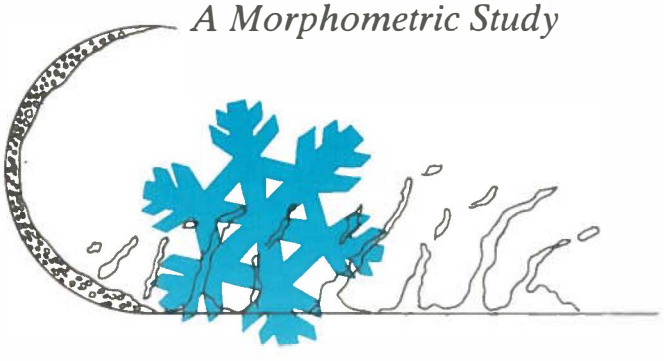
Cryosurgery in Cervical Intraepithelial Neoplasia

A Morphometric Study



H. BOONSTRA

Cryosurgery in Cervical Intraepithelial Neoplasia

Stellingen behorende bij
het proefschrift van
HENDRIK BOONSTRA

Cryosurgery in Cervical Intraepithelial Neoplasia

Groningen, 4 februari 1987

1. Weefseldestructie als behandeling van Cervicale Intraepitheliale Neoplasie dient in het aangedane gebied tot een diepte van tenminste 4 mm over een afstand van tenminste 15 mm plaats te vinden.

   Dit proefschrift.

2. Bij het behandelen van Cervicale Intraepitheliale Neoplasie met cryocoagulatie, moet er rekening mee gehouden worden dat het vriesletsel zich op de anatomische drie- en negen-uurposities langzamer ontwikkelt.

   Dit proefschrift.

3. De gemiddelde maximale diepte van de cysten van Naboth verschilt niet significant van de gemiddelde maximale diepte van de crypten in de cervix uteri.

   Dit proefschrift.

4. De naam Krukenberg tumor dient alleen gebruikt te worden voor die ovariumtumoren die aan de oorspronkelijk door Krukenberg beschreven histologische criteria voldoen en niet voor alle metastatische ovariumtumoren.

   Woodruff J.D., and Novak E.R.,
   Krukenberg tumors of the ovary.
   Obstet. Gynecol. 1960; 15:351.

5. De belofte die vaak wordt gedaan aan patiënten met een ongeneeslijke vorm van kanker, dat ze geen pijn hoeven te lijden, kan niet altijd worden waargemaakt zonder het bewustzijn van de patiënt uit te schakelen.

6. De eerste lijns gezondheidszorg zou meer gebruik moeten maken van de reeds in het ziekenhuis begonnen gespecialiseerde hulpverlening aan de patiënt met kanker in een terminaal stadium.

7. Mede door het niet herkennen van een psychogene depressie wordt deze aandoening nog te dikwijls alleen met tranquillizers behandeld.

8. De positieve waardering voor een rustige zuigeling behoort meer de moeder ten deel te vallen dan de zuigeling.

9. Voor diegenen die op levensbeschouwelijke gronden van mening zijn onder geen voorwaarde menselijk leven te mogen beëindigen, is het uitvoeren van abortus provocatus vóór en na de 12e week van de zwangerschap voor het geweten even bezwaarlijk.

10. Zolang medische tuchtcolleges zelf geen gebruik maken van onderlinge toetsing van hun werkwijze, is het moeilijk te verdedigen het aspect intercollegiale toetsing zwaar te laten wegen bij de beoordeling van zaken.

11. Het is gewenst dat in de regelgeving ten aanzien van het basisonderwijs duidelijker wordt aangegeven, in welke mate rekening is gehouden met de belangen van het kind, opdat acceptatie en uitwerking hiervan door schoolbesturen meer verantwoord mag worden geacht.

12. De kwantiteit en de kwaliteit van de hulpverlening aan het slachtoffer van een misdaad is, vergeleken met die aan de dader, in ontwikkeling achter gebleven.

13. Integratie van het christelijk geloof in het alle- en hedendaagse leven verhoogt de geloofwaardigheid van dat geloof.

14. Er is aanleiding de slotwoorden van Openbaring 6:6 'en breng geen schade toe aan de olie en de wijn' te interpreteren als een bevel de voorraad olie en wijn intact te laten voor de verzorging van gewonden (Lucas 10:34).
Holwerda D., Opbouw 1975; 19e jaargang no.44.

15. De meest linkse rechtse is meestal linkser dan de meest rechtse linkse en dient daarom als links beschouwd te worden.

16. Het voordeel van het met potlood schrijven van een manuscript valt niet uit te vlakken.

RIJKSUNIVERSITEIT TE GRONINGEN

Cryosurgery in Cervical Intraepithelial Neoplasia

A Morphometric Study

PROEFSCHRIFT

ter verkrijging van het doctoraat in de geneeskunde
aan de Rijksuniversiteit te Groningen
op gezag van de Rector Magnificus Dr. E. Bleumink
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Hendrik Boonstra

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Tweede promotor : Prof. Dr. J. Janssens

Eerste referent : Dr. J.G. Aalders
Tweede referent : Dr. J. Koudstaal

"Tis the Chyrurgions praise, and height of Art,
Not to cut off, but cure the vicious part".

Robert Herrick (1591-1674)
Hesperides, "Lenitie"

Aan Tiny, Klaas, Peter
Venje Henke, Akkelies,
Rumsiah en Sadiah.

Aan Heit en Mem
Aan Vader en Moeder

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Preface

The study described in this thesis was carried out at the Gynaecological Department of the University Hospital of Groningen (Head at that time: Prof. Dr. J. Janssens, at present: Prof. Dr. H.J. Huisjes) and at the Department of Pathology of the University of Groningen (Head: Prof. Dr. J.D. Elema).

The following hospitals and laboratories have contributed to the study by collecting hysterectomy specimens for measuring the depth of crypts:

Delfzijl: Delfzicht Hospital; Drachten: Hospital Ny Smellinghe; Groningen: Department of Gynaecology and Pathology of the Roman Catholic Hospital (RKZ); Leeuwarden: Bonifatius Hospital, Diaconessenhuis, Laboratory of Pathology; Stadskanaal: Refaja Hospital; Winschoten St. Lucas Hospital, Laboratory of Pathology.

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Contents

CHAPTER I

Introduction and purpose of the investigations

I.1. Introduction

It is generally accepted that the majority of the invasive squamous carcinomas of the uterine cervix arise from the precursors dysplasia and carcinoma in situ (CIS) (Patten 1969).

From the difference in mean age of patients with severe dysplasia, in situ cancer and invasive cancer, it can be postulated that progression to invasive cancer is a slow process (Reagan et al. 1955, Patten 1966, Vooys 1975). From the literature it is evident that the progression rate of dysplasia shows a wide range. Collected data show progression rates varying from 3.4-50.0%, regression rates from 24.3-75.0% and persistent disease from 15.4-43.9% (Burghardt 1972). Variations in the methods of investigation and diagnostic criteria are factors contributing to this wide range of results (Van Lent 1979). The progression and regression rate of dysplasia depends on the severity grade of the process. According to Patten (1966) 71.9% of the light dysplasia regress, while for moderate and severe dysplasia the regression rates are 44.2 and 16.3 percent, respectively. Of the non-spontaneously regressed severe dysplasia, approximately 66% progresses into carcinoma in situ, the remaining persists (Richart 1968, Richart and Barron 1969, Barron and Richart 1970). In a period of 5-10 years, a high percentage of the in situ carcinomas will become invasive (Van Lent 1979: 54-71%).

In the last decades the treatment of cervical epithelial abnormalities has become a matter of considerable interest. Traditional treatment of premalignant cervical disease by exconization with or without hysterectomy, has recently been considered to be radical. In particular for a group of young, often nulliparous women, preservation of fertility is particularly important (Jordan 1982). In this group of young women there is a rapid increase in the number of patients presenting with pre-invasive cervical neoplasia (Feldman et al. 1978, Van Lent 1979, Dillon et al. 1981, Singer and Walker 1982, Jordan 1982).

Although the literature is not unanimous, there is evidence that cone biopsy of the cervix is attended by complications and morbidity such as bleeding, cervical stenosis and incompetence in subsequent pregnancies (McLaren et al. 1974, Johnstone 1974, Jones et al. 1979, Weber and Obel 1979, De Blok et al. 1985). For these reasons there is an increasing need to develop an alternative therapy.

Moreover, cone biopsy has also been used diagnostically, and therefore an alternative should be found for this procedure. It resulted in a renewed interest in colposcopy and the colposcopically directed biopsy. From several investigations it is evident that the colposcopically directed biopsy and endocervical curettage can replace the diagnostic procedure of cone biopsy (Verschoof 1975, Van Lent 1979).

In the last 15 years different methods of local tissue destruction, such as diathermy, cryosurgery, cold coagulation, infrared coagulation and laser vaporisation have become available. By fulfilling specific pretreatment criteria, the results of the different methods are satisfactory and show little divergence (Van Lent 1979, Singer and Walker 1982). Cryosurgery has the advantage that it can be performed easily in the outpatient department with minimal discomfort to the patient. It resulted in a wide use of this type of local destructive treatment.

During recent years, however, an increasing number of reports about residual disease and even of invasive carcinoma of the cervix following outpatient management for apparently benign or pre-invasive disease, have been published (Kaufman and Irwin 1978, Sevin et al. 1979, Townsend et al. 1981). In most of the reported cases a proper pretreatment evaluation was not carried out. Also from other studies a low cure rate has been reported (Charles and Savage 1980a, b, Charles et al. 1981) and it

reveals that the results of cryosurgery depend on the histological grading, the linear extent of the lesion, the extent of cervical crypt involvement and the age of the patient (Kaufmann and Irwin 1978, Ostergard 1980, Hatch et al. 1981, Monaghan and Townsend 1982). Most of these factors are interrelated and the major causes of failure in cryotherapy are large size of the lesion and extensive crypt involvement, both frequently seen in older patients, (Reagan and Hicks 1953, Einerth 1978, Anderson and Hartley 1980, Monaghan and Townsend 1982, Kwikkel et al. 1985b).

It is beyond doubt that a small focal ectocervical lesion can be treated effectively by cryotherapy, but it is uncertain whether the cryolesion is extensive enough to eradicate all the pathological epithelium in cervical crypts and in the endocervical canal (Sonek et al. 1971, Einerth 1978, Sevin et al. 1979, Charles and Savage 1980a,b, Savage et al. 1982, Singer and Walker 1982).

To evaluate this, it is necessary to know the extension of the pre-invasive lesions and the extension of the cryolesion. The extension on the surface, the linear extent of the abnormal epithelium can be recorded colposcopically. This implies that there is an absolute contraindication for cryosurgery if the lesion extends into the endocervix beyond the point where it can be seen colposcopically. A fundamental problem is the extension into the cervical crypts, which cannot be seen colposcopically.

Therefore it is necessary to investigate morphometrically the maximum depth of the cervical crypts and the maximum depth of crypt involvement with pre-invasive neoplastic disease. Some investigators have performed such studies (Reagan and Hicks 1953, Przybora and Plutowa 1959, Reagan and Patten 1962, Anderson and Hartley 1980, Abdul-Karim et al. 1982). The results are divergent and not comparable because, with the exception of the work reported by Przybora and Plutowa (1959), shrinkage caused by processing tissue for histological investigation was not considered. Although it is assumed in the literature that by cryosurgery tissue destruction to a depth of 5 to 6 mm can be obtained (Crisp 1971), there is still a lack of adequate data to support this point (Singer and Walker 1982). Moreover, no standardization of cryosurgical techniques exists and therefore accurate definitions and more studies using cone or hysterectomy specimens following cryosurgery for pre-invasive disease are needed, in order to determine whether or not glandular involvement should be a contraindication to cryosurgery (Charles and Savage 1980a, b). There is also a need to standardize the cryosurgical techniques regarding the type of refrigerant, tank pressure, probe tip selection, probe temperature control, probe-tissue contact and duration and number of freezings.

I.2. Purpose of the investigations

The aim of this study is to investigate the factors influencing depth of cervical crypts, depth of crypt involvement and the extension of the cryolesion, achieved under standardized conditions.

Using this approach it should be possible, to select a group of patients with pre-invasive disease, who can be treated radically with cryosurgery.

