrespective of age.

Consideration only of the younger patient, reveals an interesting phenomenon. A significant difference, in collagen presence, in the deep field is apparent between those with proliferative, and those with secretory histology, despite no age difference between the groups. The increased collagen density in the luteal phase group, is in accord with clinical evidence suggesting a more rigid cervix in the luteal phase of the cycle (108,208). The continual synthesis, and breakdown of collagen, induced by oestrogen, is likely to be responsible for this observation (201).

Lower superficial and deep values, over the age of seventy, can possibly be explained by the group with procidentia, having more oedema, in the prolapsed cervix. With age, the collagen in

the cervix would become more stable, with more cross linkages, and would give higher values at both fields. This was found up to age 60 years. Above this age, collagen presence decreases, and this is the influence of the large proportion of women with procidentia, in this age group. By virtue of tissue oedema and cervical hypertrophy,the collagen fibres are dispersed, and this results in a decreased density of collagen, and lower values.

In the clinical context, it is the woman aged 50 to 60 years who presents the colposcopist and cytologist with the greatest problems. In this age range, cytological and colposcopic abnormalities are not uncommon, but complete examination of the transformation zone, by either diagnostic method, is rarely assured, due to the retraction of the SCJ into the endocervix. This study, indicates that at this age range, the collagen presence immediately below the SCJ is greatest. This renders the cervix less compliant, and mitigates against the success of colposcopic examination.

These data, and those from the previous chapter, will be discussed in the light of other findings of this work, in the summary.

TABLES FROM CHAPTER 8

TABLE 27

Comparison of superficial and deep values by age

AGE(yrs)	n	log sup mean(SD)	log deep mean(SD)	paired t test	confidence p
20-29	3	7.95(.62)	8.62(.12)		n.s.
30-39	24	*7.44(1.04)	8.42(.56)	5.63	.001
40-49	16	7.81(1.03)	8.56(.84)	5.47	.001
50-59	25	*8.18(.74)	8.58(.52)	4.52	.001
60-69	12	8.02(.74)	8.46(.67)	2.87	.05
70+	18	7.69(.95)	8.09(.66)	2.62	.05

*30-39 vs 50-59 mean superficial value $t=2.82$, $p<0.01$

TABLES FROM CHAPTER 8

TABLE 28

Deep, superficial ratio by age

age(yrs)	n	(deep value -28) (superficial value-28)mean (S.D.)
25-29	3	2.12(.73)
30-34	15	3.86(3.31)
35-39	9	3.64(2.08)
40-44	6	2.46(1.25)
45-49	10	2.49(1.41)
50-54	18	1.57(.87)
55-59	7	1.94(.72)
60-69	12	1.80(1.06)
70+	18	1.86(1.36)

Kendalls coefficient of rank corelation τ =.216, $p<0.0027$

TABLE 29

Comparison of values by histology

HISTOLOGY	n	log sup mean(SD)	log deep mean(SD)	mean age yrs.	
prolif.	26	7.64(1.19)	8.43(.64)	40.7)	t=2.14
secret.	11	7.83(.92)	8.79(.69)	39.6)	p<0.05
atrophic	30	7.75(.93)	8.28(.78)	60.8	
hyperplas.	9	7.84(.43)	8.38(.25)	50.1	

TABLE 30

Collagen and procidentia

	n	log sup mean(SD)	log deep mean(SD)	mean age yrs.	
PROCIDENTIA >50	18	7.79(.82)	8.08(.62)*	69.0)	t=2.72
NO PROCIDENTIA>50	37	8.08(.82)	8.55(.58)*	59.7)	p<0.01