CHAPTER II

Review of the literature

II.1. The anatomy of the uterine cervix

II.1.1. Gross anatomy

The cervix is the distal part of the uterus and is divided from the proximal part, the corpus uteri, by a fibromuscular junction, called the internal os. The cervix projects through the top of the vagina, by which the lower part of the cervix, the portio, is visible during gynaecological examination. The other, upper part of the cervix is called the supravaginal portion. The forming of the vaginal fornices is a result of the penetration of the cervix through the vagina top. The vaginal mucosa is reflected around the cervix. The mouth of the endocervical canal in the vagina is called the external os. The cervical canal has a lenght of about 30 mm and is somewhat flattened in the antero-posterior direction. The maximum width is about 7 mm. In nulliparous women the cervix is cylindrical with a circular external os. The diameter is about 25 mm. During pregnancy the cervix increases in size and becomes bulbous. Laceration during delivery results in a more or less slit shaped external os with the formation of an anterior and posterior lip. The lips can reflect so that the endocervical tissue bulges outside, the so-called ectropion. A lateral cervical tear can extend into the vaginal fornix (Singer and Jordan 1976).

II.1.2. Microscopy

In late fetal life the original columnar epithelium of the Müllerian duct covers the uterine cavity and extends downwards into the endocervical canal in the direction of the vagina.

The tissue with a glandular appearance is in fact part of a system of clefts from the endocervical surface (Fluhmann 1957, 1961). These clefts can arrange themselves as long narrow tubes, resembling as gland ducts. Occlusion of the surface openings leads to an accumulation of mucus and the formation of cysts (Nabothian).

The endocervical columnar epithelium meets the original vaginal squamous epithelium distally and is called the original squamocolumnar junction (SCJ). This junction is located somewhere in the endo- or on the ectocervix. At birth, in about 70% of cases it is located distally to the external os, in about 30% at or proximately to the external os and in 3-5% in the lateral vaginal fornices (Pixley 1976).

During late fetal life, and especially during adolescence and pregnancy an oestrogen induced growth of the cervix uteri occurs. It results in an increase of the endocervical columnar epithelium. As a consequence the columnar epithelium becomes everted and occupies a relative ectocervical position (Singer and Jordan 1976). In the most distal part of this columnar epithelium, at the junction with the squamous epithelium of the ectocervix, a stepwise change takes place from columnar epithelium to the squamous epithelium. This squamous metaplasia is a physiological process and is most active during adolescence and pregnancy. Depending on the period of life, there is a permanent conversion from columnar to squamous epithelium and not the reverse. Contrary to the theory of Fluhmann (1961), who assumed that the process was a to and fro movement, the columnar epithelium once changed to squamous epithelium does not become columnar epithelium again. The columnar epithelium undergoes an extensive metaplasia under the influences of the vaginal low pH (Lang 1955, Singer 1975, Singer and Jordan 1976). Coppleson and Reid (1975) and Singer (1975) suggest that also coitus can be a stimulus to squamous metaplasia. These authors found a larger area of squamous metaplasia in young, sexually active girls. The cause is not clear, but possibly prostaglandins play a role (Coppleson and Reid 1975, Singer 1975).

The newly formed border between the original columnar epithelium and the metaplastic squamous epithelium is called the Neo-Squamocolumnar Junction (N-SCJ). This junction is a capricious line. The area between the original SCJ and N-SCJ is the transformation zone within which the process of metaplasia takes place. At the ectocervix, three types of epithelium can be seen: The original columnar epithelium, original squamous epithelium and metaplastic squamous epithelium (figure II.1).

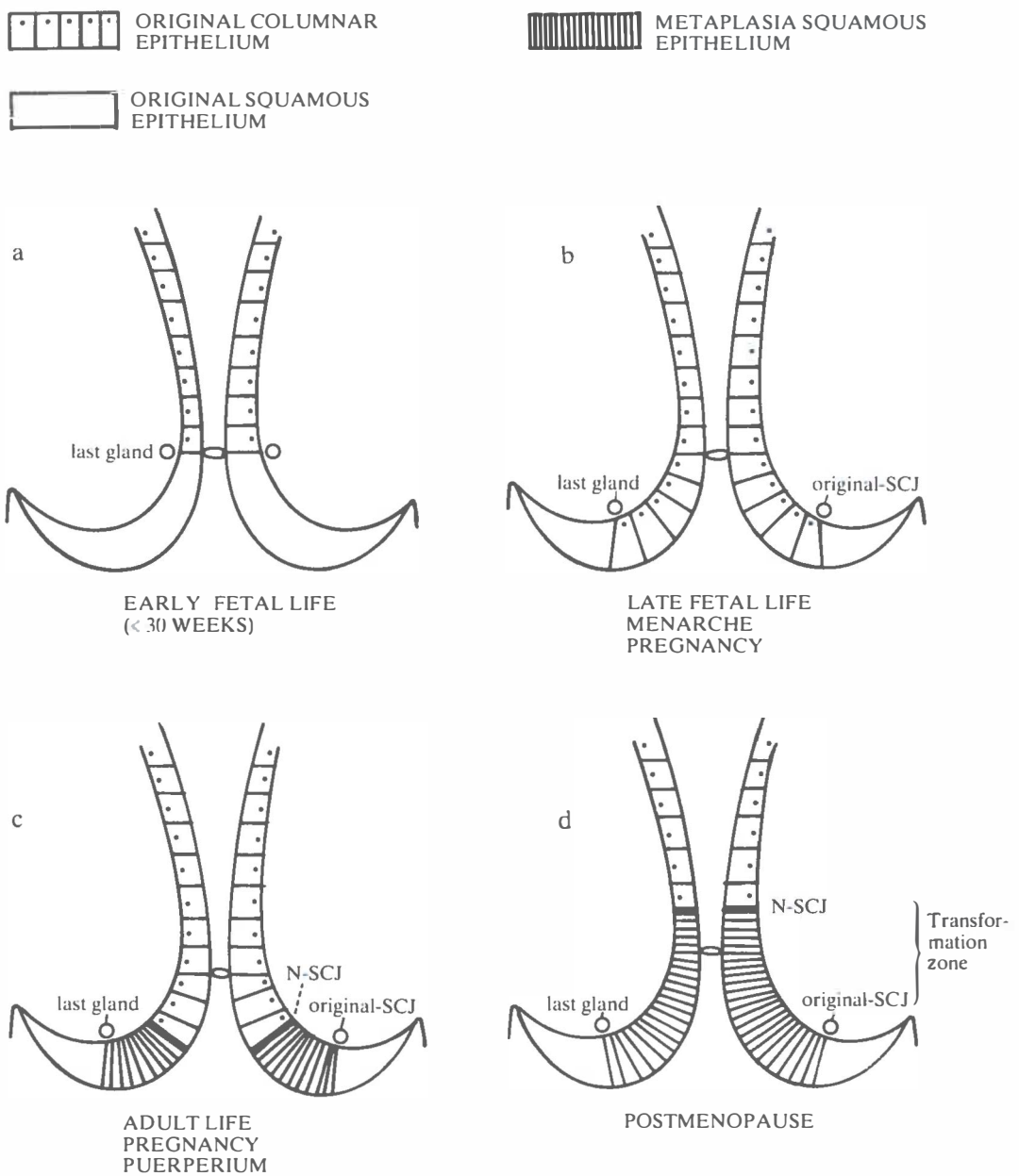

Figure II.1 The surface epithelium of the vaginal portio at various periods of life (Original-SCJ = Original-Squamocolumnar Junction; N-SCJ = Neo-Squamocolumnar Junction).

Because of the physiological process of metaplasia during reproductive years, the extent of ectocervical columnar epithelium decreases and the N-SCJ moves in endocervical direction. When the postmenopausal state has been reached, the N-SCJ is generally located endocervically.

The original SCJ, the point as far as the columnar epithelium has reached before, is marked by the most caudal crypt. This 'last gland' is a fixed point from which the extension of the endocervical mucosa can be measured (Kaufmann and Ober 1959). Burghardt and Holzer (1972) are using this point as a reference point in histological studies.

The most common topographic changes related to age and parity reported in the literature can be summarized as follows:

- In a fetus of less than 30 weeks gestation, the SCJ is most common at the external os or proximal to it (Pixley 1976) (figure II.1a).
- During late fetal life (the last 10 weeks of gestation), adolescence and pregnancy there is an eversion of the columnar epithelium to the ectocervix. The SCJ moves distally and the ectocervix is partially covered with columnar epithelium, in which the squamous metaplasia is developing. Depending on the extent of metaplastic changes, in the course of time the N-SCJ will move in the endocervical direction (Singer 1975, Pixley 1976) (figure II.1b+c).
- The postmenopausal state is characterized by extensive squamous metaplasia and atrophy of the cervix, resulting in a tendency of the N-SCJ to reverse within the endocervical canal (Crompton 1976) (figure II.1d).

II.2. Cervical Intraepithelial Neoplasia (CIN)

II.2.1. Introduction

A complete literature review about atypical cervical epithelium, is beyond the scope of this thesis. Matters will be discussed only as far as they are of importance for this study. Therefore attention will be given to its definition, topography and epidemiology. In connection with the present study, the topographically literature study has been focussed on morphogenesis, surface distribution, linear extension and endocervical crypt involvement of the pre-invasive cervical disease. Finally a limited review will be given about diagnosis and treatment.

II.2.2. Definition of CIN

Many definitions have been used to describe severe dysplasia (SD) and carcinoma in situ (CIS). Generally it refers to a disturbance of differentiation. In 1961, a definition of carcinoma in situ and dysplasia was recommended and adopted by the International Committee on Histological Terminology for lesions of the uterine cervix:

> *"Only those cases should be classified as carcinoma in situ which, in the absence of invasion, show a surface epithelium in which, throughout its whole thickness, no differentiation takes place.*
>
> *The process may involve the cervical glands without hereby creating a new group. It is recognized that the cells of the uppermost layers may show some flattening. The very rare case of an otherwise characteristic carcinoma in situ which shows a greater degree of differentiation belongs to the exception for which no classification can provide.*
>
> *All other disturbances of differentiation in the squamous epithelial lining, the glands or covering of the surface is to be classified as dysplasia. They may be characterized as a high or low degree, terms which are preferable to suspicious and non-suspicious, as the proposed terms describe the histological appearance and do not express an opinion." (Wied 1962).*

In spite of the apparent comprehensiveness of this definition, it seems to be too restricted. Only the disturbance in the structure has been considered and not the cellular atypia, on which the cytologic screening has been based (Van Lent 1979). Burghardt (1983) has also stated that this definition is far too narrow. In his opinion a large variety of epithelial lesions exists, like more or less differentiated types of dysplasia. Carcinoma in situ also shows various types of differentiation. The original definition of the International Committee on Histological Terminology only includes a very undifferentiated form to be termed as carcinoma in situ.

The invasive cancers also show several types of differentiation and according to Burghardt (1983), these do correspond with the different types of differentiation of the pre-invasive lesions, whether it is dysplasia or in situ cancer. That means, in his opinion, that dysplasia is not only a precursor of a carcinoma in situ, but might be considered as a counterpart of extremely highly differentiated invasive cancers (Burghardt 1983).

It is generally accepted that the epithelial lesions, which morphologically can be distinguished in mild, moderate and severe dysplasia and in various differentiated types of in situ cancer, form part of intraepithelial, potentially malignant lesions with an increasing degree of seriousness.

In the gynaecological literature there is an increasing tendency to use the term Cervical Intraepithelial Neoplasia (CIN) introduced by Richart (1968). It is defined as a spectrum of intraepithelial lesions, that begins as a generally well-differentiated intraepithelial neoplasia, usually qualified as mild dysplasia, and ends with undifferentiated in situ carcinoma (Ferenczy 1977).

In the literature the spectrum of epithelial alterations has, according to the number of undifferentiated malignant cells occupying the cervical epithelium, been quantitatively classified into three categories: CIN grade I. grade II and grade III. CIN grade I corresponds to the classic very mild to mild dysplasia, CIN grade II to moderate dysplasia and CIN grade III to severe dysplasia and carcinoma in situ (Ferenczy 1977).

II.2.3. Topography of (pre-)malignant cervical disease

II.2.3.1. Introduction

Many topographic studies concerning the distribution of dysplasia, carcinoma in situ and invasive carcinoma of the cervix have been carried out. The goal of that type of studies is to obtain knowledge about:

- the morphogenesis of pre-invasive and invasive diseases,
- the distribution and extension of pre-invasive lesions on the surface as well as in the endocervical crypts.

The difficulty in topographic studies is that there is no well-defined anatomical point of reference on the cervix. Fennell (1955) used the squamocolumnar junction as a reference point. This point is not acceptable because it moves relative to the anatomical external os. The anatomical external os used by Kaufman and Ober (1959) is also too precarious. The same applies to the reference point prescribed by Przybora and Plutowa (1959), who stated that the external os is the dividing border between the portion visible through the speculum and the portion concealed in the canal. This border is not a line but a ring-like area in the lower canal.

Coppleson and Reid (1967) however pointed out that the speculum opens the cervical canal. so that the cervix as seen in vivo and as seen in the fixed specimen may differ. Burghardt (1972) and also Abdul-Karim et al. (1982) used the last endocervical gland as the reference point. Reagan and Patten (1962) referred to a point where a vertical line passing through the axis of the cervical canal transected a horizontal line passing through the outermost portion of the portio vaginalis. These various reference points are illustrated in figure II.2.

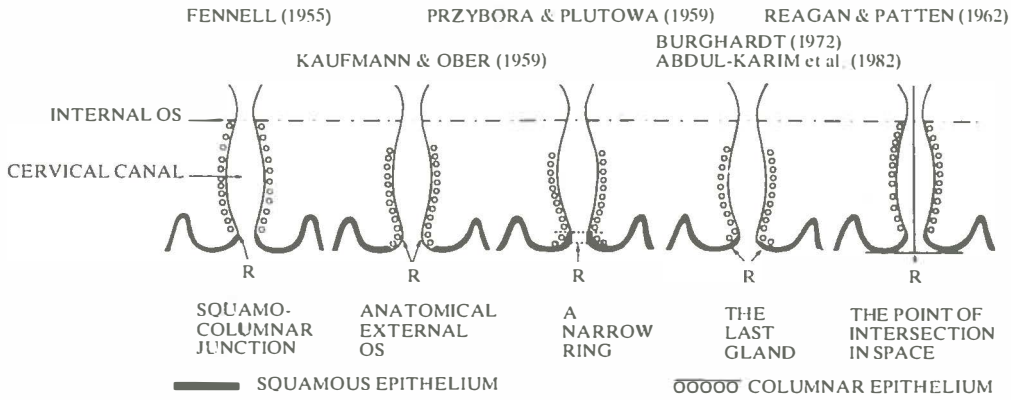

Figure II.2 Points of reference (R), used in topographic studies of the cervix (Modified from Langley and Crompton 1973).

II.2.3.2. Topography and morphogenesis of (pre-) malignant cervical disease

Not only the relation of the transformation zone and the squamocolumnar junction to the reference point, was studied by Reagan and Patten (1962), Reagan (1964) and Patten (1969), but also the histological characteristics such as metaplasia, reserve cell hyperplasia, dysplasia, carcinoma in situ and different types of invasive cancer. In their studies, the indication 'left' to the reference point, means distal to the reference point, i.e. in an ectocervical direction, while right corresponds with proximal, that is in an endocervical direction (see Fig. II.3).

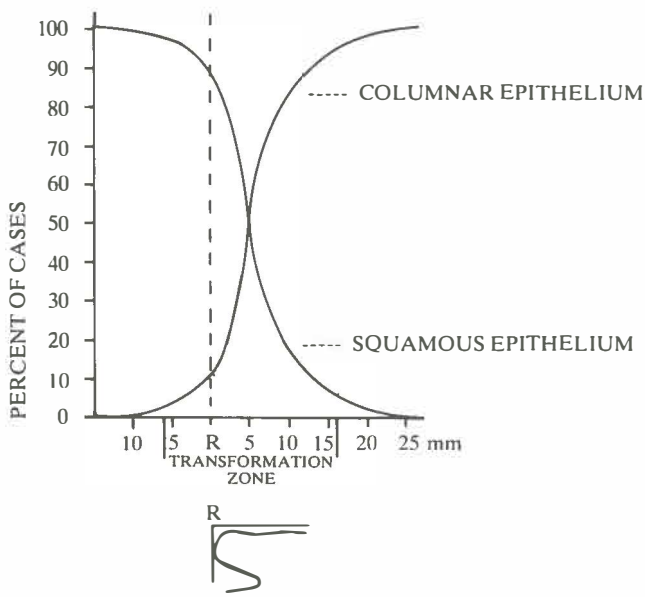

Figure II.3 The distribution of the stratified squamous and simple columnar epithelium in relation to the reference point and the extent of the transformation zone in 140 normal cervices (Modified from Reagan and Patten 1962).

It was evident that the exact site at which the conversion from columnar epithelium to stratified squamous epithelium occurred, was subjected to considerable variation. The transformation zone, a bandlike area near the junction was situated in 95% of 140 normal cervices, within a zone from 6 mm to the left 16 mm to the right of the reference point. The center of the transformation zone was located 5 mm to the right of the reference point, in the surrounding of the external os. In 50% of the cases it was covered by stratified epithelium and in an equal number covered by columnar epithelium (figure II.3).

They found that reactions such as dysplasia, carcinoma in situ and changes associated with immature squamous metaplasia and reserve cell hyperplasia were related to the transformation zone. This is in agreement with others who stressed that CIN occurs in areas of transformation on the portio (Richart 1968, Coppleson and Reid 1967). In one third of the 90 patients with dysplasia, the dysplastic reaction occurred to the left of the reference point, corresponding to the localization of mature stratified squamous epithelium. The remaining two thirds were located at the right side of this arbitrary point. The localization of one half of the latter corresponded with the distribution of mature stratified epithelium and the other half with immature squamous metaplasia.

Maximum dysplastic involvement occurred at the center of the transformation zone, 5 mm to the right of the reference point, corresponding to the approximate location of the external os (figure II.4).

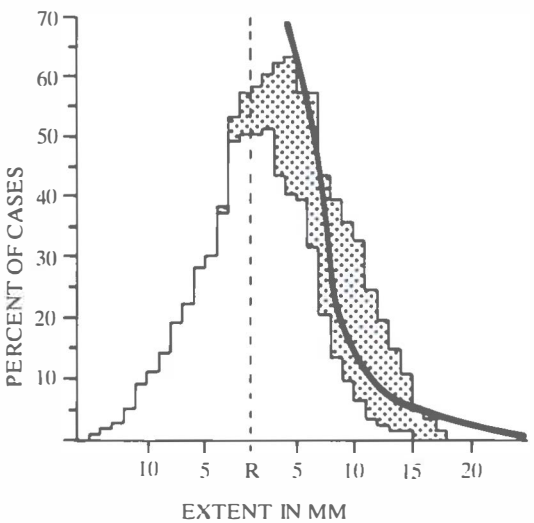

Figure II.4 The distribution of dysplasia of the cervix in relation to the reference point. Dysplasia related to mature stratified squamous epithelium is depicted by the unhatched area. The hatched component represents dysplasia involving metaplastic epithelium (Modified from Patten 1969).

In 98% of the 102 examined cervices the carcinoma in situ was situated to the right of the reference point in relation to the endocervical canal and comparable to the distribution observed for immature squamous metaplasia and reserve cell hyperplasia, which in turn were related to the columnar epithelium area (see figure II.5).

Moreover, there proved to be two histological types of carcinoma in situ: a large and a small cell type. The former was most commonly observed. In general, the large cell type was located distally to the location of the small one. The same could be observed for the immature squamous metaplasia and the reserve cell hyperplasia. Therefore the distribution of the large cell in situ carcinoma variant corresponded to immature squamous metaplasia and the small one to reserve cell hyperplasia.

Carcinoma in situ, in the area of immature squamous metaplasia near the transformation zone, partly overlapped the distribution of dysplasia (see figure II.5). If dysplasia and carcinoma in situ coexisted in the uterine cervix the latter was usually situated more proximally.

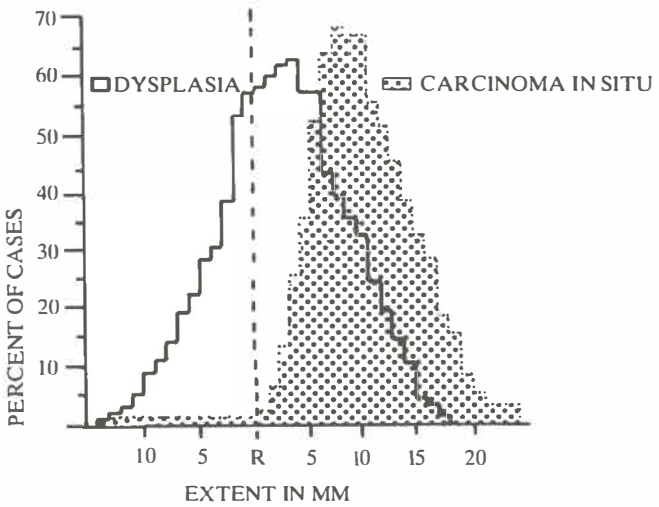

Figure II.5 The over-all distribution of dysplasia of the cervix as compared to the distribution of carcinoma in situ. The latter is depicted by the hatched distribution. (Modified from Reagan and Patten 1962).

The distribution of the invasive carcinomas was of interest. The keratinizing cancers were found within the distribution area of the keratinizing dysplastic reaction, the large cell non-keratinizing cancers in the same area as the large cell carcinoma in situ.

Unfortunately in the study of Reagan and Patten (1962) no small cell malignant tumours of limited extent were present for study. When advanced, their localization indicates that these tumours are located more proximally in the cervical canal comparable with reserve cell hyperplasia.

In the same detailed study Reagan and Patten (1962) supported their hypothesis by analyzing 200 cervical neoplasms in relation to the overlying epithelium. In 80% of the keratinizing cancers the overlying epithelium demonstrated a dysplastic reaction. In large-cell non-keratinizing tumours, a dysplastic reaction was overlying in 16.3% and a carcinoma in situ in 83.7%. Of the small cell tumours, 98% was encompassed by in situ carcinoma and only one case by dysplasia (table II.1).

Table II.1
The surface epithelium in relation to 200 cervical neoplasms (Reagan and Patten 1962).

type of tumour	no. of patients	overlying surface epithelium dysplasia	ca. in situ
keratinizing	55	54 (98%)	1 (2%)
non-keratinizing			
-large cell	104	17 (16%)	87 (84%)
-small cell	41	1 (2%)	40 (98%)

Furthermore they found an average age of 40.22 ± 13.45 years for the patients with keratinizing tumours and 46.46 ± 14.81 years for patients with large cell non-

keratinizing tumours. The difference in average age between these two groups of patients runs parallel with the difference in average age between patients with dysplasia and with carcinoma in situ. Wentz (1966), who found more or less the same results as Reagan and Patten, demonstrated that the overlying surface epithelium in large-cell non-keratinizing tumours merely was associated with dysplasia and not with in situ carcinoma as found by Reagan and Patten (1962). Wentz (1966) has utilized this evidence to support his hypothesis that non-keratinizing cancers originate from a metaplastic type dysplasia. This suggests that morphological differences found in this type of dysplasia are determined by the type of cell in which the dedifferentiation has occurred (Vooys 1975).