FIGURE 4 -COLLAGEN UNDER POLARISED LIGHT

photomicrograph

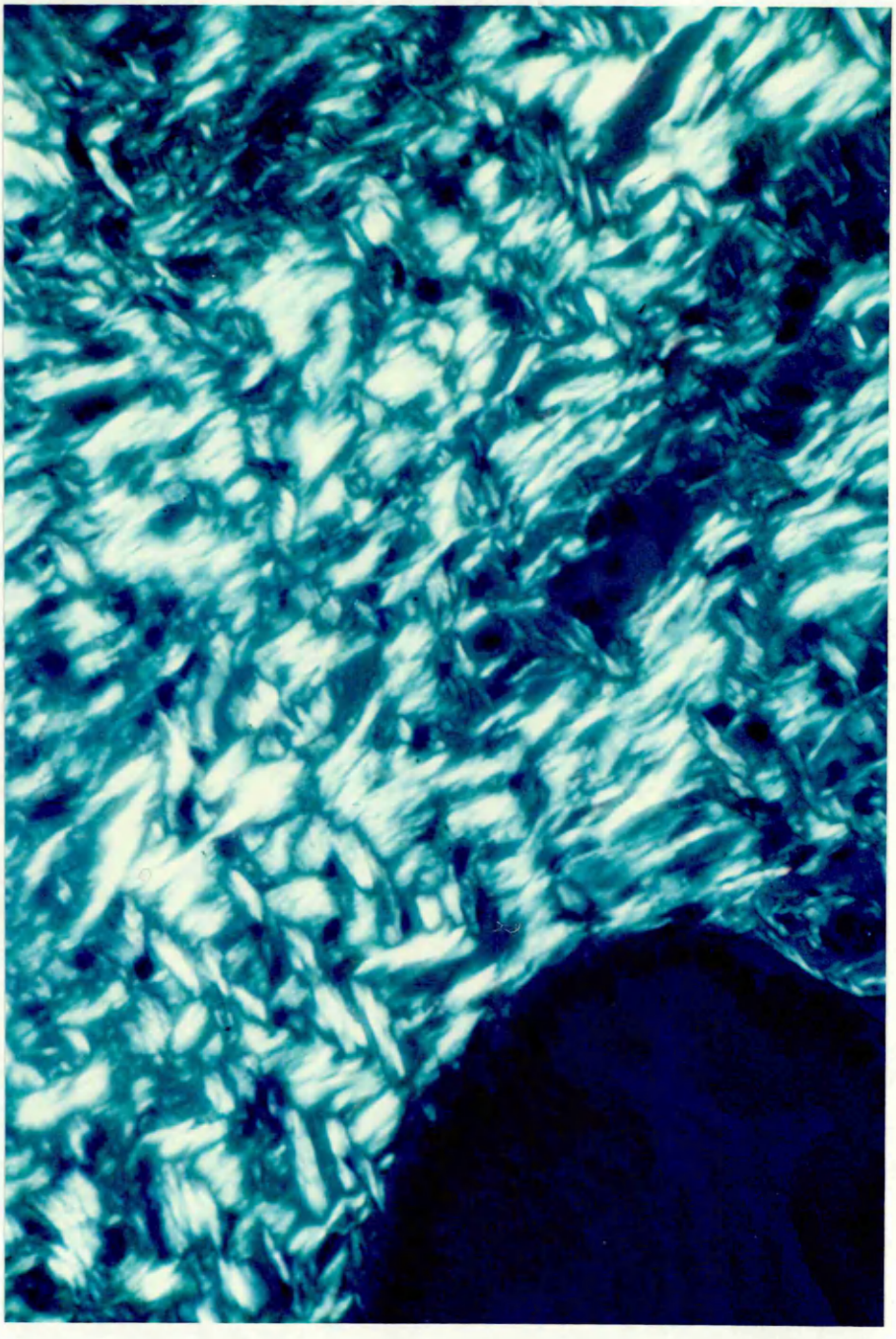


FIGURE 5 -IMAGE ANALYSER, MICROSCOPE AND CAMERA

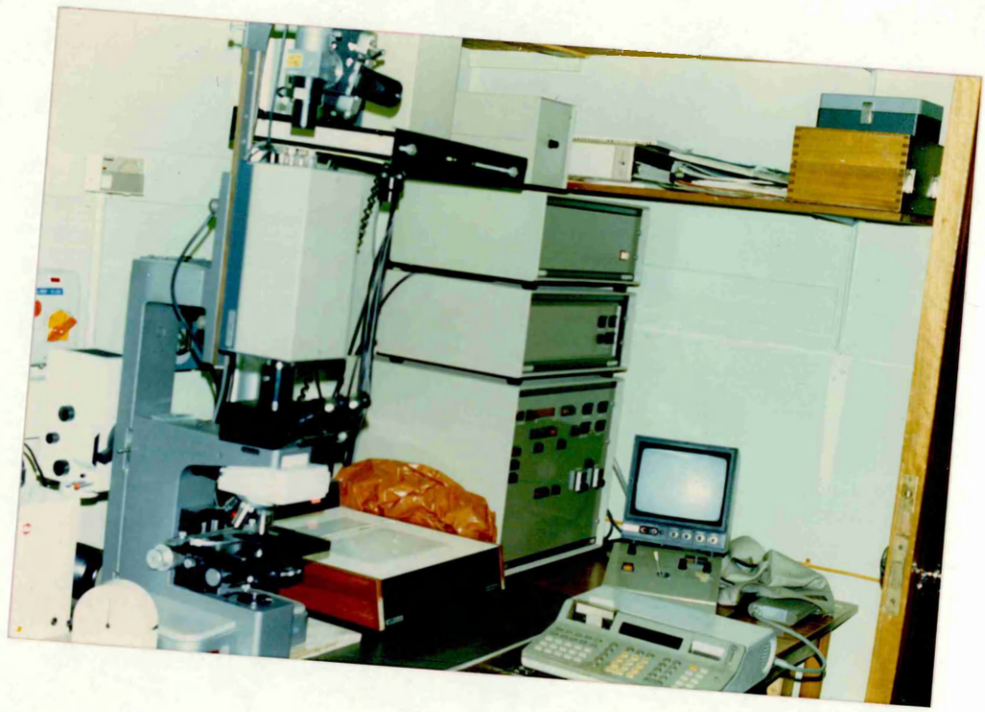


FIGURE 6 -IMAGE ANALYSER

figure 7

Comparison of superficial and deep values by age

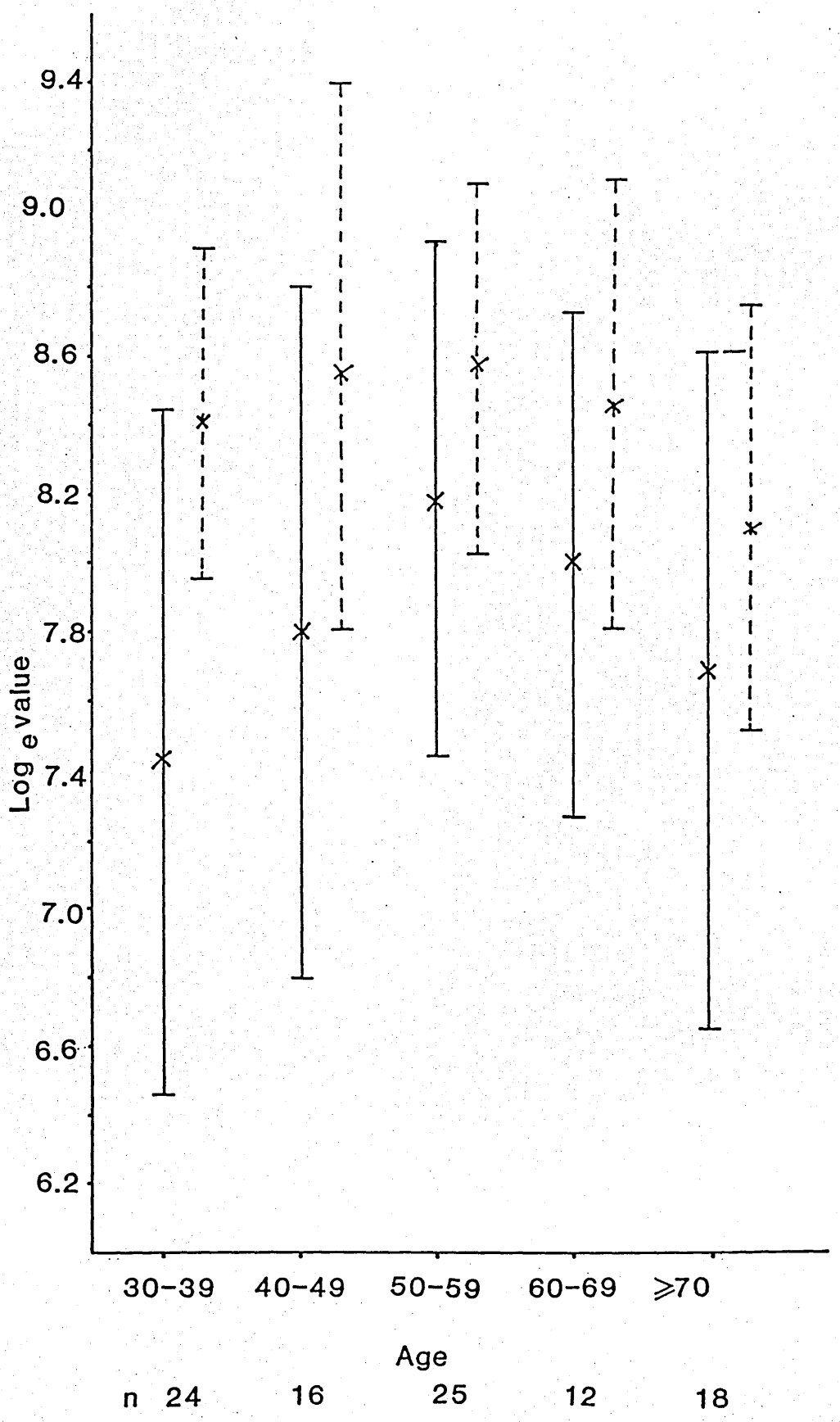
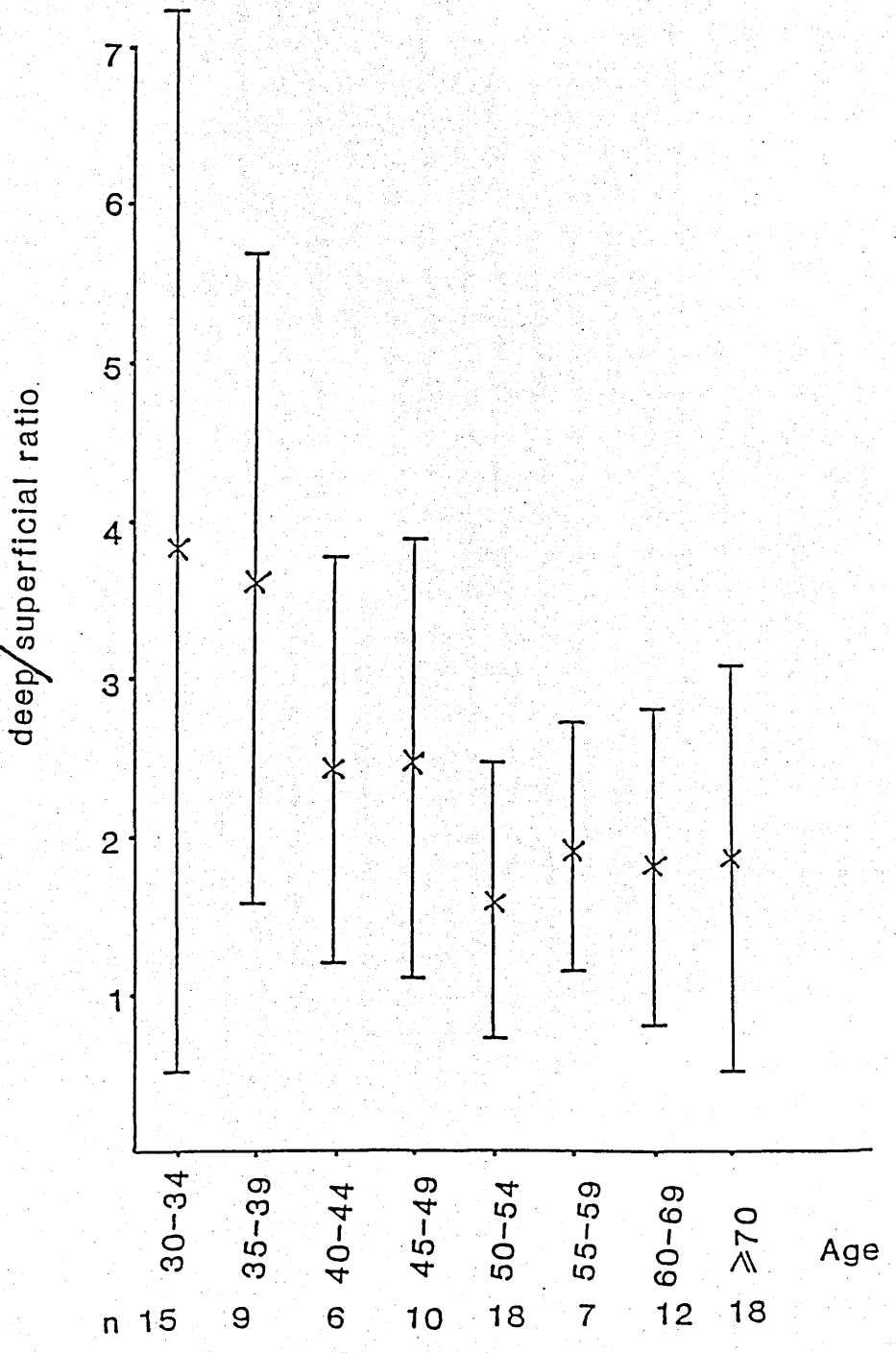


figure 8

Collagen content ratio by age



ELECTRON MICROSCOPY OF CERVICAL STROMA

Three punch biopsies were obtained, one from a premenopausal woman,one from a postmenopausal woman, and one from a postmenopausal woman taking HRT. The fresh specimens were transported to the pathology laboratory where Dr. Rod Denholm performed electron microscopy on the specimens. The intention was to scan the collagen in the stroma,to see if there was any appreciable difference in the appearance of the collagen, especially with reference to cross linkages. When the electron photomicrographs were compared,there was no obvious difference in the quality of the collagen,in the three biopsies. As this was a time consuming and expensive task, it was felt that there was little point in pursuing this investigative line.

SUMMARY TO SECTION 3

The aim of the work reported in this thesis, has been to illustrate the differences in the cervix uteri of the older woman,to that of the younger woman.

The work in chapters two and three, has shown that colposcopy is of less value in the older woman, than younger women, but HRT will increase this value. Doubts as to the effectiveness of cervical cytology in the older woman were raised. Other authors, have also had doubts, as to the effectiveness of screening the older woman (209), but these doubts are mainly founded on the reluctance of the postmenopausal patient, to attend for screening. Other worries on the ease of obtaining and interpreting a smear, have also been expressed (151). The finding, that there is a failure of exfoliation in the older woman with CIN, has not had wide exposure or analysis.

It is, however, felt that failure of cervical cytology to detect lesions in the older patient, is due to lesions being endocervical and not available to cytological sampling. Special spatulae have been designed to sample the endocervix, and these are undergoing evaluation at present (23). There is no doubt, that the SCJ retracts into the endocervix with age. Two studies, in chapters 7 and 8, have endeavoured to find changes, in the cervical stroma, which occur with age, which may account, at least in part, for this clinical observation.

In addition to the relocation, of the SCJ within the cervical canal with ageing, there is a change in the consistency of the cervix, which renders manipulation painful and difficult. This

is partly responsible for limitations in screening. In extreme cases, shrinkage of the stromal elements, can render the cervical os stenosed and even occluded. The firmness of the cervix in the postmenopausal woman, rendered a suspicion that there was either a decrease in tissue water, or an increase in collagen density, or both. Changes in cervical water occur both within the menstrual cycle, and during pregnancy and labour. A change in cervical water seemed likely, as the hormonal milieu changed at the menopause. This was confirmed by measurement of dry and wet biopsies, and the critical event to this change, was the menopause itself, and not age as such. Administration of HRT quickly reverted the water content to that of the premenopausal woman, while having little effect on the collagen content. The cervical water, deeper in the cervical stroma, was similar in pre- and postmenopausal women, and the cervical core itself, appears less influenced by circulating hormones.

This observation, in part explains, why the cervix is softer and more maleable in the younger woman, and also why the SCJ is more frequently visualised in women taking HRT.

Observations on the collagen content, were made using two different approaches, one biochemical, and the other histological. The measurement of hydroxyproline as an indicator of the collagen content, is standard practice. It is generally held that hydroxyproline, accounts for about 10% of the amino acid content of collagen. Its other advantage, is that it is present almost exclusively in collagen, and only as a trace in other proteins. It therefore correlates closely with collagen content. Hence many authors have used this measurement in

cervical studies (111,112,119). A correlation was observed between advancing age, and increasing hydroxyproline dry weight ratio. This indicates that the collagen density is increasing with age, at this site, just below the epithelium. There was the theoretical possibility, that the thicker epithelium, in the younger patient, would decrease the collagen content in punch biopsies. It was felt, that by including only biopsies of a reasonable size, this effect would be minimised. The findings of increasing collagen with age, were confirmed by the histological study.

This study used a different approach, and this had not been used for this purpose before. Collagen, being birefringent, refracts light in three dimensions, when viewed under polarised light. This can easily be detected by an image analyser. This technique has previously been described (204), but to use this as a quantitative test, for comparing collagen contents, necessitated all measurements, being performed under the same conditions, at the same time. Results were obtained which bore out the findings of the biochemical study. From both studies, there appears to be a linear relationship, between the increase in collagen density, in the area immediately beneath the basement membrane of the epithelium, and advancing age. Both studies found very similar collagen presence, deeper in the cervical stroma. There was, however, a significant difference in the values obtained from the women with secretory endometrial histology, to those with proliferative histology, despite having the same mean age. This confers an explanation, on the clinical observation of increased firmness of the cervix, in the luteal

phase.

The ratio, of the superficial to deeper field, was valuable in showing that the cervix became more heterogeneous for collagen, with age. In the younger woman, the cervix became more collagen dense, with penetration into the stroma. In the older patient this was less pronounced.

Indubitably, endogenous hormones are affecting the cervical stroma at times outwith pregnancy, and observations on the presence of oestrogen receptors in the non pregnant patient would affirm this (113).

This work, has attempted to explain the clinical observation, of difficulty in visualisation of the SCJ in the older woman. That the SCJ recedes into the canal is not in doubt, and the clinical effects of this, on the effectiveness of the screening programme for cervical cancer, have been suspected. It has been shown that, at the SCJ there is a structural change in the cervix, with age. There is a decrease in cervical water, and an increase in collagen density, both rendering the cervix less amenable to the manipulation that adequate cytology and colposcopy demands.