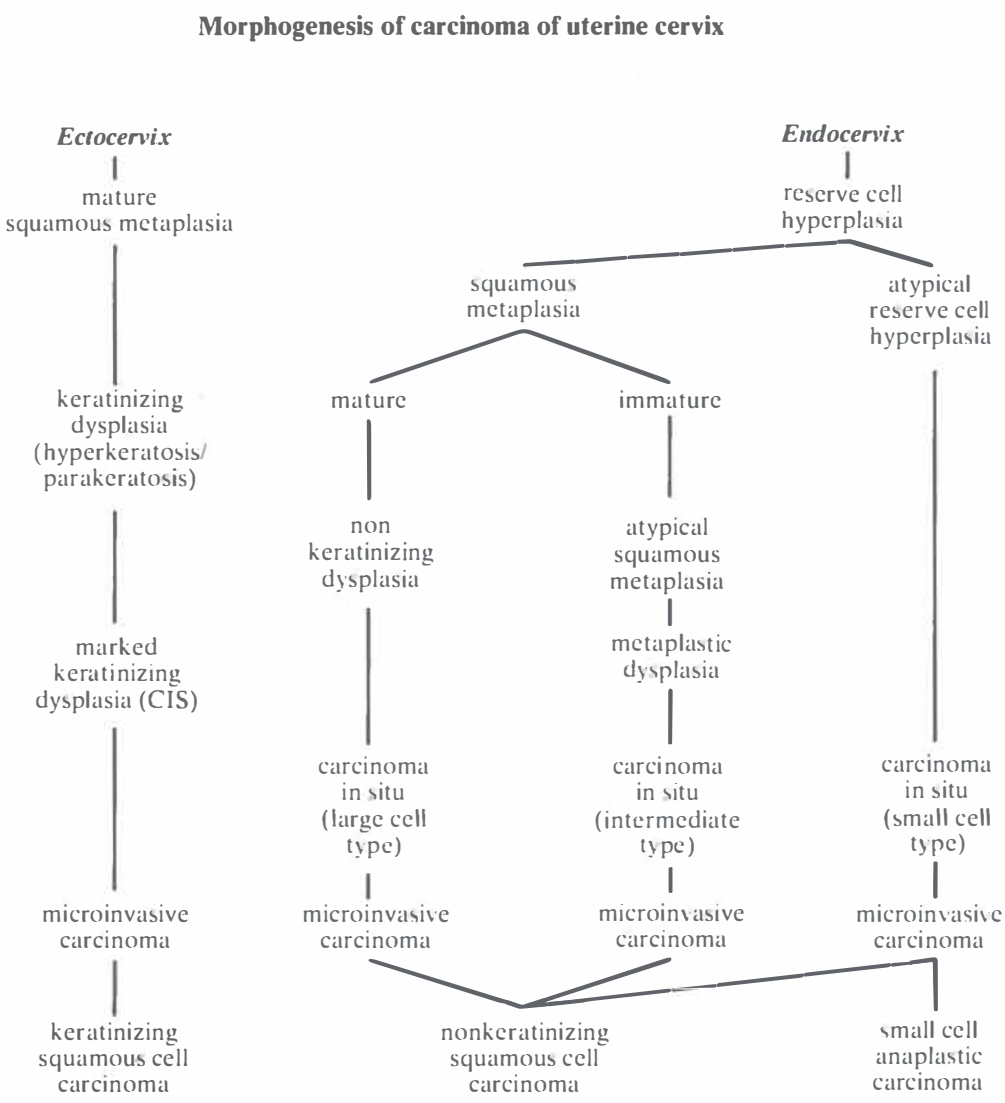

Figure II.6 Mechanisms of morphogenesis of invasive carcinoma of the uterine cervix. (From Patten 1976).

Patten (1969) stated that this surface reaction might be better described by the term atypical squamous metaplasia. In figure II.6 the various mechanisms of morphogenesis of invasive cervical cancer are summarized.

Burghardt (1972) used the last cervical gland as a reference point in topographic studies. He found the same distribution of the premalignant lesions as Reagan and Patten (1962). Dysplasia was located peripherally of carcinoma in situ and the more differentiated carcinoma in situ more peripherally than the lower differentiated types.

Moreover Burghardt and Holzer (1972) stated that the localization of pathological epithelium of various differentiation depends on the type of matrix on which the atypia develops. Epithelial lesions peripherally to the glandular field, probably develop in the original squamous epithelium by means of basal hyperplasia. Lesions proximal to the last gland develop in metaplastic squamous epithelium, or in areas with reserve cell hyperplasia. As a consequence each different type of pathological epithelium is situated in limited fields. As the last gland is the point representing the division border between the original columnar and squamous epithelium, this border will not be transgressed by different types of pathological epithelium. Indeed Burghardt (1983) found this only in 3% of the cases. Based on the localization of dysplasia he concluded that this dysplastic epithelium must not only be considered as a precursor of carcinoma in situ, but that part of it is simply a highly differentiated type of a carcinoma in situ and precursor of highly differentiated squamous cell cancer. Another part of the so-called dysplasia may regress and Burghardt (1983) states that this part represents nothing but unspecific hyperproliferations without any relation to carcinogenesis.

In addition, from the literature there is evidence that invasive cancer originate from squamous epithelium without a prior in situ phase. Ashley (1966) has postulated that there are two biologically different forms of cervical cancer: a slow-growing type, which may be preceded by carcinoma in situ, and a rapidly growing type, which occurs later in life and is not preceded by carcinoma in situ. Bangle et al. (1963) is of the same opinion. He noticed from his carefully performed topographic-histological study in the keratinizing tumors sometimes an almost normal overlying epithelium. From this he concluded that invasive cancer can develop from normal squamous epithelium.

Finally in a model an illustration is given of the probable relationships between the various abnormalities of the cervical epithelium (figure II.7. Modified from Langley and Crompton 1973).

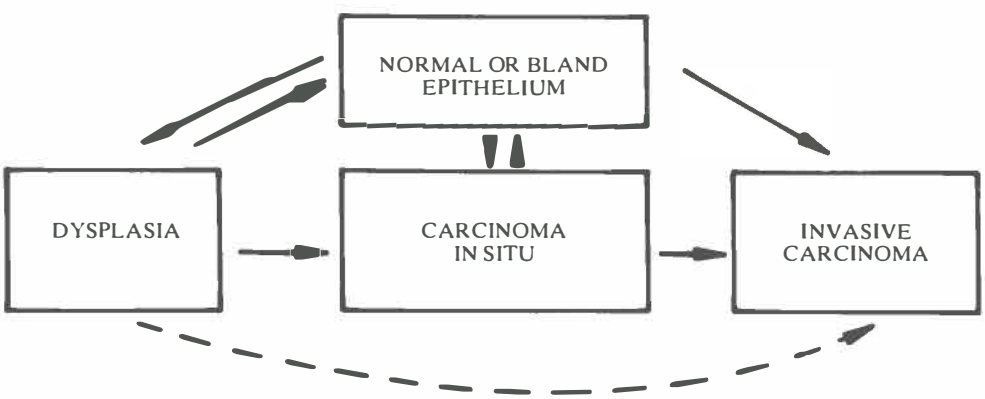

Figure II.7 Model illustrating the probable relationships between the various abnormalities of cervical epithelium. (Modified from: Langley and Crompton 1973).

II.2.3.3. Distribution and extension of CIN

Local tissue destruction as treatment for premalignant cervical disease can only be applied when information is available about its distribution and extension within the cervical canal and on the portio and the extension into the endocervical crypts. On this subject several studies have been performed.

II.2.3.3.1. Surface distribution

Przybora and Plutowa (1959) studied 100 cases of carcinoma in situ lesions and lesions with early invasion. In their analysis they corrected for the effect of tissue shrinkage by technical processes. All lesions were related to the region of the external os, this being their reference point (see figure II.2). In 44% both the portio vaginalis and the endocervical canal had been involved. In 33% the lesion was situated on the portio vaginalis and in 12% into the endocervical canal. In 10% of the cases the lesions were limited to the region of the external os. In one exceptional case, the in situ carcinoma was located in a cervical polyp. With the exception of one patient with carcinoma in situ in the vaginal fornix, the portio, the cervical canal and the uterine cavity, in none of the patients did the cancerous epithelium reach further than 2 cm within the endocervical canal, while 1 cm was exceeded only in 21% of the cases. The vaginal fornices were involved with in situ carcinoma in 5%. Unfortunately Przybora did not refer to the concomitant presence of dysplasia.

Also Gusberg and Moore (1953) and Johnson et al. (1964) only referred to the distribution of in situ lesions. Gusberg found that intraepithelial cancer was related to the squamocolumnar junction in 55%; 8% was on the vaginal portio, but all within ½ cm of the squamocolumnar junction. The rest, i.e. 37% was located in the cervical canal. Only 4% was located more than ½ cm from the squamocolumnar junction. Johnson et al. (1964) reported that in 98%, carcinoma in situ was located completely or partially within the transitional zone. Abdul-Karim et al. (1982) found in a morphometric study that 87.2% of the CIN involved the transformation zone, 9.7% were located higher in the endocervical canal and 3.1% on the ectocervix. Due to an upward retraction of the transformation zone with advancing age, in women over 51 years of age, 28.6% of CIN is present high in the cervical canal. Based on their experience, Gusberg and Moore (1953) point out the important clinical aspects of multicentric origin in some of the intraepithelial cancer lesions. According to Richart (1976) CIN may occur in multiple foci by transition from normal columnar epithelium through squamous metaplasia to CIN. However, he states that all these multiple foci are within the transformation zone.

The anterior lip of the portio is more frequently involved by carcinoma in situ and dysplasia than the posterior lip. Richart (1968) reports that 33% of the carcinoma in situ and 26% of the dysplasia occurred on the anterior lip alone whereas only 11% of the carcinoma in situ and 17% of the dysplasia were on the posterior lip alone. Both lips were involved in 22% of the patients with carcinoma in situ and in 9% of the patients with dysplasia. It was circumoral in 16% of the patients with CIS and in 5% of those with dysplasia.

In an excellent topographic study Reagan and Patten (1962) and Patten (1969) did distinguish between the distribution of dysplasia and carcinoma in situ. As already mentioned previously, 33% of the dysplastic changes were situated in ectocervical direction, while 98% of the in situ carcinomas were found in relation to the endocervical canal. In an overlapping zone, corresponding to the transformation zone, both dysplasia and in situ carcinoma were found.

Burghardt (1972) observed about 21% of the dysplasia proximally within the canal and about 25% on the ectocervix. The remaining 54% was found between it, in the region of the external os and the last gland. The majority of carcinoma in situ (44%) was located within the glandular field of the cervical mucosa in the canal. In 21% it was located on the ectocervix and the rest (35%) between these regions. The in situ carcinoma was mostly located in the upper part of the canal, in contrast to dysplasia

which never involved the upper parts of the canal. On the whole Burghardt found that dysplasia is localized more distally than carcinoma in situ and more differentiated carcinoma in situ more distally than the lower differentiated types. These findings are in keeping with those of Reagan and Patten (1962). On the other hand Abdul-Kamir et al. (1982) dit not find a correlation between the location and the severity of CIN.

In summary the following observations have been made:

- In the majority of cases the distribution of dysplasia and carcinoma in situ are associated with either the transformation zone or the squamocolumnar junction (Gusberg and Moore 1953, Przybora and Plutowa 1959, Reagan and Patten 1962, Jonhson et al. 1964, Abdul-Karim et al. 1982).
- Only in a few cases has in situ carcinoma been found to be isolated from the transformation zone: Gusberg and Moore (1953) 4% in the endocervical region; Johnson et al. (1964) 2% in the ectocervical region; Abdul-Karim et al. (1982) 9.7% in the endocervical region and 3.1% in the ectocervical region. For this reason Gusberg points out the clinical importance of the multicentric origin of the pre-invasive lesion. Richart (1976) however, states that multifocality rarely occurs only within the transformation zone.
- Involvement of the vaginal fornix by pre-invasive lesion was present in a low percentage (Przybora and Plutowa 1959: 5%, Jordan 1976: 3-4%).
- The anterior lip is an area of prediliction for pre-invasive ectocervical disease (Richart 1968).
- Dysplasia is localized towards the ectocervix and more distally than carcinoma in situ, of which the majority is found within the endocervical canal (Reagan and Patten 1962, Burghardt 1972, 1983).
- The more differentiated carcinomas in situ, even the more differentiated pre-invasive lesions are usually located more peripherally than the lesser differentiated types (Reagan and Patten 1962, Burghardt 1972, 1983).

II.2.3.3.2. Linear extension

With the exception of the case in which in situ carcinoma involved the whole cervix and the uterine cavity, Przybora and Plutowa (1959) found that the most extensive carcinoma in situ lesion, although showing early invasion, was spread on the portio over a distance of 3.6 cm and within the canal over 1 cm, thus involving a total of 4.6 cm. The total maximal extension of in situ lesions without invasion was almost the same. The greatest distance within the canal was 2 cm. The least extensive in situ cancers were 0.12 cm and were located in the region of the external os.