APPENDICES

APPENDIX 1

APPENDIX TO CHAPTER 2

n=50 42 returned after 2 months therapy/ 8 no return

KEY

COLUMN 1 age(years)

2 postmenopausal time(months) 1= still menstruating

3 treatment a conjugated equine oestrogens/norgestrel-29

b oestradiol valerate/levonorgestrel-4

c oestrone/norethisterone-6

d oestradiol/oestriol/norethisterone-1

e conjugated equine oestrogens-2

4 E2 value pmol/L - not available

5 smear maturation value 1,2or 3 before/during

6 SCJ, + seen,++ easily seen, - not seen before/during

7 haemorrhages 0,1 or2 before/during

8 epithelium 0,1,2 or3 before/during

9 Lugols iodine 0,1,2 or 3 before/during

10 mucus 0,1,2or3 before/during

11 initial score

12 score on therapy

13 change in score

1	2	3	4	5	6	7	8	9	10	11	12	13
50	84	a	150	2/2	-/-	0/0	2/3	1/1	0/3	6	14	8
57	36	a	150	1/3	-/-	2/2	2/3	1/1	2/3	16	20	4
55	60	a	150	2/2	-/-	1/2	2/3	2/2	0/2	11	20	9
37	15	a	-	2/2	-/+	0/1	3/3	0/1	0/2	6	15	9
52	6	a	150	2/3	-/-	2/2	2/3	1/3	0/3	12	24	12
53	7	a	150	2/2	+/+	2/2	2/3	1/3	2/3	16	24	8
51	6	a	150	2/2	-/-	0/2	1/3	1/2	1/1	6	18	12
49	9	a	150	2/2	-/-	2/2	3/3	0/1	0/1	12	16	4
41	60	a	237	2/3	-/++	2/2	3/3	2/2	2/3	20	22	2
54	36	a	-	1/2	-/+	2/2	3/3	0/2	0/1	12	18	6
50	72	a	150	1/3	-/+	2/2	2/3	0/1	0/2	10	18	8
48	1	a	150	2/2	-/+	2/2	3/3	2/2	2/2	20	20	0
48	3	a	793	2/3	-/+	2/2	3/3	2/2	1/1	18	18	0
46	1	a	540	2/3	+/+	2/2	2/2	3/3	3/3	22	22	0
51	4	a	150	2/3	-/+	0/2	2/3	2/3	0/2	8	22	14
50	108	a	150	1/2	+/++	1/2	3/3	1/2	1/1	13	18	5
51	30	a	150	2/2	-/-	2/2	0/1	2/2	1/3	12	18	6
53	36	a	150	1/3	-/-	0/2	2/3	0/1	1/2	6	18	12
45	12	a	409	2/2	-/+	1/2	3/3	2/2	2/3	17	22	5
37	72	a	150	2/3	+/+	1/2	3/3	2/3	1/3	15	24	9
49	36	a	150	1/3	-/++	0/2	1/3	1/2	2/3	8	22	14
51	24	a	150	1/2	-/-	2/2	1/2	0/2	0/2	8	18	10
63	144	a	150	3/3	-/++	1/2	2/3	1/2	0/2	9	20	11
45	1	a	150	2/2	+/+	2/2	3/3	2/2	1/2	18	20	2
52	1	a	150	2/3	+/+	2/2	2/3	2/2	2/3	18	22	4
53	18	a	-	2/2	-/-	2/2	2/3	2/2	0/1	14	18	4
50	1	a	542	3/2	++/++	2/2	2/2	1/2	3/3	18	20	2
49	1	a	150	3/3	-/+	2/2	2/3	1/1	0/3	12	20	8
50	120	a	150	2/2	-/+	1/2	3/3	1/2	0/1	11	18	7
53	3	b	150	2/3	-/-	2/2	1/3	1/1	1/3	12	20	8
50	24	b	456	3/2	-/-	2/2	2/2	0/2	0/0	10	14	4
49	4	b	150	3/3	-/-	2/2	3/3	2/2	1/2	18	20	2
52	6	b	150	2/2	+/+	2/2	3/3	1/2	2/3	18	22	4
51	24	c	150	1/3	+/+	2/1	3/3	0/1	1/2	14	15	1
52	12	c	150	1/2	+/+	1/2	0/2	1/2	2/2	9	18	9
51	24	c	150	2/2	-/-	2/2	3/3	2/2	0/2	16	20	4
54	48	c	-	2/3	-/+	2/2	1/2	1/2	0/2	10	18	8
45	1	c	1060	2/3	-/++	2/2	2/3	2/2	1/2	16	20	4
52	18	c	171	2/2	-/+	2/2	1/3	0/2	0/1	8	18	10
49	24	d	150	2/2	+/++	2/2	2/3	1/1	1/3	14	20	6
51	6	e	150	1/3	-/+	1/2	2/3	0/2	0/3	7	22	15
50	6	e	542	3/3	-/-	0/1	2/3	1/2	1/2	8	17	9
51	60	-	150	2	-	2	0	2	0	10	-	-
51	72	-	-	2	-	2	1	2	0	12	-	-
50	60	-	150	1	-	0	3	1	0	8	-	-
54	24	-	150	2	-	0	2	1	1	8	-	-
53	60	-	-	2	-	1	1	1	0	7	-	-
48	18	-	150	3	-	2	1	0	0	8	-	-
52	48	-	179	1	-	2	3	0	0	12	-	-
56	72	-	150	2	+	2	1	2	1	14	-	-

APPENDIX 2

COLPOPHOTOGRAPHS FROM PATIENTS IN CHAPTER 2

As mentioned earlier the quality of these colpophotographs was not always ideal but they are shown to illustrate the findings of this chapter.

Patients receiving HRT

Slides 20-21 50 year old occasional menstruation

Slide 20 before HRT, thinned epithelium, visible Squamo-columnar junction (SCJ), mucus present

Slide 21 on HRT Good uptake iodine

Slides 22-23 50 year old LMP 5 years before

Slide 22 before HRT Mucus, haemorrhages, SCJ not visible

Slide 23 before HRT poor uptake Lugols iodine

Slides 24-25 52 year old LMP 18 months before

Slide 24 before HRT thin epithelium, SCJ not visible

Slide 25 before HRT virtually no uptake iodine

Slides 26-27 51 year old LMP 6 months before

Slide 26 before HRT thin epithelium, haemorrhages

Slide 27 before HRT patchy uptake iodine

Slides 28-29 45 year old irregular menstruation

Slide 28 on HRT metaplasia, mucus, thick epithelium

Slide 29 moderate uptake iodine

Slide 30 50 year old LMP 6 years before

Slide 30 on HRT SCJ visible with forceps, previously not visible

Slides 31-32 48 year old LMP 3 months before

Slide 31 before HRT thinned epithelium, SCJ not visible

Slide 32 SCJ on HRT SCJ visible with forceps

Slides 33-34 52 year old LMP 18 months before

Slide 33 before HRT thin epithelium, SCJ not seen

Slide 34 on HRT thicker epithelium

Slides 35-36 49 year old LMP 2years before

Slide 35 on HRT squamous metaplasia, mucus

Slide 36 on HRT SCJ seen with forceps

Slide 37 57 year old LMP 3 years before

Slide 37 before HRT thin epithelium, mucus, SCJ not seen

Slides 38-40 54 year old LMP 3 years before

Slide 38 before HRT cervical polyps, no mucus, SCJ not seen

Slide 39 before HRT poor uptake iodine

Slide 40 on HRT better uptake iodine

Slides 41-42 52 year old LMP 5 years before

Slide 41 before HRT thin epithelium, haemorrhages, SCJ seen without endocervical forceps

Slide 42 during HRT endocervical prolapse, SCJ easily seen, thicker epithelium, mucus

Slide 43 during HRT good uptake iodine

APPENDIX 3

This appendix supplies evidence of CIN in the four women from the study in Chapter 3.

The referral letter to Dr. Malcolm Anderson is shown. This identifies the four patients by name. Dr. Anderson's reply is shown, indicating that he agrees with the diagnosis in all patients, and he goes further to suggest, that in Patient Martin the abnormality may even be CIN 3.

After these letters, there is a short section devoted to each patient. This takes the form of identifying the patient from table 6, giving a colposcopic diagram, followed by photocopies of original cytology, which is negative, and histology which shows CIN. At the end of each patients' section there follows photomicrographs of histology, and in some cases colpophotographs or cytology photographs.

PATIENT 1- TABLE 6- MARTIN

54 YEARS OLD- LMP 4 YEARS BEFORE

A) COLPOSCOPIC DIAGRAM

B) FIRST SMEAR REPORT- NEGATIVE

C) SECOND SMEAR REPORT- NEGATIVE

D) PUNCH BIOPSY REPORT- CIN 1-2

E) FIGURE 9 COLPOPHOTOGRAPH SHOWING ATZ WITH AWE

F) FIGURE 10 NEGATIVE CERVICAL CYTOLOGY FROM PATIENT 1.

G) FIGURE 11 PHOTOMICROGRAPH SHOWING CIN 2 FROM PATIENT 1.

H) FIGURE 12 PHOTOMICROGRAPH SHOWING CIN 2 FROM PATIENT 1.

PATIENT 4- TABLE 6- CRAWFORD

51 YEAR OLD- LMP 5 YEARS BEFORE

A) COLPOSCOPIC DIAGRAM

B) FIRST SMEAR REPORT- NEGATIVE

C) PUNCH BIOPSY REPORT- CIN 1

D) FIGURE 13 COLPOPHOTOGRAPH (RATHER POOR) SHOWING TINY RIM OF AWE.

E) FIGURE 14 PHOTOMICROGRAPH SHOWING CIN 1/ EPITHELIAL ATYPIA FROM PATIENT 4.

PATIENT 5- TABLE 6- WALLACE

50 YEAR OLD- LMP 9 YEARS BEFORE

A) COLPOSCOPIC DIAGRAM

B) FIRST SMEAR REPORT- NEGATIVE

C) COLPOSCOPIC DIAGRAM AFTER SUTURE REMOVED SHOWING AWE AND MOSAIC

D) SECOND SMEAR - NEGATIVE

E) PUNCH BIOPSY REPORT- CIN 1

F) FIGURE 15 COLPOPHOTOGRAPH SHOWING MCDONALD SUTURE BUT NO AWE

G) FIGURE 16 PHOTOMICROGRAPH SHOWING CIN 1 FROM PATIENT 5.

PATIENT 11- TABLE 6- KEENAN

37 YEAR OLD- LMP 6 YEARS BEFORE

A) COLPOSCOPIC DIAGRAM

B) FIRST SMEAR REPORT- NEGATIVE

C) PUNCH BIOPSY REPORT- CIN 1

D) FIGURE 17 PHOTOMICROGRAPH SHOWING CIN 1 FROM PATIENT 11.

Ref. ADGR/EM
Ref.

Yorkhill, Glasgow G3 8SH
Telephone: 041-339 8888

4th July, 1984

Dr. M.C. Anderson,
Senior Lecturer,
Histopathology Department,
Samaritan Hospital for Women,
Marylebone Road,
LONDON,
NW1 5YE.

Dear Dr. Anderson,

These are the slides I spoke to you about in Bristol. These few patients were among 50 patients about to receive hormone replacement therapy. Cytology had never previously been abnormal and there was no indication to colposcope other than as a screening tool.

We thought all four smears were negative. Martin was thought to have CIN 2, Keenan and Wallace CIN I and Crawford we were not convinced had CIN. Your opinion is very much appreciated.

Could you please return these slides when you have finished with them.

Thank you again.

Yours sincerely

Anthony D.G. Roberts
Registrar to Dr. J.W. Cordiner

THE SAMARITAN HOSPITAL FOR WOMEN

MARYLEBONE ROAD
LONDON NW1 5YE

DEPARTMENT OF HISTOPATHOLOGY AND COLPOSCOPY

Senior Lecturer and Honorary Consultant
M. C. Anderson F.R.C. Path.

Telephone 01-402 4211
Ext. 138

MCA/ce

20 August 1984

Dr A D G Roberts
Reistrar in Gynaecology
Queen Mother's Hospital
Yorkhill
Glasgow
G3 8SH

Dear Dr Roberts

Thank you for sending me the slides on these four postmenopausal patients about whom we spoke in Bristol.

As we discussed on the telephone, I agree with your diagnoses on all of them. In fact, I would suggest that the abnormality in Martin is at the upper end of the CIN 2 range and, in the crypts, may be CIN 3. I have not reviewed the cytology as I am not a cytologist.!

I am returning the slides.

With kind regards

Yours sincerely

M C Anderson

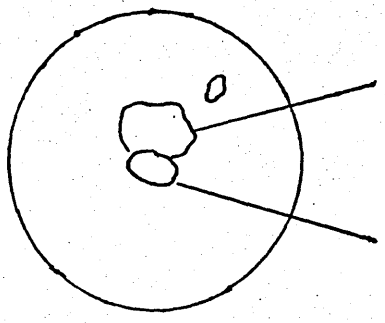
PATIENT 1 MARTIN

PATIENT 1- TABLE 6 - MARTIN

54 YEAR OLD - LMP 4 YEARS BEFORE

Negative cytology

Histology CIN 2 (3)



SMEAR

BIOPSY

TAKEN

HRT PRESCRIBED

FIRST SMEAR REPORT

USE BALL PEN FIRMLY

HEALTH BOARD

GYNAECOLOGICAL CYTOLOGY

02-08-LABORATORY No. C 83/0

09-24-Surname MARTIN

25-38-Christian Name

Clinician DR Roberts

39-80-Address

	D	M
09-14-Birthdate	19	0
15-20-Date of Examination	07	0

21-24-Name and Full Postal Address of G.P. DR. Robertson W. Cent. C.

25-28-Sender Dr Roberts Dbo Sym Clinic

29-34-Hospital Record No.

35-REPEAT SMEAR, Yes 1

36-44-Last Cytology No.

46-Single 1 Married 2 Widowed 3 Divorced 4

47-48-No. of Pregnancies 2

49-Pregnant Yes 1; No 2

50-51-Post Natal Weeks.

52-53-Age at Menopause

Date of L.M.P. 4 Yrs ago.

60-Oral Contraception: Nil 1; Past 2; Current 3

61-Radiation, Yes 1

62-Hormone Treatment, Yes 1

63-Cervix: Healthy 1; Benign 2; Suspicious 3

64-Smear Type: Cervical 1; Vaginal 2; Other

Clinical Diagnosis and Comments:

(1) VAC. SMEAR for KPI — on HRT.

(2) Cervical smear (colposcopy abnormal).

Pathologist's Comments:

Smear (1) Mild dyskaryosis. Superficial fg. 60%/40%

Smear (2) A few cells inflamed — no dyskaryotic cells seen in this smear.

65-Results: Negative / Suspicious / Positive / Unsatisfactory

66-Advice: Repeat / Referral to

67-68-Recall in

Pathologist's Signature

Date 11/7/83

SECOND SMEAR REPORT

USE BALL PEN FIRMLY

HEALTH BOARD

GYNAECOLOGICAL CYTOLOGY

08-LABORATORY No. 8?

09-24-Surname: Martin

25-38-Christian Name:

39-80-Address:

Clinician: COLPOSCOPY

09-14-Birthdate: D 1 9 M 0

15-20-Date of Examination: 6 4 8

21-24-Name and Full Postal Address of G.P.:

25-28-Sender: DR ROBERTS 1

29-34-Hospital Record No.:

35-REPEAT SMEAR. Yes (1)

36-44-Last Cytology No.: F 3 0 9 7 2

46-Single 1; Married (2); Widowed 3; Divorced 4

47-48-No. of Pregnancies: 2

49-Pregnant: Yes 1; No (2)

50-51-Post Natal Weeks:

52-53-Age at Menopause:

Date of L.M.P.:

60-Oral Contraception: Nil 1; (Past 2); Current 3

61-Radiation. Yes 1

62-Hormone Treatment. Yes 1

63-Cervix: Healthy 1; Benign 2; Suspicious 3:

64-Smear Type: Cervical 1; Vaginal 2; Oth...

Clinical Diagnosis and Comments: Prev normal Cytol but CIN 1-2 on biopsy - repeat

65-Results: (Negative) Susp... Positive Unsat...

66-Advice: Repe... Refer...

Pathologist's Comments:

Normal

Pathologist's Signature

Date 6/7/83

67-68-Recall in: ...

Rec

PATHOLOGY REPORT - CIN 1-2

WESTERN INFIRMARY/GARTNAVEL GENERAL HOSPITAL PAT

Lab. No.: Name: MARTIN, 54 Hosp. No.:

Initialled as seen: Phys./Surg.: Dr. Roberts Ward: G10 WIG

Punch biopsy of cervix

These are scrappy fragments.
Microscopy shows extensive squamous metaplasia of endocervical glands, with focal mild dysplasia (CIN I-II).

18

Robin Reid

Reid

SNOP 83 7600

SPECIMEN	DATE OF OPERATION	DATE OF REPORT
Punch biopsy of cervix	7.7.83	11.7.83

FIGURE 9 -COLPOPHOTOGRAPH FROM PATIENT MARTIN SHOWING AN ATZ WITH AWE

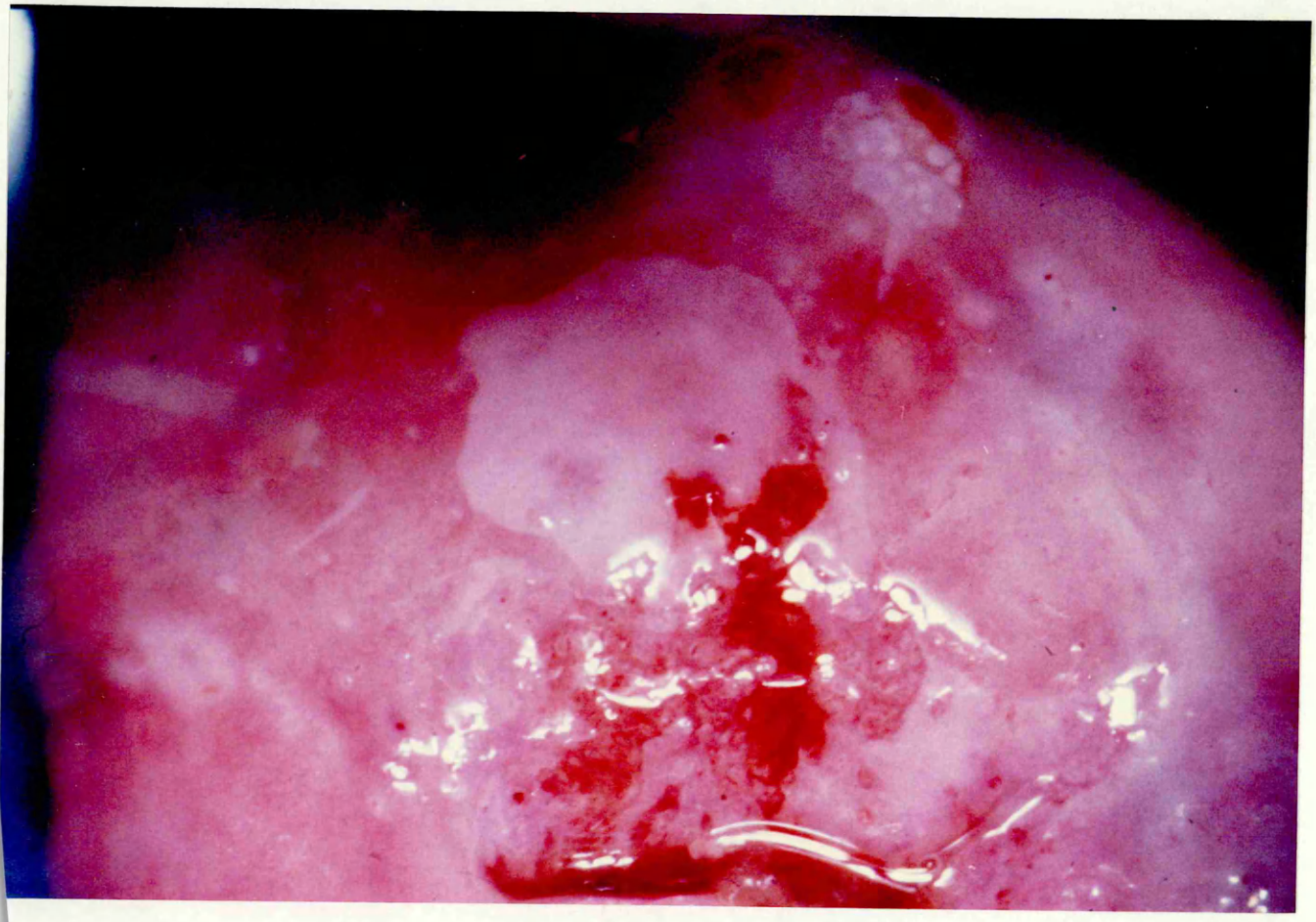
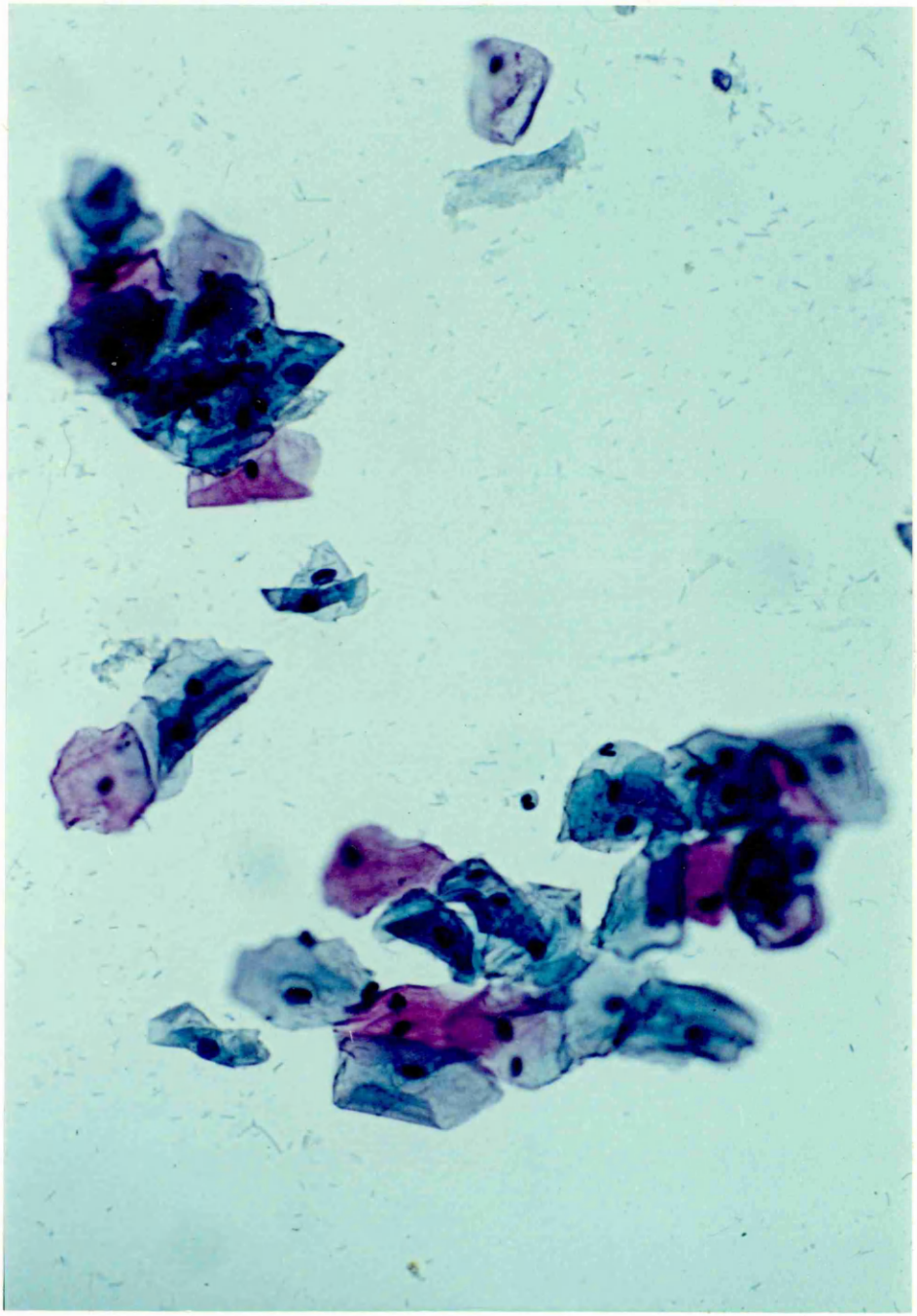
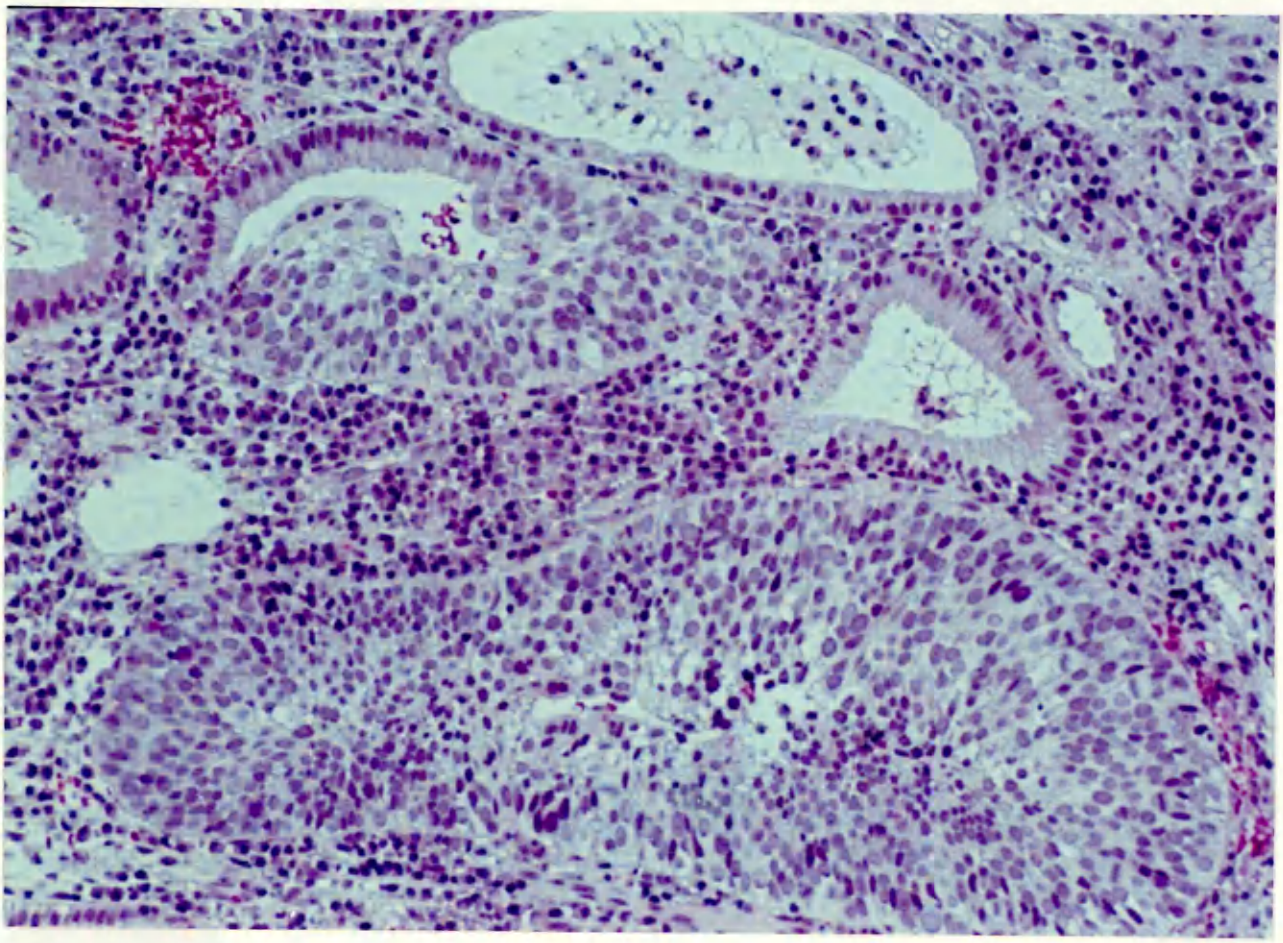