Reagan (Reagan and Hicks 1953, Reagan and Patten 1962) reported for dysplasia an average linear extension of 9.02 mm and for carcinoma in situ 6.3 mm. Although

Table II.2: The average linear extension of dysplasia and carcinoma in situ in relation to age. (Modified from: Reagan and Hicks (1953) and Reagan and Patten (1962)

age in years	dysplasia mean	dysplasia S.D.	carcinoma in situ mean	carcinoma in situ S.D.
10-19	6.00	±0.00		
20-29	7.07	±2.99	5.5	unknown
30-39	8.54	±4.64	5.8	unknown
40-49	8.80	±4.01	7.0	unknown
50-59	11.00	±0.32	7.1	unknown
60-69			6.6	unknown
mean	9.02	± 4.37	6.30	± 3.21

Abdul-Karim et al. (1982) only compared the difference in extension between CIN grade I, II and III and did not distinguish dysplasia and carcinoma situ, he found that the extent of the lesion increased with the severity. For CIN grade III the mean linear extent was 7.60 ± 4.32 mm.

As shown in table II.2 (Modified from Reagan and Hicks 1953, Reagan and Patten 1962) the average linear extension increased with advancing age. The smallest lesions were more common in younger patients. This is in accordance with the recent literature (Abdul-Karim et al. 1982).

Although invasion can be found in small in situ lesions, most frequently however, it appears in extensive in situ carcinomas. Contrary to Gusberg and Moore (1953), Przybora and Plutowa (1959) believe that the external os of the portio is the site in which invasion begins most frequently; the cervical canal is the least involved site, which is in agreement with the observation that invasion starts in the majority of cases from the surface growth, not from extension into the glands (Przybora and Plutowa 1959). On the other hand Burghardt and Holzer (1980) reported a case of invasive cancer in which growth started from the base of an involved endocervical gland without having reached the surface of the ectocervix or the endocervical canal.

In summary:

- The mean linear extent of CIN III lesions is ± 7.6 mm (Abdul-Karim et al. 1982).
- With advance in years, there is an increase in the linear extent of the lesion.
- Invasion mostly takes place in extensive lesions.

II.2.3.3.3. Endocervical crypt extension

In Przybora's study crypt involvement with carcinoma in situ was present in 66 of 79 patients (83.5%). Fifteen percent of the patients in the study of Przybora showed only minimal involvement of the crypt, whereas 85% had measurable extension in the crypts. The maximum extension was 5 mm (Przybora and Plutowa 1959), and 95% of the pure in situ carcinoma did not penetrate deeper into the stroma than 3 mm. Reagan and Hicks (1953) reported for carcinoma in situ a mean maximum depth of involvement of 1.6 mm. In the case of dysplasia this was 0.73 mm (Reagan and Patten 1962). In general the crypts were more deeply involved with the undifferentiated type of carcinoma in situ, while more differentiated lesions were located more superficially (Reagan and Hicks 1953).

This is in keeping with Abdul-Karim et al. (1982) who reported a significant difference in the mean depth among different grades of CIN. Moreover there was a significant correlation between the depth of crypt involvement and the linear extent of the lesion. Reagan and Hicks (1953) showed that the maximum depth of involvement with carcinoma in situ, increased with advancing years upto the age of 59; after that age there was a decrease.

This agrees with the work of Anderson and Hartley (1980). They measured the mean depth of the uninvolved crypts and those involved with CIN grade III. In 88,6% crypt involvement was found. This percentage did not appear to depend on age. From a mean depth of involvement of 0.64 mm in the 15-20 year group there was a gradual increase in depth of crypt involvement to a mean value of 1.61 mm in the 46-50 year age-group, decreasing after the age of 50. Of all patients the overall mean maximum depth of the crypts, involved with CIN grade III amounted to 1.24 mm. The maximum depth was 5.22 mm (table II.3). The mean maximum depth of uninvolved crypts was 3.38 mm with also evidence of increasing depth up to 50 years and a decrease after that age (Anderson and Hartley 1980).

Abdul-Karim et al. (1982) also found an increase in crypt involvement with increasing age. He reported a mean depth of CIN III crypt involvement of 1.35 mm. The mean depth of the normal, uninvolved crypts proved to be 2.94 mm. A summary of these literature data about the depth of uninvolved and involved crypts is collected in table II.3.

Table II.3: Depth of crypts and crypt involvement by CIN grade III

author	uninvolved crypts		involved crypts		
	mean maximum depth (mm)	maximum depth (mm)	type of premalig-nancy	mean maximum depth of involve-ment (mm)	maximum depth of involve-ment (mm)
Reagan and Hicks (1953)	not recorded	not recorded	CIS	1.6	not recorded
Przybora and Plutowa (1959)	not recorded	not recorded	CIS	not recorded	5.0
Reagan and Patten (1962)	not recorded	not recorded	SD	0.73	not recorded
Anderson and Hartley (1980)	3.38	7.83	CIN III	1.24	5.22
Abdul-Karim et al. (1982)	2.94	not recorded	CIN III	1.35	not recorded

II.2.4. Epidemiology

The number of young patients with CIN has increased during the last decade (Dillon et al. 1981, Jordan 1982). From 1975-1980, a period of 5 years, 22% of the patients referred to the Colposcopy Clinic of the Johns Hopkins Hospital for evaluation of abnormal cytology, were under the age of 20 (Dillon et al. 1981).

From 1969 to 1972 only one patient under 20 years of age was referred for the same reason (Thompson et al. 1972). This extreme increase in the number of young patients with CIN is also in agreement with Feldman et al. (1978), who reported that in a period from 1974-1977, 16% of the patients with abnormal smears, referred to them were teenagers. Twenty percent of those had severe dysplasia or carcinoma in situ. Grönroos et al. (1980) found the same for dysplasia. In 1968-1969 4.7% of all cases of dysplasia were 19 years or younger. In the years 1976-1977 this percentage was gradually increased to 39.3%. From the Walton Report of the cervical cancer screening programs in Canada (1976), it can be seen that the incidence of in situ carcinoma peaks in the age group 25 to 29 years and gradually declines to nearly zero at older ages. In the youngest age group, 20 to 24 years, there is already an appreciable incidence. The report states that this peak in incidence at an earlier age is an expression of the changing characteristics of the disease in the population. Jordan (1982) states that the number of patients presenting with CIN has more than doubled in the last decade and many of these patients are young. For the Dutch population, Van Lent observed the same in the gynaecologic department of the University Hospital in Leiden (Van Lent 1979).

From several reports it is evident that sexual intercourse at an early age is a risk factor for developing cervical intraepithelial neoplasia. This variable is highly correlated with multiple sexual partners, early marriage, age of childbearing, parity, veneral infections, prostitution, low social-economic circumstances etc. (Rotkin 1962, 1973, Christopherson and Parker 1965, Rawls et al. 1976, Feldman et al. 1978, Van Lent 1979, Grönroos et al. 1980, Dillon et al. 1981, Singer 1982).

Although the increase of incidence of CIN in young patients can be explained by a more intensive screening program, it remains questionable whether the altered pattern of sexual and reproductive behaviour in the last decades has not also contributed to it (Henson and Tarone 1977, Macgregor 1978, Dillon et al. 1981).

In this context the role of viral infections must be considered. There is evidence that

Human Papillomavirus (HPV) infections may have an etiologic role in the cervical cancers. It appeared that 80 to 90 percent of dysplasias of all grades and cervical carcinomas contain HPV DNA sequences. Molecular hybridization studies have revealed that milder forms of dysplasia contain a large number of HPV types (e.g. types 6, 11 but also types 16, 18 and 31). In contrast only types 16, 18 and 31 have so far been identified in severe dysplasia, CIS, and invasive cancers. These data suggest that the oncogenic potential of the dysplastic lesions depends on the HPV type and that mild dysplasia containing 'low-risk' HPV types (6 and 11) have little or no malignant potential, whereas mild dysplasia containing 'high-risk' types (16 and 31) may be pre-malignant (Brescia et al. 1986).

The results of the study of Eglin et al. (1982) support also an association between Herpes Simplex Virus-type 2 (HSV-2) and cervical squamous carcinoma. They demonstrated, by in situ hybridisation, HSV-2 specific RNA in a high proportion of such lesions. Although there are a few studies which have not found a significant association between HSV-2 and cervical cancer, the majority of sero-epidemiological surveys report a higher prevalence or titre of antibody to this virus-type in women with preinvasive and invasive carcinoma of the cervix (Skinner et al. 1982).

In conclusion, neoplastic transformation is probably determined by specific viral infections, but, in addition, requires initiation by some other carcinogenic stimulus.

II.2.5. Diagnosis

The rapid increase in the number of patients having CIN was the main reason to replace traditional methods of treating CIN, such as cone biopsy or hysterectomy, by methods of local destruction, because hysterectomy is overtreatment for most patients and this is also true in many cases of cone biopsy. Moreover, in selected cases, colposcopically directed biopsy and endocervical curettage (ECC) has allowed us to eliminate cone biopsy as a diagnostic procedure. Many studies have been performed about the reliability of the colposcopically directed biopsy and the ECC in diagnosing disease of the uterine cervix.

Studies comparing the histology of colposcopically directed biopsies and the histology in the cone or hysterectomy specimen, revealed that in about 75-85% the morphology was the same (Van Lent 1979: 82.1%, Jafari and Sansguiri 1978: 73.5%, Verschoof 1975: 86.4%). In about 12.5% the pathology in the subsequent surgical cone or hysterectomy specimen showed more advanced disease than in the colposcopically directed biopsies (Van Lent 1979).

These figures apply only to cases in which the endocervical canal is free of neoplastic disease. In case the endocervical curettage (ECC) is positive and the colposcopy unsatisfactory, the correlation between these diagnostic methods drops to about 50-60% and frequently more severe abnormality, even invasive carcinoma, is found in the surgical specimen (Jafari and Sansguiri 1978, Van Lent 1979). The ECC was thought to be an integral part of the evaluation of premalignant disease of the uterine cervix (Townsend et al. 1970, Shingleton et al. 1976, White et al. 1976). Recently, however, several authors have questioned the necessity of an ECC as a routine procedure during colposcopy (Talebian et al. 1977, Swan 1979, Javaheri and Fejgin 1980, Rochelson and Krumholz 1983). They state that elimination of the endocervical curettage does not decrease the diagnostic accuracy in patients who have a satisfactory colposcopic examination and is not necessary in patients who have unsatisfactory examinations because those patients should undergo diagnostic conization. On the other hand, by several authors the ECC is still considered important to establish a more accurate outpatient diagnosis and avoid missing an invasive cancer (Townsend et al. 1981, Drescher et al. 1983, Hatch et al. 1985, Kwikkel 1985a). Urcuyo et al. (1977) concluded that the endocervical curettage was unnecessary in satisfactory colposcopical examinations, but helped to prevent underdiagnosis of invasive cancer in unsatisfactory examinations. The latter view is supported by Hatch et al. (1985), by stating that it is not only helpful in patients with lesions which are not totally visible, but most of all in patients with no observable lesions.

In conclusion, if colposcopy is satisfactory, the accuracy of these above quoted diagnostic methods is as good as cervical conization. It follows that if a local destructive method is considered in the treatment of CIN, it is imperative to observe the following criteria:

- The patient should be seen and assessed by an expert colposcopist.
- The colposcopist should be able to see the entire area at risk, i.e. the transformation zone.
- Invasive carcinoma must be excluded by colposcopically directed biopsy.
- The local destructive therapy must be applied by the colposcopist himself.
- There must be a good cytological and colposcopical follow-up.

On the basis of the review of the literature it is for the time being highly advisable to carry out an endocervical curettage. But it must be realized that the final diagnosis is only partly reached by these clinical, diagnostic methods. The final diagnosis is based on a histopathological examination. Although it is well-known that there is a considerable intra- and inter-observer variation with respect to the histological diagnosis.

It would be of great importance to be able to distinguish between progressive and non-progressive CIN-lesions or, in other words, between preneoplastic abnormalities, the real precursors and between the lesions which are only an expression of infection or another kind of epithelium disturbance. Only the real precursors are at risk and need an intensive evaluation and treatment. Spriggs and Boddington (1980) showed that cases with poorly differentiated cells in the smear more frequently progress. Fu et al. (1982) stated that the progressive and non-progressive lesions are indistinguishable by morphological criteria. In contrast, by measuring the nuclear and cytoplasmic cell area and nuclear cytoplasmic area ratio, Boon et al. (1985) were able to subclassify morphologically the smears in two groups. A group with immature and a group with mature dysplastic cells and on the basis of this morphometric classification a prediction could be made about the progressive and regressive properties. Nasiell et al. (1979) analysed in a microspectrometer the DNA-distribution pattern in patients with moderate cervical dysplasia. He did not find a significant difference between lesions which regressed to normality and lesions which progressed to in situ carcinoma.