FIGURE 10-NEGATIVE CERVICAL CYTOLOGY FROM PATIENT MARTIN





PATIENT MARTIN CIN x200

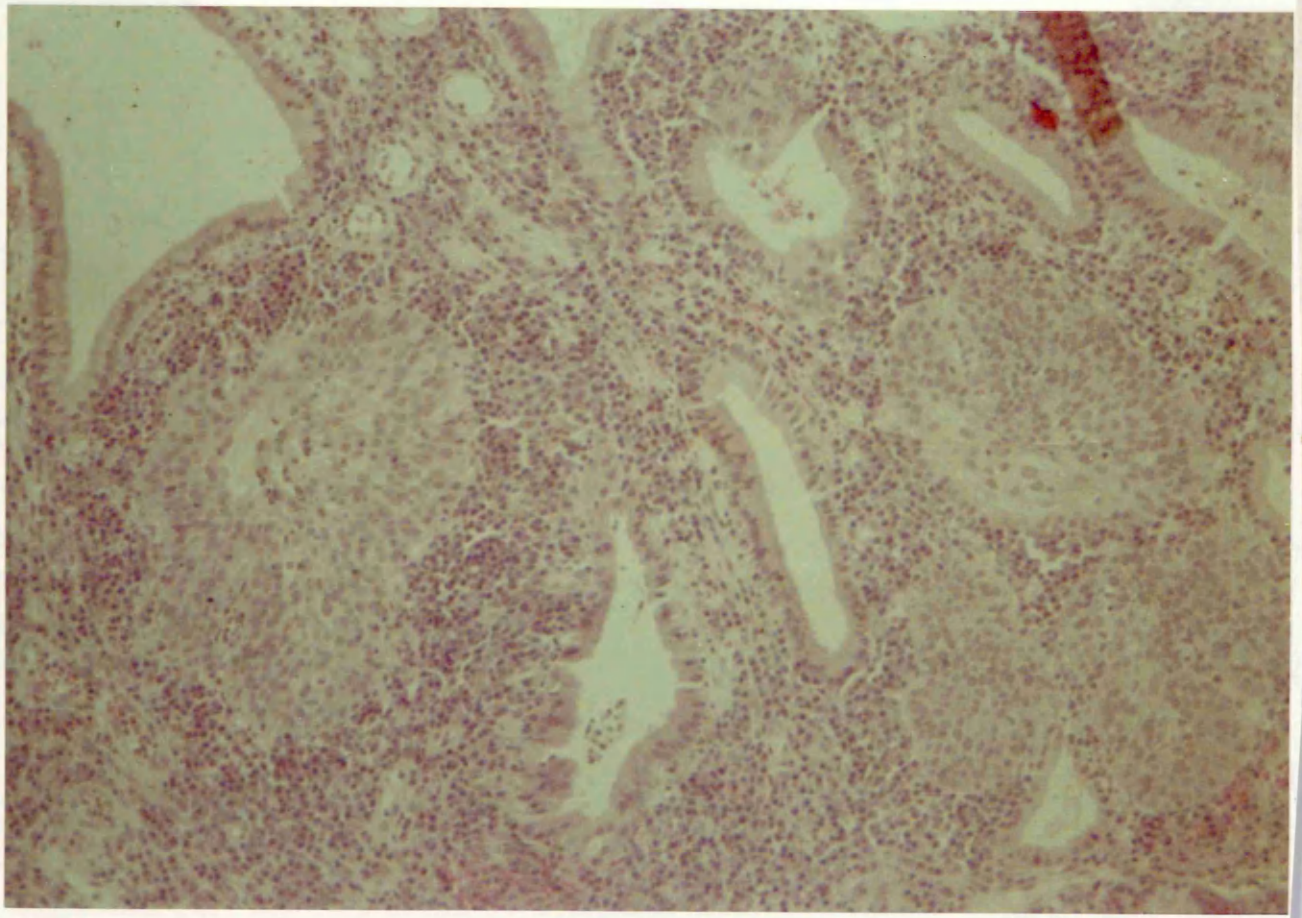


FIGURE 11-PHOTOMICROGRAPH FROM PATIENT MARTIN SHOWING CIN IN GLAND x 200

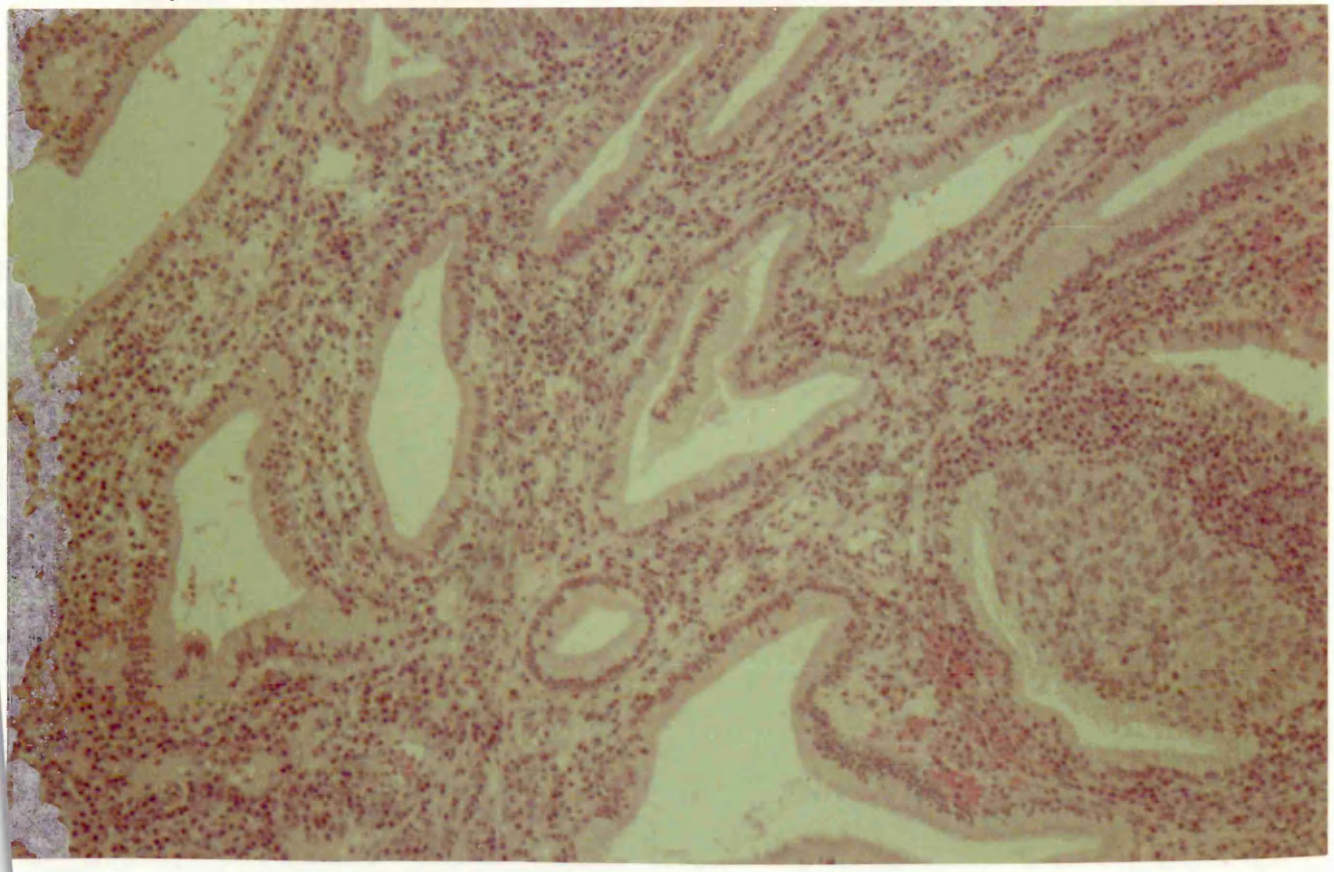
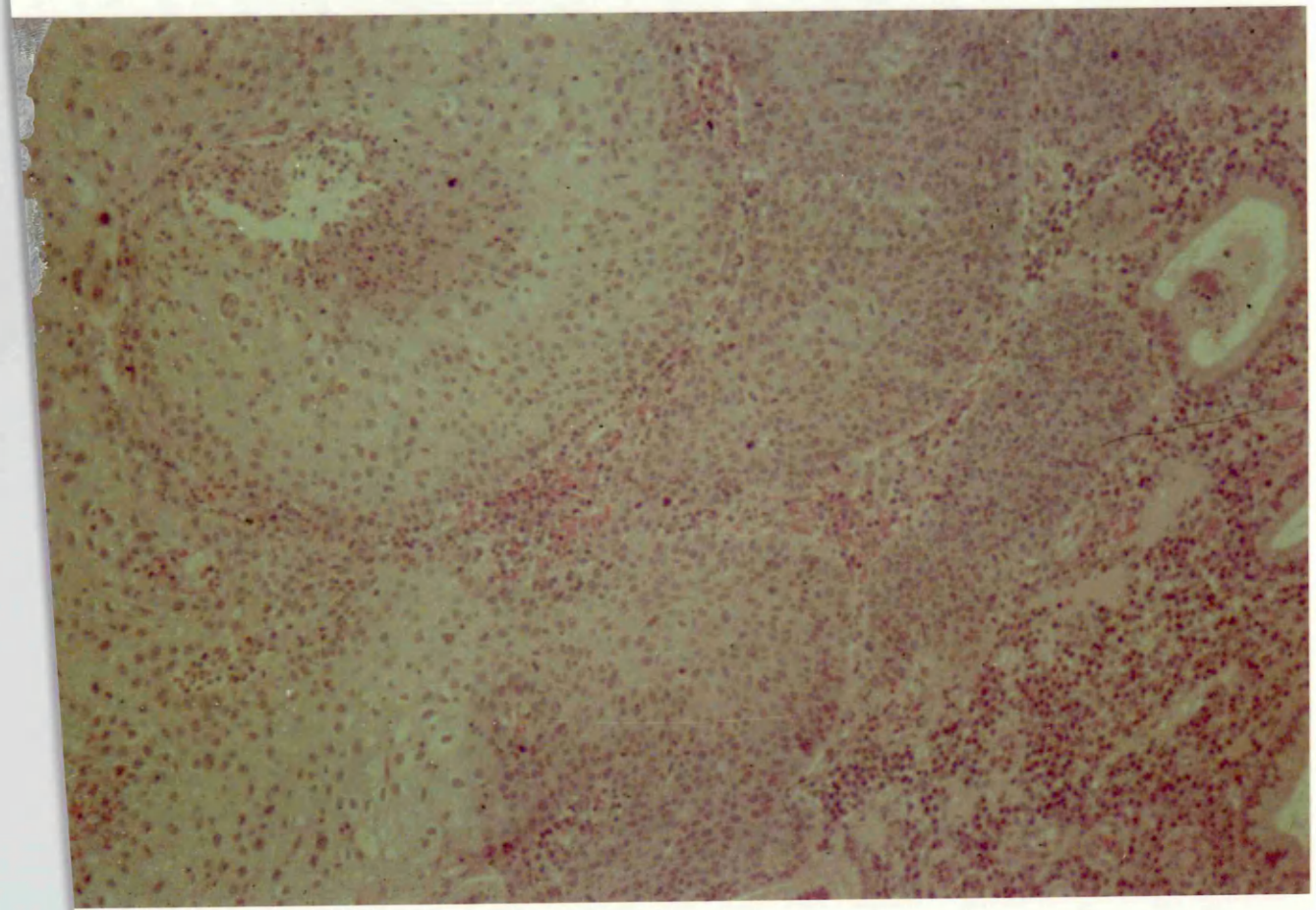


FIGURE 12-PHOTOMICROGRAPH FROM PATIENT MARTIN SHOWING CIN 2 x 200



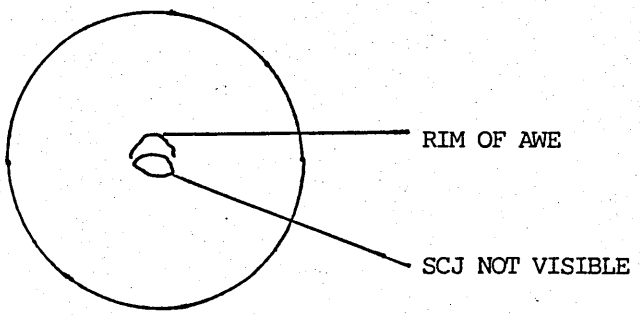
PATIENT 4 CRAWFORD

PATIENT 4- TABLE 6 - CRAWFORD

51 YEAR OLD - LMP 5 YEARS BEFORE

Negative cytology

Histology CIN 1/ATYPIA



SMEAR TAKEN

BIOPSY TAKEN

HRT COULD NOT BE TOLERATED

SMEAR AND BIOPSY REPORTS OVER

FIRST SMEAR REPORT

USE BALL PEN FIRMLY

HEALTH BOARD

GYNAECOLOGICAL CYTOLOGY

02-08-LABORATORY No. C

09-24-Surname Crawford

25-38-Christian Name

Clinician Dr A. Roberts

39-80-Address

	D		M		Y	
09-14-Birthdate	2	5	0	2	3	3
15-20-Date of Examination	0	5	0	4	8	4

21-24-Name and Full Postal Address of G.P.

25-28-Sender WD GIO

29-34-Hospital Record No.

35-REPEAT SMEAR, Yes 1

36-44-First Cytology No.

46-Single 1; Married 2 (circled); Widowed 3; Divorced 4

47-48-No. of Pregnancies 0

49-Pregnant: Yes 1; No 2 (circled)

50-51-Post Natal Weeks

52-53-Age at Menopause

Date of L.M.P. 5 years ago

60-Oral Contraception: Nil 1; Past 2; Current 3

61-Radiation, Yes 1

62-Hormone Treatment, Yes 1

63-Cervix: Healthy 1; Benign 2; Suspicious 3; Malignant 4

64-Smear Type: Cervical 1; Vaginal 2; Others 3 (Specify)

Clinical Diagnosis and Comments: NOT ENDO I Cervical
II Vaginal — for Hormone status please.

65-Results: Negative 1 (circled); Suspicious 2; Positive 3; Unsatisfactory 4

66-Advice: Repeat 1; Referral to Gyn. 2

Pathologist's Comments: 1) Normal appearances
2) Very scanty smear — inconclusive

67-68-Recall in [ ] months

Pathologist's Signature

Date 21/5/84

JMcC/11101 IRHB C1c

PATHOLOGY REPORT CIN 1

WESTERN INFIRMARY/GARTNAVEL GENERAL HOSPITAL **PATHOLOGY**

Lab. No.: Name: CRAWFORD Hosp. No.:

Initialled as seen: Phys./Surg.: Dr. Roberts Ward: G9, WIG

Punch biopsy of cervix

Microscopy of this fragment of tissue shows traumatised tissue with mild dysplasia of squamous epithelium (CIN I). There is no evidence of malignancy.

18

J.C. Willox

Joanna B. Willox

I.L. Brown

SNOP 83 7600

SPECIMEN:	DATE OF OPERATION:	DATE OF REPORT:
Punch biopsy of cervix	05.04.84	11.04.84

FIGURE 13-COLPOPHOTOGRAPH FROM PATIENT CRAWFORD SHOWING AWE

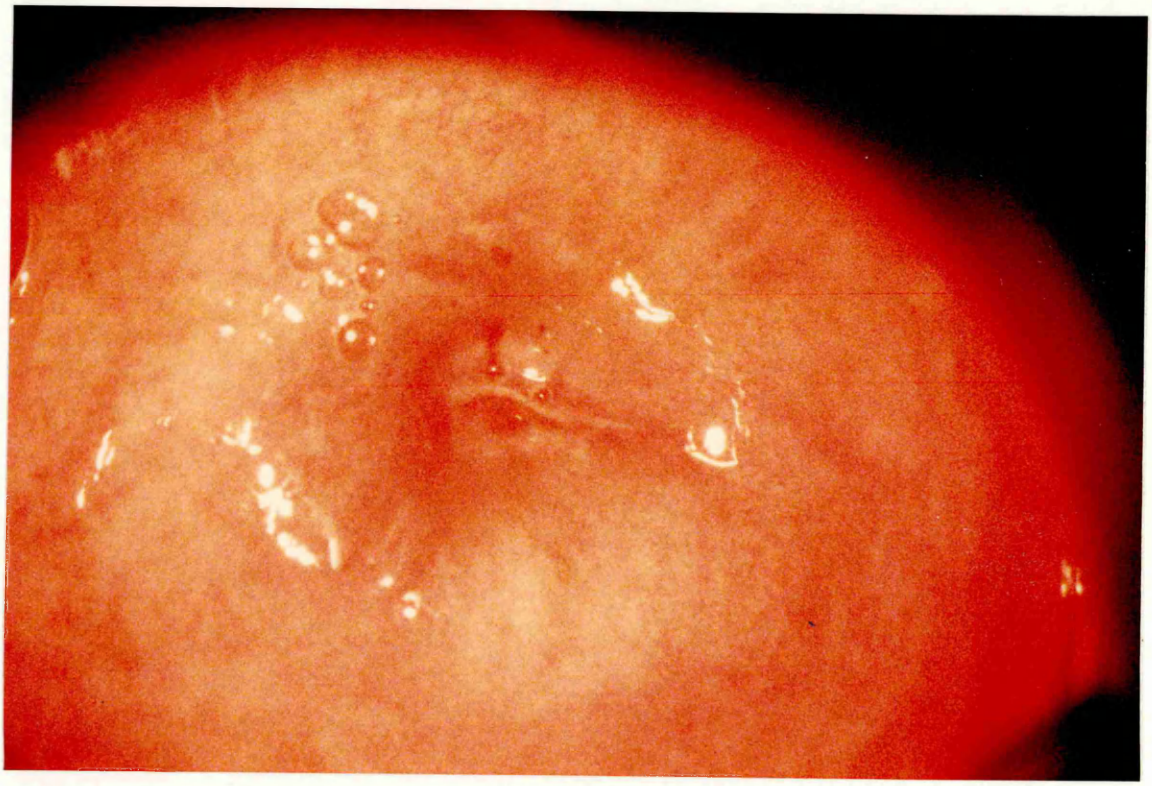
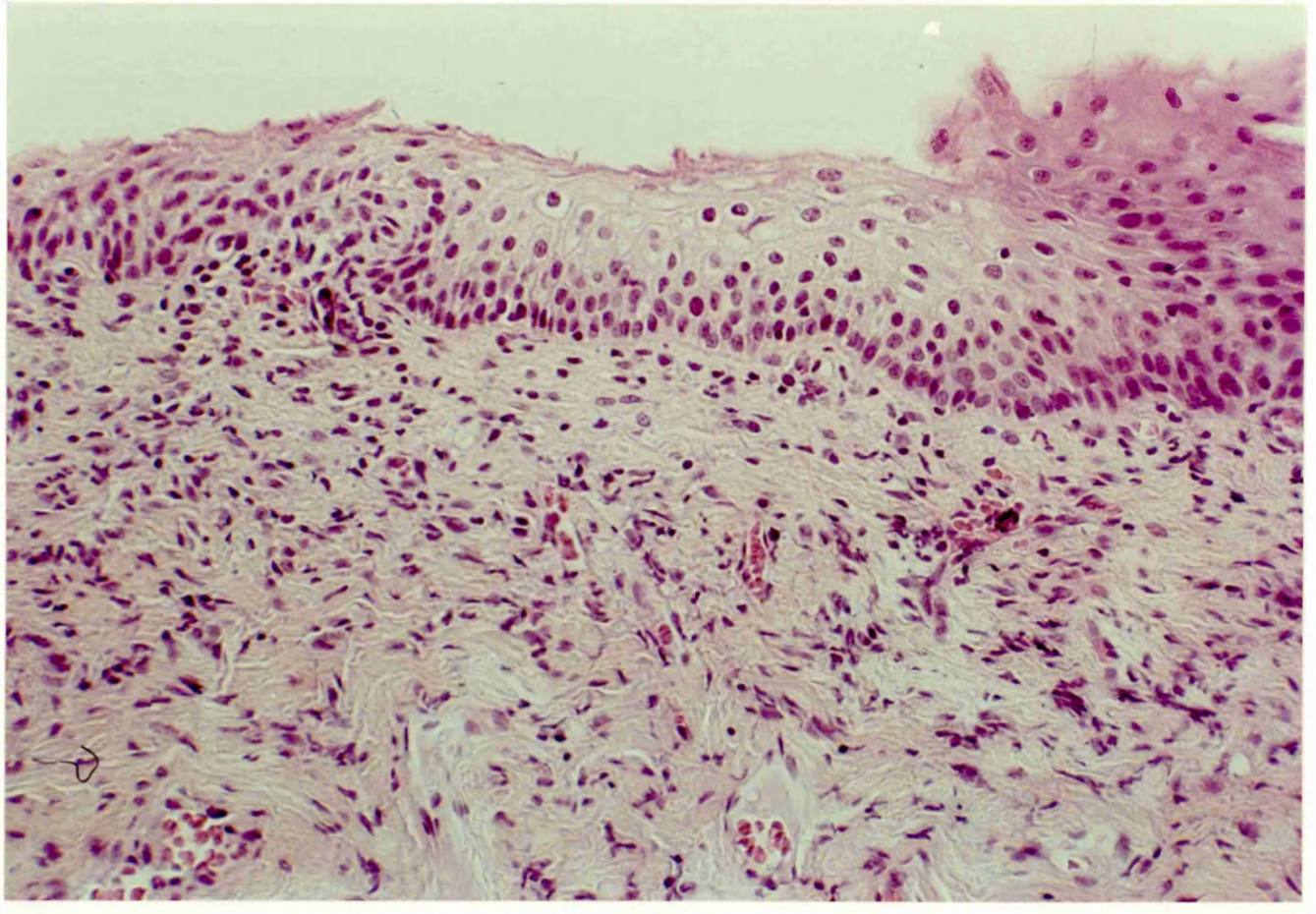


FIGURE 14-PHOTOMICROGRAPH FROM PATIENT CRAWFORD SHOWING CIN 1/ EPITHELIAL ATYPIA **x 250**



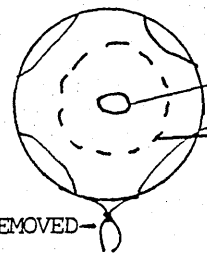
PATIENT 5 WALLACE

PATIENT 5 - TABLE 6 - WALLACE

50 YEAR OLD - LMP 9 YEARS BEFORE

NEGATIVE CYTOLOGY HISTOLOGY CIN1

FIRST VISIT



SCJ SEEN IN ENDOCERVIX

INFLAMMED METAPLASTIC TISSUE

McDONALD SUTURE REMOVED

HRT PRESCRIBED

SMEAR TAKEN

FIRST SMEAR REPORT — HEALTH BOARD

USE BALL PEN FIRMLY — GYNAECOLOGICAL CYTOLOGY

02-08-LABORATORY No. C

09-24-Surname: WALLACE

25-38-Christian Name:

Clinician: COLPOSCOPY CLINIC Dr KAUR

39-80-Address:

09-14-Birthdate: D 31 M 01 Y 13

15-20-Date of Examination: 29 01 3 84

21-24-Name and Full Postal Address of G.P.

25-28-Sender: Dr KAUR Colposcopy Clinic

29-34-Hospital Record No.:

35-REPEAT SMEAR, Yes 1:

36-44-First Cytology No.:

46-Single 1; Married 2; Widowed 3; Divorced 4

47-48-No. of Pregnancies: 7

49-Pregnant: Yes 1; No 2

50-51-Post Natal Weeks:

52-53-Age at Menopause:

Date of L.M.P. 9 yrs ago

60-Oral Contraception: Nil 1; Past 2; Current 3

61-Radiation, Yes 1

62-Hormone Treatment, Yes 1

63-Cervix: Healthy 1; Benign 2; Suspicious 3; Malignant

64-Smear Type: Cervical 1; Vaginal 2; Others 3 (Specify)

Clinical Diagnosis and Comments: ~~NO CIN~~ NO CINS SEEN

1) Cervical Smear

2) Vaginal Smear for hormone status please

65-Results: Negative 1; Suspicious 2; Positive 3; Unsatisfactory 4

66-Advice: Repeat 1; Referral to Gyn. 2

Pathologist's Comments: 1) CERVICAL SMEAR - INFLAMMATORY EXUDATE PRESENT

2) VAGINAL SMEAR CONSISTS LARGELY OF INTERMEDIATE SQUAMES AND PARABASAL CELLS. OCCASIONAL SUPERFICIALS PRESENT

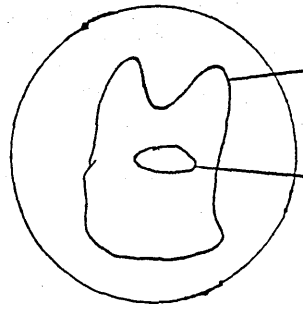
Pathologist's Signature

Date 03 04 84

67-68-Recall in: months

JMEC 11101 IBHB C1c

SECOND VISIT GOOD EFFECT FROM HRT



WELL DEMARCATED AWE AND MOSAIC

SCJ SEEN EASILY

SMEAR TAKEN

BIOPSY TAKEN

SECOND SMEAR REPORT — HEALTH BOARD

USE BALL PEN FIRMLY — GYNAECOLOGICAL CYTOLOGY

02-08 LABORATORY No. C

09-24 Surname: Wallace

25-38 Christian Name:

39-80 Address:

Clinician: COLPOSCOPY CLINIC

09-14 Birthdate: D 31 M 07 Y 33

15-20 Date of Examination: 24 05 84

21-24 Name and Full Postal Address of G.P.:

25-28 Sender: Colp

29-34 Hospital Record No.:

35 REPEAT SMEAR. Yes 1

36-44 Last Cytology No.:

46 Single 1 / Married 2 / Widowed 3 / Divorced 4

47-48 No. of Pregnancies: 3

49 Pregnant: Yes 1; No 2

50-51 Post Natal Weeks:

52-53 Age at Menopause:

Date of L.M.P.: ON HRT

60 Oral Contraception: Nil 1; Past 2; Current 3

61 Radiation, Yes 1

62 Hormone Treatment, Yes 1

63 Cervix: Healthy 1; Benign 2; Suspicious 3; Malignant 4

64 Smear Type: Cervical 1; Vaginal 2; Others 3 (Specify)

Clinical Diagnosis and Comments:

NO SMEAR (1) Cervical Smear

(2) Vaginal for Hormone Status

65 Results: Negative 1 / Suspicious 2 / Positive 3 / Unsatisfactory 4

66 Advice: Repeat 1 / Referral to Gyn. 2

67-68 Recall in: months

Pathologist's Comments:

1) Normal Squames Present.

2) Almost exclusively Intermediates Squames

Pathologist's Signature: M

Date: 25/5/84

IRHB C

PATHOLOGY REPORT - CIN 1

WESTERN INFIRMARY/GARTNAVEL GENERAL HOSPITAL — PATHOLOGY

Lab. No.: Name WALLACE, Hosp. No.:

Initialled as seen: Phys./Surg.: Dr Roberts Ward G9 Gyn WIG

Punch biopsy of cervix

Microscopy shows squamous metaplasia and focal mild dysplasia (CIN I) of the cervical squamous epithelium.