Fu et al. (1981) however, also using microspectrophotometric determinations showed that euploid or polyploid lesions are more likely to regress (91%) and only 9% of those lesions persisted. Of the aneuploid lesions 81% persisted as CIN, 12% progressed to invasive carcinoma and only 7% regressed. The presence of abnormal mitosis was the most reliable histologic criterion for aneuploidy. This was supported by his finding that all ordinary cervical condylomas without atypia, had a diploid or polyploid nuclear DNA-distribution, while 45% of the dysplastic atypical condylomata were aneuploid lesions and 55% diploid or polyploid (Fu et al. 1982).

In contrast to cells containing a diploid number of chromosomes, all polyploid and aneuploid cellular population contain atypical cells. However, even a minimally aneuploid lesion produces significant cytological atypia. In general the higher the degree of polyploidy and the higher the degree of deviation in aneuploidy, the greater the cytological atypia (Richart et al. 1981).

In conclusion, most cervical cancer precursors have an abnormal chromosomal content and nuclear DNA-distribution. Although some polyploid lesions might be precursors as well, the majority of them appear not to progress. Therefore, the best basis to select the progressive lesions, is to prove the presence of aneuploidy by cytophotometric analysis.

II.2.6. Treatment

For many years, hysterectomy with or without a vaginal cuff and surgical excision by cone biopsy has been the treatment for CIN. These traditional methods give excellent results (Van Lent 1979). However, hysterectomy is overtreatment for most patients

with CIN, particularly for young women for whom it is important that fertility is preserved. Although the literature is not unanimous, there is evidence that conization is also attended by complications and morbidity e.g.: haemorrhage, stenosis of the cervix, fertility disturbance and cervical incompetence in subsequent pregnancies (McLaren et al. 1974, Jones et al. 1979, Van Lent 1979, Larsson 1983, De Blok et al. 1985). That implies that even cone biopsy is an overtreatment for young women with a desire for subsequent pregnancies.

Therefore if the pretreatment diagnostic criteria have been fulfilled, particularly in that group of young patients, the therapeutic cone biopsy should be replaced by destructive treatment methods.

For the sake of safety and completeness, the contraindications to apply local destruction of CIN are summarized once more:

- The neo-squamocolumnar junction and the pre-invasive lesion is not completely visible and is extending into the endocervical canal.
- The size of the pre-invasive lesion is too extensive.
- The biopsy of the cervix reveals:
  micro- or macroinvasion;
  adenocarcinoma (in situ).
- The endocervical curettage indicates any neoplastic disease.
- Discrepancy exists between cytological, colposcopical and histological diagnosis. In that case the origin of this discrepancy should be traced by repeated colposcopy.

Tissue destruction can be obtained in various ways:

- Diathermic electrocoagulation.
- Cold coagulation (Semm).
- Infra-red coagulation.
- Laser vaporization.
- Cryocoagulation.

Cartier (1977) has used the diathermic loop in the management of the pre-invasive cervical lesions. In the first place it is used diagnostically and deep representative biopsies can be taken with the loop. Secondly it has been successfully used in the treatment of CIN. In contrast to other techniques, diathermy loop excision does not destroy the tissues and offers the possibility to confirm that the lesion has been removed completely and to identify micro- or macro-invasive carcinoma, unrecognised in biopsies.

The results of treatment by all these methods do not differ significantly and each type has its supporters, advocating specific advantages. Chanen and Rome (1983) claimed a primary cure rate of 98% with diathermic electrocoagulation. The mean success rate is about 85% (Berget and Lenstrup 1985). Occasionally the electrocoagulation is complicated by postoperative haemorrhage and infection. A disadvantage of electrocoagulation is that sufficiently deep destruction only can be achieved under general anaesthesia. Moreover, electrocoagulation can cause severe scar tissue reaction on the surface of the cervix, causing the late complication of cervical stenosis, which may result in prolonged menstrual bleeding and severe dysmenorrhea. Duncan (1981) obtained a cure rate of about 94% with the Semm 'cold' coagulator. There is virtually no experience with infra-red coagulation in cases of pre-invasive cervical disease.

Laser vaporization and cryocoagultion are most popular as destructive methods and have been used by many workers for quite a long time. Wright and Davies (1981) compared these two treatment modalities for all types of CIN and found a higher percentage of persistent disease in the cryo-treated (14.5%) than in the laser-treated group (3,1%). Considering CIN grade III only, it is worthy of note that the percentage of failure in the cryo-treated group was much higher than in the laser-treated group (25.0% and 7.7% respectively). A study by Townsend and Richart (1983) yielded opposite results, a 7% failure rate for cryotherapy and a 11% failure rate for laser

therapy. In a randomized study Kwikkel et al. (1985 b) found a difference in success rate in favour of cryotherapy compared with laser (86% versus 71%).

In CIN-lesions measuring less than 3 cm in diameter without extension into the endocervix, Ferenczy (1985) obtained similar low failure rates (4 to 5%), regardless of the histological grade and using laser or cryotherapy. The complication rate of cryotherapy was low compared to laser. In view of these data, he recommended cryotherapy for less than 2.5 cm large CIN-lesions and laser therapy for lesions larger than 3 cm and with limited endocervical extension.

With a depth of vaporization of 5-7 mm, Jordan and Mylotte (1982) obtained a cure rate of 88.4% in CIN III and 91.4% in CIN I, II and III. He stated that the 'laser failures' are caused by inadequate depth of vaporization and poor patient selection. All of this occurred in the early days of his study and at the moment cure rates of about 96-97% can be reached. In literature a mean success rate of ± 90% can be found (Berget and Lenstrup 1985). With laser vaporization pre-invasive lesions can be treated selectively. However, a lot of experience in colposcopy and laser surgery is mandatory. The whole transformation zone is usually treated. Unfortunately tissue for histological examination is lost after laser evaporation. To overcome that problem, a proper cylindrical excision can be performed by laser (Wright et al. 1984).

The main disadvantage of the laser is that its initial cost is very high. Therefore, cryosurgery, because of its efficiency, safety and low cost, is at present more widely used. The mean success rate for cryocoagulation in the treatment of CIN grade III is about 85%, while Bryson et al. (1985) and Trimbos and Van Lent (1982) reported a cure rate of about 93%. In some studies, much lower cure rates were found: Charles and Savages (1980a) 55.6% and Wright and Davies (1981) 75% (table II.4, Berget and Lenstrup 1985). Townsend et al. (1981) and Sevin et al. (1979) reported 66 and 8 cases respectively of patients who were referred to their clinic because of developing invasive cancer after cryosurgery.

Table II.4: Cryosurgical treatment results of grade III cervical intra-epithelial neoplasia (CIN III)

authors	year	type of neoplasia	no. of patients	cure rate
Kaufmann & Irwin	1978	Severe dysplasia	98	82.7%
		Carcinoma in situ	28	78.6%
Popkin et al.	1978	Severe dysplasia	44	96.0%
		Carcinoma in situ	31	91.0%
Charles & Savage	1980a	Severe dysplasia	9	55.6%
Ostergard	1980	Severe dysplasia	28	92.9%
		Carcinoma in situ	18	61.2%
Benedet et al.	1981	Severe dysplasia	136	87.0%
		Carcinoma in situ	229	86.0%
Charles et al.	1981	Severe dysplasia	17	82.4%
		Carcinoma in situ	5	80.0%
Javaheri et al.	1981	Severe dysplasia	45	83.8%
		Carcinoma in situ	15	84.6%
Wright & Davies	1981	CIN III	44	75.0%
Trimbos & Van Lent	1982	CIN III	102	93.0%
Townsend & Richart	1983	CIN III	53	90.0%
Creasman et al.	1984	CIN III	259	82.3%
Arof et al.	1984	CIN III	53	72.9%
Bryson et al.	1985	CIN III	422	92.9%
Kwikkel et al.	1985b	CIN III	12	92.0%

Literature studies reveal that the cause of the failures can be found in the following parts of the procedure:

Positive endocervical curettage (ECC). Kaufmann and Irwin (1978) demonstrated that if the ECC was positive for neoplastic disease, the failure rate for cryocurgery

was 20.8% in contrast to 5.8% in the case when the ECC was negative.

Inadequate colposcopy. Townsend et al. (1981) reported about 66 patients referred to their clinic with invasive cancer following out-patient evaluation and therapy for cervical disease. Thirty-three patients underwent cryosurgery or hot cautery without colposcopy and 17 did not have endocervical curettage. Three patients had a positive ECC, from 8 patients no biopsies were taken and finally, 9 patients had an adenocarcinoma of the cervix. A proper treatment evaluation was carried out in only 5 patients.

Sevin et al. (1979) analyzed 8 patients with invasive cancer of the cervix after cryosurgery. They obtained similar results and concluded that inadequate pretreatment evaluation, poor technique or incomplete follow-up, can lead to poor results.

Location and linear extent of CIN lesion. In paragraph II.2.5. the importance and consequence of a positive endocervical curettage has been discussed. It is a well-known fact that conservative treatment only can be considered if the CIN lesion is entirely visible and the ECC negative. Arof et al. (1984) however, demonstrated that in the presence of endocervical extension, even in case the CIN lesion is entirely visible and the ECC negative, the success rate of cryosurgery drops to 64%. These findings have been confirmed by Creasman et al. (1984).

Cryosurgery treatment failures also tend to occur when the CIN lesion is located in scarred folds on the cervix at 3 and 9 o'clock positions, frequently seen after deliveries (Popkin et al. 1978, Benedet et al. 1981, Monaghan and Townsend 1982, Bryson et al. 1985).

By many authors the size of the lesion was noted to be a factor of great importance in the failure rate of cryocoagulation (Townsend 1979, Townsend and Richart 1983, Walton et al. 1980, Monaghan and Townsend 1982, Arof et al. 1984, Bryson et al. 1985). Townsend (1979) reported a failure rate of 7% for small lesions (smaller than 1 cm), a 14% failure with moderate sized lesions, and a 42% failure rate for the lesions covering most of the ectocervix. Arof et al. (1984) found that for lesions covering one quadrant or less a 99% cure rate was noted. For lesions one to two quadrants in size, there was a cure rate of 93%. When lesions covered three or four quadrants of the cervix, only 61% were cured. Bryson et al. (1985) did not find a significant difference between the cure rate in lesions involving one quadrant or less and those involving more than one quadrant. Nevertheless he did observe that in patients with cryosurgical treatment failures only 20% had small lesions while 80% had large lesions.

Histological grading. Evidence from the more recent literature suggests that the severity of the CIN is a cause of failure in the treatment with cryocoagulation. In different studies, it was found that failure rates in CIS and probably severe dysplasia are greater than for the lower degrees of dysplasia (Kaufmann and Irwin 1978, Popkin et al. 1978, Charles et al. 1981, Benedet et al. 1981, Hatch et al. 1981, Wright and Davies 1981). Hatch et al. (1981) reported for CIN I and II a failure rate of 10% while for CIN III a failure rate of 20% was noted. The results of this study confirmed Ostergard's report (1980). He found a failure rate of 6.3% and 7.5% in CIN grade I and II respectively. In severe dysplasia and carcinoma in situ together there was a failure rate of 19.6%. However, there was a great difference in failure rate between patients having severe dysplasia and carcinoma in situ. In patients classified as having severe dysplasia the failure rate was 7.1%, which was equal to that of patients with CIN grade I and II. In the group with carcinoma in situ the failure rate was 38.8%, based only on 18 of 344 patients evaluated. In spite of that small number Ostergard states that cryosurgery is no longer an appropriate and effective treatment for more advanced CIN III lesions. In Bryson's study (1985) a difference in persistence rate was also found between severe dysplasia (5.2%) and carcinoma in situ (12%). This difference could be explained by the fact that carcinoma in situ had larger lesions, a finding which is in accordance with the supposition that the size of the lesion is related

to histological grade and that increasing severity of dysplasia is attended by larger lesions (Monaghan and Townsend 1982, Creasman et al. 1984, Arof et al. 1984, Bryson et al. 1985).

Endocervical crypt involvement. After cryocoagulation Stafl and Mattingly (1973) found after healing in 25% of the cases small Nabothian cysts or residual gland openings suggesting an insufficient depth of destruction. Sevin et al. (1979) demonstrated that after cryosurgery an invasive carcinoma can develop beneath normal squamous epithelium. Hatch et al. (1981) reported in their study that in the case of extensive crypt involvement, the failure rate was significantly higher than in minimal crypt involvement (38.4% and 20.8% respectively).