18

M. Seywright

M Seywright

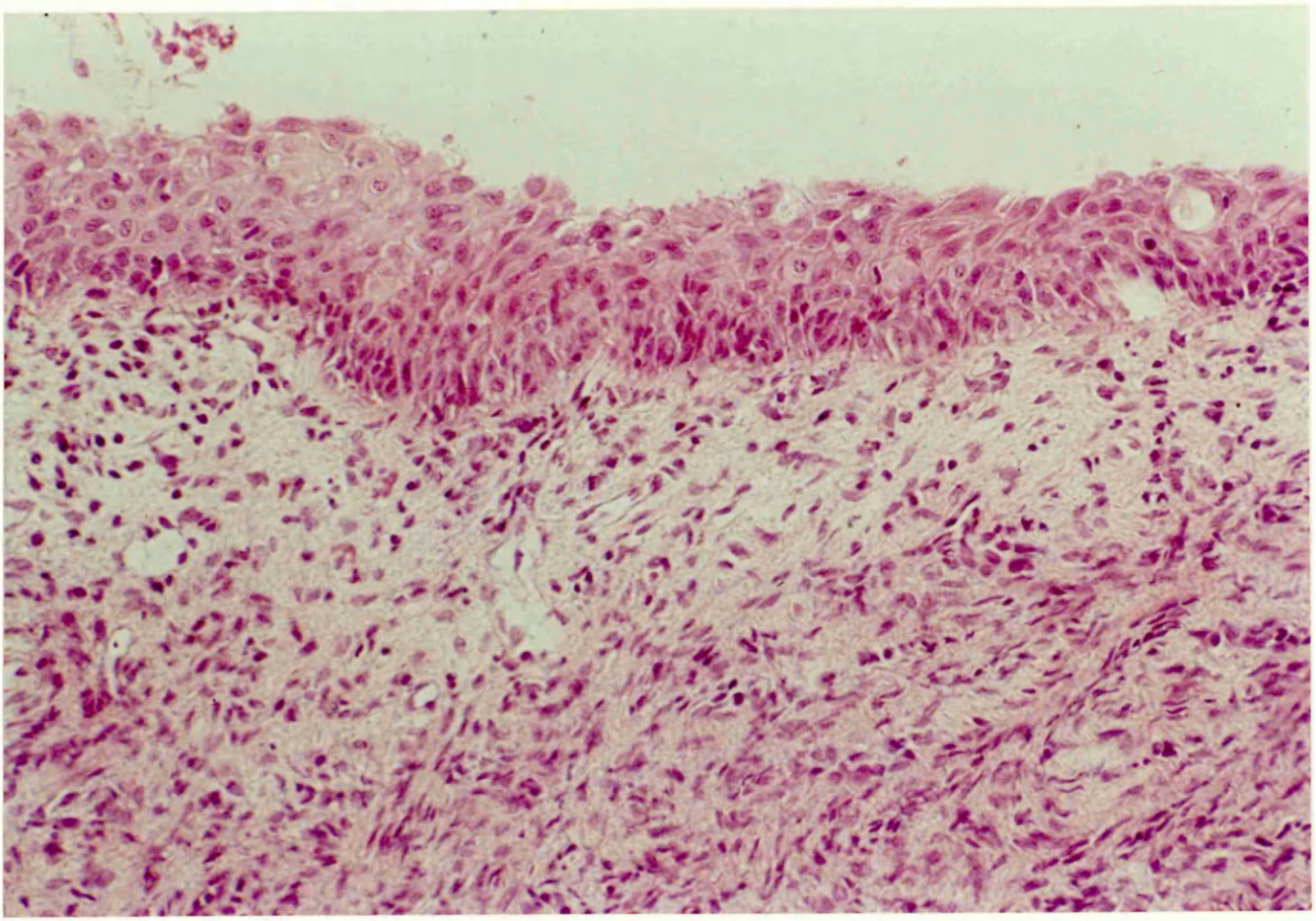
SNOP 83 7600

SPECIMEN	DATE OF OPERATION	DATE OF REPORT
Punch biopsy of cervix	24.5.84	1.6.84

FIGURE 15-COLPOPHOTOGRAPH FROM PATIENT WALLACE SHOWING MCDONALD SUTURE BUT NO AWE

FIGURE 16-PHOTOMICROGRAPH FROM PATIENT WALLACE SHOWING CIN 1

x250



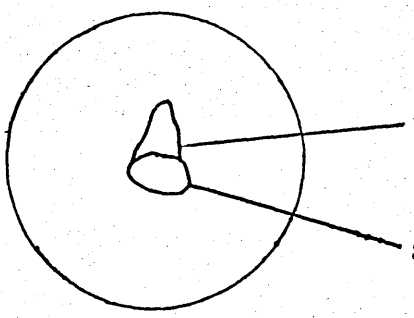
PATIENT 11 KEENAN

PATIENT 11 - TABLE 6 - KEENAN

37 year old - LMP 6 years before

Negative cytology

Histology CIN 1



SMEAR TAKEN

BIOPSY TAKEN

FIRST SMEAR REPORT

USE BALL PEN FIRMLY

HEALTH BOARD

GYNAECOLOGICAL CYTOLOGY

02-08 LABORATORY No. C

09-24-Surname KEENAN

25-38-Christian Name

Clinician COLPOSCOPY CLINIC DR ROBERTS

39-80-Address

09-14-Birthdate D 29 M 10 Y 46

15-20-Date of Examination 17 16 84

21-24-Name and Full Postal Address of G.P.

25-28-Sender Colp clinic

29-34-Hospital Record No.

35-REPEAT SMEAR. Yes 1

36-44-Last Cytology No.

46-Single 1 Married (2) Widowed 3 Divorced 4

47-48-No. of Pregnancies 3

49-Pregnant: Yes 1; (No 2)

50-51-Post Natal Weeks

52-53-Age at Menopause 311

Date of L.M.P. 6 yrs

60-Oral Contraception: Nil 1; Past 2; Current 3

61-Radiation. Yes 1

62-Hormone Treatment. Yes 1

63-Cervix: Healthy 1; Benign 2; Suspicious 3; Malignant 4

64-Smear Type: Cervical 1; Vaginal 2; Others 3 (Specify)

Clinical Diagnosis and Comments:

A = (1) NO CIN (1) Conical (2) Vaginal Smear for Hormonal status

B = (2)

65-Results: (Negative) 1; Suspicious 2; Positive 3; Unsatisfactory 4

66-Advice: Repeat 1; Referral to Gyn. 2

67-68-Recall in months

Pathologist's Comments: A CERVICAL SMEAR - MILD INFLAMMATORY EXUDATE IS PRESENT

B = VAGINAL SMEAR CONTAINS BOTH INTERMEDIATE AND SUPERFICIAL SQUAMES

Pathologist's Signature

Date 11 06 84

IRHB C1c

PATHOLOGY REPORT

WESTERN INFIRMARY/GARTNAVEL GENERAL HOSPITAL **PATHOLOGY**

Lab. No.: Name: KEENAN, Hosp. No.:

Initialled as seen: Phys./Surg. Dr Roberts Ward: G10 WIG

Punch biopsy of cervix
Microscopy shows one small focus of mild epithelial dysplasia (CIN I) and a mild cervicitis.

18

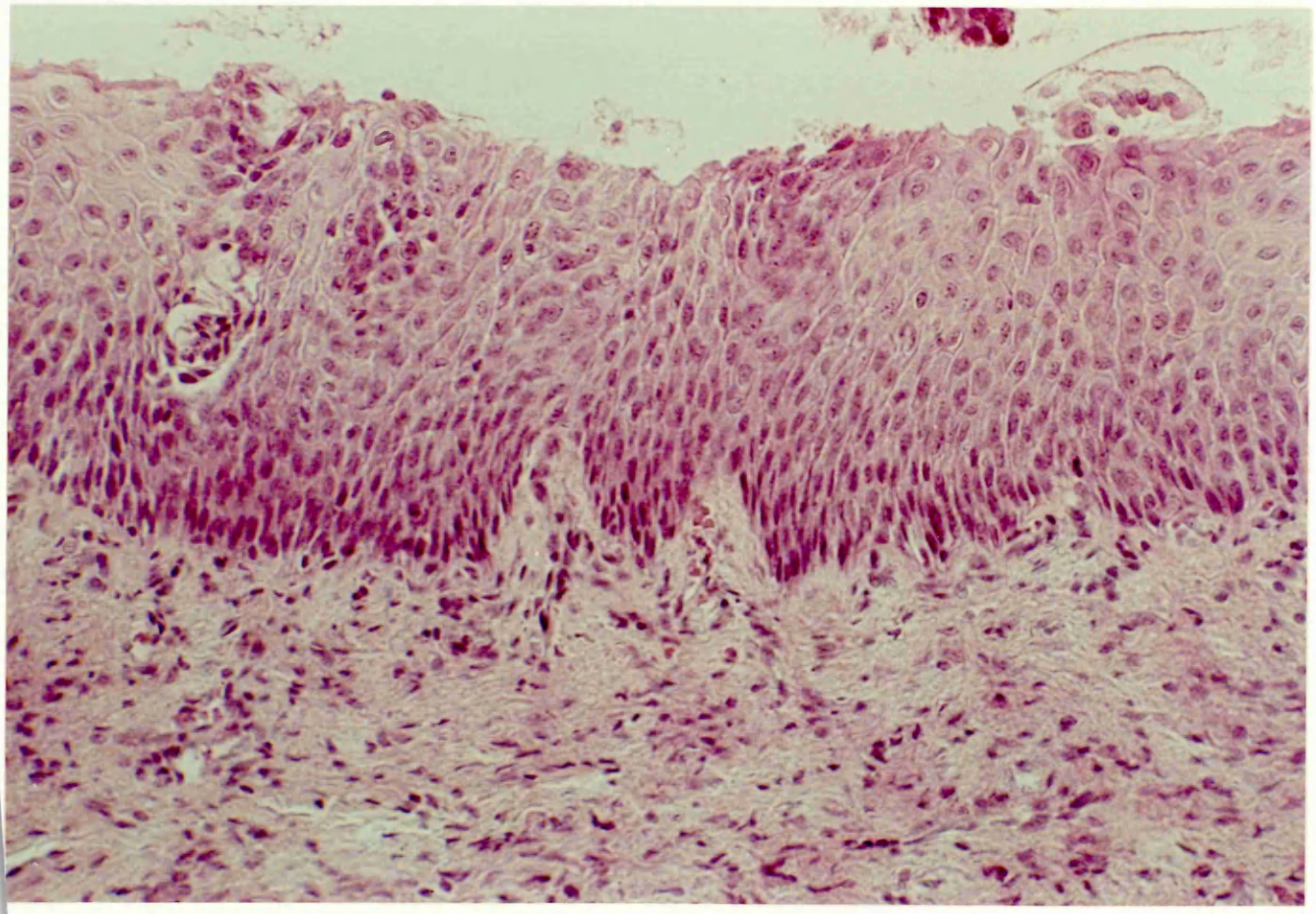
A McPhaden

I A R More

SNOP 83 7600

SPECIMEN	DATE OF OPERATION	DATE OF REPORT
Punch biopsy of cervix	7.6.84	11.6.84

FIGURE 17-PHOTOMICROGRAPH FROM PATIENT KEENAN SHOWING CIN 1
x 250



APPENDIX 4

NOTES ON THE COMPLETION OF THIS THESIS

During the collection of data for this thesis, it was decided that suitable information would be stored, and analysed, by a microcomputer. I felt that if the computer was situated at home, then greater access to the data would be available, and consequently more time for analysis. For this purpose, I purchased a microcomputer and learned BASIC.

Patient information from the studies in chapters four, five, and eight, was held in a simple database, and these data were examined by a programme that I devised. The principle behind this method of data analysis is very simple; Information was held in an alphanumeric string, this is a sequence of digits or numbers, which are held in an array such as this;

13287362	patient 1
15387221	patient 2
12376233	patient 3

Any piece of information can be coded a number, for example the first number of the string may be parity, the second and third, age, and the fourth, the organism isolated, or the specific measurement. Patient 1 therefore, was para 1, age 32, and had organism 8, say C.trachomatis isolated. Analysis of the data, can be carried out by 'questioning' the string. By asking the string from patient 1 what is the fourth character, and counting if it is 8, and then passing on to string 2 and continuing the count, the number with 8 as the fourth character i.e. with C. trachomatis, is quickly summated. Quick answers to

fairly complex questions could be obtained.

Any number of conditions could be made to make the questions very specific such as; if the fourth character is 3, and the fifth character is 6, and the eighth character is 4, and the twelfth character is 5, then count the second and third character,i.e. age. By such specific application of the database to the data, it was uncomplicated to obtain prevalence of specific conditions, and mean ages, parities etc.

To work in conjunction with this database, some colleagues and I wrote programmes to perform statistics directly on the data held in the arrays. Statistical programmes for standard deviations, standard errors, t tests, chi square tests, factorials, logarithmic transformation, and nonparametric tests such as Mann Whitney U test and Kendals coefficient of rank correlation, were all devised and applied directly to the data.

These methods,enabled the data to be analysed much quicker, than by manual methods. Furthermore, interesting correlations could be quickly checked, and statistical analysis applied.

The nature of a thesis, makes it very suitable for being written on a word processor. A letter quality daisywheel printer, fast data storage and retrieval system, and a word processing programme were added to the microcomputer. The main benefit of this system,was that I could write, and rewrite,at home in the evening, and a hard copy could be obtained after each draft. Not only did this teach me to type, it avoided the repeated use of secretarial time. The whole system including all software cost less than £500, and did not require a special video display unit, a humble television proving adequate.

There is, however, a balance in all things, and this is no exception. Once the system was operational it ran smoothly, but the teething problems with hardware, and especially programming, were considerable. There were problems with file handling and error trapping which, in the absence of specialised help, took a considerable time to correct. In terms of time saved over manual methods, by using a computer to such a large extent, the issue is fairly marginal. However, the experience of becoming even a little 'computer literate' is an investment for the future.

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O.P.C.S. Monitor,(1985), MB1, 85/1.
O.P.C.S. Monitor,(1984), MB1, 84/1.

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