This finding confirms suggestions from others that a number of cryosurgical failures may be due to cervical crypt involvement (Charles and Savage 1980a). Savage et al. (1982) convincingly showed a highly significant difference in failure rate between patients with and without glandular involvement (9.3% versus 27.0%), for all degrees of CIN.

Age of the patient. Anderson and Hartley (1980) found that up to the age of fifty, a gradual increase in the depth of the crypts, both involved and uninvolved, occurs. After the age of fifty a decrease is observed. Other investigators did not only find an increase of CIN crypt involvement, but also an increase of the linear surface extension of the CIN lesion with increasing age (Reagan and Hicks 1953, Abdul-Karim et al. 1982).

The study of Einerth (1978) revealed that in young patients the CIN-lesion frequently is situated on the ectocervix and less frequently in the endocervical crypts. It seems logical that local destructive treatment is more suitable for young CIN patients.

Freeze technique. Insufficient freezing can arise from less than ideal equipment, such as lack of temperature gauges to monitor the freezing temperature, lack of suitable probes, incorrect refrigerant, inadequate tank pressure. The pressure within the tank must be adequate for achieving low temperatures for optimal freezing conditions (Disaia and Creasman 1984).

The use of a water-soluble lubricant on the probe provides a better contact between the probe and the cervix, especially in irregular scarred cervices which is common in the parous patient (Ostergard 1980, Monaghan and Townsend 1982, Disaia and Creasman 1984, Bryson et al. 1985).

However, lack of a standardized freezing technique is the main problem in treating CIN by cryosurgery. There is a lack of knowledge about the duration of freezing and the corresponding depth extension of the cryolesion. Crisp (1971) stated that the destruction after cryosurgery can reach a depth of 5-6 mm.

Creasman et al. (1973) using a 3 minute single freeze, obtained a cure rate of 51.5%, while using a double freeze (3-5-3 minutes) the cure rate increased to 81.3%. Charles and Savage (1980a,b) and Charles et al. (1981) observed similar results. With 5 minutes single freeze the cure rate was 53.3%, while 83.7% of the patients were cured after a double freeze of 3-3-3 minutes. On the other hand, Benedet et al. (1981) used a single three minutes freeze and obtained results that were comparable with any double-freeze results, produced to date. Recently several studies have been published in which a significant lower failure rate was achieved after a double freeze, compared with the single freeze (Schantz and Thormann 1984, Creasman et al. 1984, Bryson et al. 1985).

Discussion. Many studies have been carried out to analyze the effect of age, grade of CIN, size of lesion, type of freeze (single versus double) endocervical crypt involvement on the rate of persistent disease. The question is, which factor will be of prognostic significance? Creasman et al. (1984) using multiple logistic regression analysis, stated that freezing techniques and grade of CIN are significant prognostic

factors. The size of the lesion seemed to be an important risk factor. However, evaluation of the grade and size simultaneously, indicates that the grade of the disease is significantly associated with the rate of persistence after adjustment for the size of the lesion.

Creasman et al. (1984) and Arof et al. (1984) also analyzed the association between size and grade of CIN, and found that the larger lesion is significantly more likely to be found with a higher grade of CIN. The observation in many studies that large lesions have a higher persistence rate after cryosurgery, can be explained by the association between the size of the lesion and the grade of CIN. The increase of failure rate with increasing size of the CIN-lesion is due to the higher grade of CIN (Arof et al. 1984, Creasman et al. 1984). These facts are consistent with the results of Bryson et al. (1985) who also used multivariate analysis techniques. Other investigators have also stated that lesion size has little influence on the failure rate (Popkin et al. 1978, Kaufmann and Irwin 1978, Ostergard 1980, Hatch et al. 1981).

On the other hand it has been suggested that the size and the distribution of the lesion are more critical than the severity of the CIN (Townsend 1979, Walton et al. 1980, Monaghan and Townsend 1982, Kwikkel et al. 1985b). These opposite results have been confirmed statistically by Arof et al. (1984). When grade alone was observed there was a significant correlation between severity of CIN lesion and cure rate. When adjustment was made for the size of the lesion no significant contribution to cure rate could be observed for the grade of the CIN lesion. However, comparing lesion size to cure rate, a significant relation was seen regardless of the severity of the CIN.

These divergent results can be explained by the fact that the statistical analysis has been carried out in non randomized, non stratified studies.

The finding in Arof's study that patients with endocervical extension of the CIN lesion show a much lower cryosurgical cure rate than those without endocervical involvement (64% versus 85%) is important. This low success rate was not apparently affected by size and grade of the CIN lesion.

In most of the above mentioned studies the superiority of the double freeze over the single freeze technique in eradicating pre-invasive disease has been stated, although it was not evaluated in a prospective randomized study. Schantz and Thormann (1984) did carry out a prospective randomized study of the efficacy of the single and double freeze techniques in CIN I and II lesions. They found a statistically significant difference in recurrence rate comparing single and double freeze technique treatment (16.4% single freeze versus 6.2% double freeze). The recurrence rate after single freeze was particularly striking in patients with increasing size of the CIN lesion. Conflicting results make it difficult to determine whether the single freeze is truly inferior to the double freeze technique. A long single freeze technique used by Wright for CIN grade I and II, produced similar results to a short double freeze (Wright and Davies 1981).

Bryson et al. (1985) did not find a higher incidence of crypt involvement in the group of patients with persistent disease after cryosurgery than in the original untreated group of patients. For that reason he concluded that endocervical crypt involvement is not a significant risk factor. Therefore he concluded that CIN lesions involving the endocervical crypts do not necessarily remain untreated when freezing has been applied correctly.

Conclusion. The prognostic significance of potential risk factors, i.e. patient selection, age of patient, lesion size, location of disease on the cervix, grade, crypt involvement and type of freeze, should be analysed in a prospective, randomized, stratified study. However, with regard to these prognostic factors in treating CIN with cryosurgery, the following observations can be made:

- Inadequate patient selection leads to irrevocable treatment failures.
- More severe CIN lesions are accompanied by a more extensive linear extension on the ectocervix as well as in the endocervical canal (Monaghan and Townsend 1982,

Abdul-Karim et al. 1982, Creasman et al. 1984, Arof et al. 1984, Bryson et al. 1985).
- More severe CIN lesions are attended by more frequent and deeper endocervical crypt involvement (Reagan and Hicks 1953, Abdul-Karim et al. 1982, Savage et al. 1982).
- With increasing age there is an increase in CIN crypt involvement and crypt depth (Reagan and Hicks 1953, Anderson and Hartley 1980, Abdul-Karim et al. 1982).
- With increasing age, there is an increase in the linear extension of the CIN lesion (Reagan and Hicks 1953, Abdul-Karim et al. 1982).
- Extension of a CIN lesion up into the endocervical canal, even if the lesion is entirely visible and the endocervical curettage negative, results in a lower cryotherapy success rate (Arof et al. 1984).
- The single-freeze technique results in a lower cryotherapy success rate especially with large lesions (Schantz and Thormann 1984).

Summarizing: Cryotherapy for the treatment of CIN is most suitable in young patients with small, less severe CIN I and II lesions. Patients with more advanced disease (CIN grade III) especially those presenting with large size lesions, endocervical extension and crypt involvement, have a higher failure risk following cryosurgical treatment, chiefly when a single-freeze technique has been used.

On the other hand one has to take into account the statement of Bryson et al. (1985): "*The operator may have more influence over persistence rates than do the variables – type of freeze, lesion size, grade and endocervical gland involvement* –".

II.3. Cryosurgery

II.3.1. History and development

The pioneer of refrigeration techniques was James Arnott. Besides the use of refrigeration anesthesia, he also pursued the therapeutic effects of local freezing in the treatment of cancer. In 1845 he employed ice cold salt solutions in special containers and applicators, and achieved local temperatures as low as − 12°F (Tytus 1968, Paloucek et al. 1968).

Cryotherapy was first used in gynaecological cancer by Openchowski (1883), who circulated iced saline through the vagina of a woman with a large pelvic neoplasm. Reduction in tumor size was noted. After a brief period of enthusiasm, the clinical application of local freezing was, for the time being, forgotten. In 1938 Temple Fay, frustrated by the problem of palliation in patients with inoperable cancer, renewed interest in the therapeutic effects of local cooling. His first patient was a woman with intractable pain due to recurrent cervical carcinoma with a rectovesicovaginal fistula. During five weeks he applied a continuous circulation of ice water at a constant temperature of 36°F deep in the vaginal mass. After a short time the patient was and remained pain-free for about five months, while the tumour decreased in size, and histologically local tumour cell degeneration was noticed. Thus encouraged, Fay extended such treatment to other patients with advanced malignancies and in 1938 he subjected his first patient with recurrent and metastatic carcinoma of the breast to hypothermia of 90°F during four days. Initially the patient was pain-free, but died ultimately of her disease. More than 100 cases of total body cooling have been reported by Fay (Tytus 1968, Paloucek et al. 1968). In 1940 Weitzner treated patients with chronic cervicitis by inserting a rod of carbon dioxide directly into the cervical canal and had very good results. Many years later (1961) this treatment method was repeated by Bobrow (Paloucek et al. 1968).

In the same year 1961, Cooper, a neuro-surgeon, produced a break-through in the field of refrigeration technique, by developing a cryosurgical system, using liquid nitrogen as the refrigerant with a temperature potential of − 196°C (Cooper 1965, Paloucek et al. 1968). With this instrument Cahan (1964) described three cases of experimental intrauterine freezing prior to hysterectomy and suggested that this

application method might have value in conception control, abnormal uterine bleeding and in the treatment of certain uterine cancers. In 1965 Cooper himself reported successful palliation with cryosurgery in liver, genito-urinary system, lung, lymph nodes and head and neck tumors. He found that all living tissue subjected to a temperature of −20°C or below for one minute or longer will undergo cryogenic congelation and necrosis. At the same time Cahan (1965) performed experiments with animals and described clinical application of cryosurgery in many benign and malignant conditions including in situ carcinoma of the endocervix.

Using the same equipment as Cooper, but with a modification of the cryo-probe, adapted for the cervix, Collins et al. (1967) presented a report on 100 patients treated for chronic cervicitis. In the following years an increasing number of studies were published about cryosurgical treatment of benign cervical and vulvar disease, such as chronic cervicitis and condyloma acuminatum of the vulva (Ostergard et al. 1969, Ostergard and Townsend 1969, Townsend et al. 1971). In the meantime some authors reported about the cryosurgical palliation of advanced genital disease, such as terminal carcinoma of the cervix and advanced malignancy of the vulva. By means of cryosurgery a decrease in tumor size and control of hemorrhage could be achieved (Crisp et al. 1967, Lash 1972, Wallach 1976).

In the same, above quoted study, Crisp et al. (1967) reported experimental cryotherapy for in situ cancer of the cervix. In the following years many studies have been published about this topic (Crisp et al. 1970, Kaufmann and Conner 1971, Kaufmann et al. 1973. Townsend and Ostergard 1971, Creasman et al. 1973, Tredway et al. 1972, Underwood et al. 1976, Einerth 1978, Popkin et al. 1978). In 1971 a symposium was organized about cryosurgery in the treatment of abnormal cervical lesions. As a result of the increasing use of cryosurgery, there has been significant progress in the development of equipment and techniques.

Two systems of refrigerant instruments, operating basically on a different principle, are available. In the first system cooling takes place by evaporating liquidized gas. Circulating liquid nitrogen passes through interchangeable probes, each one completely vacuum insulated except for the tip. At the tip the liquid nitrogen evaporates and cools the probe. With this instrument a steady temperature of −196°C can be reached. The second system relies on the Joule-Thomson effect, namely, that when a compressed gas, is allowed to expand suddenly through a small aperture, a considerable drop in temperature results. Different types of gas can be used, such as freon, carbon dioxide, and nitrous oxide. The depth of cooling varies with the size of the aperture and the degree of compression of the gas and the resultant rate of expansion. Using nitrous oxide temperatures in the range of −80°C generally can be attained.

II.3.2. Cryobiology

A number of hypotheses have been suggested to answer the question how freezing leads to cell death. Water is the major component of living tissue, and functions as a solvent and as a structural part of proteins and other cellular molecules. These functions can be modified by freezing which results in inducing irreversible dysfunction of the tissue. It has been supposed that the following mechanisms underlie these modifications:

Thermal shock: Slow rates of freezing without ice formation do not damage the cells. However, a rapid fall in temperature above or below zero can be decidedly injurious and is referred to as thermal shock. The injury may be caused by damage to lipoprotein complexes in cell membranes (Lovelock 1957). Not every cell has the same susceptibility to thermal shock (Mazur 1965, Fraser and Gill 1967a).

Crystallization and dehydration: When slow freezing rates are employed, ice crystals form mainly extracellularly. Using rapid freezing rates and lower temperatures extracellular as well as intracellular and even intranuclear ice crystal formation occurs. The intracellular ones are almost uniformely fatal to the cells (Gill and Frazer 1968). However, although cells commonly survive distortion by extracellular ice crystals

forming, they can die after cryo-application by dehydration in the absence of obvious intracellular crystallization. Due to the extracellular crystallization, water is withdrawn from the intracellular compartment, resulting in a disturbance of the intracellular milieu such as dehydration, denaturation of cell proteins, changes in the pH and toxic concentration of electrolytes. The transfer of intracellular water results in collapse of the cells and the cells are further compressed by the growing extracellular mass of ice. Finally as ice formation progresses, the effect on cell viability is catastrophic (Rinfret 1968, Gill and Fraser 1968).

Summarizing it can be stated that if cells are cooled sufficiently slowly, they will dehydrate and will not freeze intracellularly. If they are cooled sufficiently rapidly there is no time for the water to leave the cell and the cells will dehydrate less and will freeze intracellularly. For this phenomenon the surface-to-volume ratio of the cell, i.e. the size of the cell and the permeability of the cell to water, are important biological parameters. The greater the surface-to-volume ratio and the higher the permeability, the higher the cooling rate required to produce intracellular ice (Mazur 1965).

However, it is possible that if cells are cooled very rapidly, the resulting intracellular ice crystals are very small and incomplete, in fact too small to be noxious. In such a case the warming velocity could have a profound effect on survical. If the increase in temperature is slow, the small, unstable crystals may be converted to crystals of damaging size; but if the cells are warmed rapidly, the unstable crystals can melt before they have a chance to grow and to become harmful for the cell. This conversion of smaller crystals to larger ones is termed recrystallization and it also has a lethal effect on the cells (Mazur 1965).

Thawing has another additional effect on injuring the cells. In the thawing phase, especially when it happens slowly, the dehydrated and hyperosmolar cells, with injured leaking cell membranes, reabsorb water from the extracellular space. By way of that rehydration mechanism the cells swell and burst (Farrant 1971).

From several of the above quoted studies it is evident that a rapid freeze and a slow thawing are most lethal to cells. On the other hand Fraser (1975) states conversely that a rapid freeze is most likely to be associated with a proportional survival. Besides the freezing rate, the freezing temperature is also of great importance (Fraser and Gill 1967a). Cooper (1965) found that all living tissue subjected to a temperature of $-20°C$ or below for 1 minute or longer will undergo cryogenic congelation and necrosis. This was supported by others (Stone et al. 1969, Fraser and Gill 1967a, b). Stone et al. (1969) showed that there is an increased lethality to cells when sustained in a critical zone of $-10°C$ to $-40°C$ for two minutes, while freezing to temperatures below this can result in increased cell survival. Stone et al. (1969) emphasized that a double freeze-thaw cycle causes a greater decrease in viability in cancer cells than in benign cells.

Other studies have revealed a difference in resistance against freezing between different cell types. Cancer cells are killed faster than cells derived from skin (Stone et al. 1969). In animal studies, mammary cancers were more responsive to freezing than osteogenic sarcomas (Cahan 1965). Tumour cells cultured in a fluid medium seemed to be more resistant to cold, the same applies to mesenchymal cells (Fraser and Gill 1967a). It is apparent, therefore, that individual cells vary in their ability to withstand thermal stresses. This indicates that the effects of freezing may be unpredictable and quoting Fraser and Gill (1967a):

"The effects of cold may understandably produce different results in the same tumour under differing environmental conditions, in different tumours under similar conditions, and even in different areas of the same tumour under the same external environmental conditions".

Locally in the ice ball the environmental conditions are such, that a small proportion of the cells are able to survive the initial thermal injury. This low level of survival is dependent on the cooling temperature and rate, with more cells being killed at both slower and faster rates, leaving an intermediate rate giving more survival (Farrant 1971). This is obviously not in accordance with the clinical observations of Fraser and

Gill (1967b) who microscopically examined frozen lesions and observed that, in contrast to in vitro conditions, the final picture is that of uniform cell death.

It appears likely, therefore, that there are two phases to the freezing injury. There is an initial phase of direct thermal injury and within a few hours the second phase, namely ischaemic infarction, starts. The hypothesis that cellular anoxia is the ultimate lethal mechanism is supported by histological examination of frozen lesions and the almost complete absence of surviving cells when the tissue is sampled 24 hours after freezing (Gill et al. 1970). More direct evidence is found in circulation experiments reported by Zacarian et al. (1970) who observed that the vessel walls were injured and that within 24 hours after freezing the microcirculation has been arrested due to thrombus formation. The ischaemia caused by capillary obstruction and vascular stasis plays the major role in cellular death and Fraser and Gill (1967b) and others (Cooper 1965, Cahan 1965) regarded the ischaemic infarction as the factor responsible for the completion of cell destruction in the frozen area.

II.3.3. The histological changes after freezing

The earliest changes consist of an area of uniformly altered cells, sharply demarcated from the surrounding tissues. Directly after thawing a vascular dilatation and an intense congestion of the small vessels with erythrocytes can be noticed. Within the injured area, there is evidence of cellular fragmentation and a marked increase of the intercellular spaces. These spaces have been filled with extravasated red blood cells as a result of vascular stasis and damage to vessels. At this stage the cells are shrunken, showing pyknotic nuclei, and the intracellular details are obscured. Shortly after freezing there is a distinct band, some 10-30 cells wide, which separates the frozen from the non-frozen tissue. This line of demarcation can be seen histologically, has a width of 0.1 mm or less and corresponds exactly to the border of the damaged area, where the temperature has dropped to $-15°C$. The cells in this zone are more eosinophilic and have coarser cytoplasmic granularity. In this area the nuclei are smaller, without pyknosis as seen in the frozen area, more basophilic and have an increase in the number and size of chromatin clumps. After 6 hours this intermediate zone is again less distinct and 12 hours later it has disappeared, necrotic cells lying side by side with apparently normal cells.

Later on in the frozen area the changes resemble those seen with an ischaemic infarction, namely the dilatation and sludging progresses to thrombus formation in vessels and finally disintegration of the vessel wall. During the following 24 hours there is a rapidly increasing margination and diapedesis of cells through the vessel walls, building up cellular infiltration. That infiltration is composed mainly of polymorphic granulocytes, but also of lymphocytes and plasma cells and confined primarily to the margin of the lesion (Townsend et al. 1968, Fraser 1975).

II.3.4. Growth and size of the cryolesion

The cryolesion grows around the tip of a cryoprobe till a state of thermodynamic equilibrium is attained between the temperature of the tip and the temperature within the surrounding tissue. In ideal circumstances, that is to say in tissue of uniform composition and thermal characteristics and with a uniform temperature of the environment, the shape of the ultimate cryolesion is spherical.

In the ice ball a thermal gradient exists with a low temperature near the probe, gradually increasing to the freezing point of the tissue as the ice boundary is approached. From there the temperature gradient continues to rise until the ambient temperature of the tissue is reached (Fraser and Gill 1967b, Gill et al. 1970). However, the circumstances are not consistent and a lot of variables play an important part in the size and growth of a cryolesion. That is the main reason why it is difficult to predict the size and extension of a cryolesion and Gill et al. (1970) stated that mathematical equations are impractical for general clinical application. These mathematical models have been set up by some investigators (Walder 1966, Barron 1968, Cooper and

Trezek 1970) and recently Wilchins et al. (1980) started to investigate whether a mathematical model was applicable to a biologic system.

The growth and the ultimate size of the cryolesion is related to a number of variables. Gill et al. (1970) and Fraser (1975) investigated the influence of these variables and the results of those studies will be discussed in more detail. They have demonstrated that there is a direct linear relationship between the size of the probe and the size of the lesion. The same applies to the temperature. The maximum dimensions of a cryolesion is constant for a certain size and temperature of the probe. The relation between size of the cryolesion and duration of freeze is logarithmic and consequently in the first minutes of the freezing procedure the growth rate of the cryolesion is very fast, followed by a decreasing growth rate. Within 15 minutes 80-90% of its maximum diameter has been reached and after that rapid initial growth, it proceeds at a much slower rate, with only small increases in dimension. Finally after a long freezing time (± 2 hours) the ultimate maximum has been reached. The growing rate of the cryolesion in the initial rapid phase will be greater as the cryoprobe temperature is progressively lowered.

However, applications of repetitive freeze-thaw cycles at the same site, seemed to accelerate the freeze effect through the tissue and it produces a gradually increasing volume of frozen tissue with each consecutive freeze. This phenomenon can be explained by an increasing thermal conductivity of tissues previously stressed by a freezing injury (Gill et al. 1968). In their classical study Gill et al. (1968) also clearly demonstrated that with each successive application the additional volume of tissue involved is reduced until, after five to seven applications, a new maximum effect is obtained. The size of this new maximum is determined by the probe and the probe temperature and is almost twice the size of the maximum effect which can be produced by a single freeze of unlimited duration, using the same probe and temperature (Fraser 1975).

One exception was observed by Gill et al. (1968). Repetitive freeze cycles lasting only 1 minute, performed with a probe temperature below −120°C, produce a smaller maximum volume of frozen tissue than a prolonged single application does. The explanation of that fact is that the full freezing effect at lower temperatures is not given sufficient time to express itself when only short cycles of probe application are used. Therefore the effect of repetitive freezing is most effective when a double freeze (freeze-thaw-freeze) technique is used and when cycles are used, each one lasting long enough, to exploit the greater part of the available freezing effect, produced by a certain probe temperature.

The degree of contact between the probe and the surface of the tissue, a variable also studied by Gill et al. (1970), seemed to be important for the size of the cryolesion. A number of additional variables related to the tissue to be frozen, including the density, the thermal conductivity and diffusivity, the osmolarity of the tissue and its ambient temperature could theoretically influence the size and the growth of the cryolesion. Although Gill et al. (1970) have demonstrated that influence, in practice however, the tissues of the human body are relatively constant in their thermal properties and their influence on the performance of a cryoprobe is therefore negligible (Fraser 1975).

However, there are some exceptions. Due to their lower thermal conductivity, skin and fat are isolating tissues and it may be that, due to this, the expansion rate of freezing through these tissues is diminished. It is possible that bone tissue also has exceptional behaviour. Another exception is the presence of a major heat sink such as a large blood vessel. Such a large blood vessel is constantly transporting heat to a specific area and may influence the expansion of the cryolesion at that spot, resulting in an indent in the cryolesion at the site of the vessel. That influence will go on until the vessel and its contents are frozen solid (Fraser 1975).

CHAPTER III

Cervical tissue shrinkage by formaldehyde fixation, paraffin wax embedding, section cutting and mounting*

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III.1. Introduction

With the reintroduction of the colposcope as an aid in the diagnosis of cervical intraepithelial neoplasia (CIN) the various methods for local treatment of these lesions have become more and more important. When using tissue destructive methods, it is necessary that the destruction is extensive enough to eradicate all the pathological cells, particularly those located in the endocervical canal or in the glandular crypts. In the case of cryotherapy, there is a lack of adequate data as to the effective depth of tissue destruction (Charles and Savage 1980b, Singer and Walker 1982). To guarantee that destruction by cryocoagulation is deep enough to embrace the lesions in their entirety, one ought to know the maximum depth of the glandular crypts, the maximum crypt involvement by CIN and the 3-dimensional extension of the cryolesion obtained under standardized conditions. Before using cryosurgery as a treatment for CIN, we decided to investigate these problems morphologically.

The processing of tissue for morphological investigation results in shrinkage, which should be taken into consideration when slides are compared with living tissue, for example in histometric studies (Hopwood 1982). For this reason we decided to investigate the amount of shrinkage caused by tissue processing.

III.2. Material

The cervices of patients who underwent a hysterectomy for benign disease were used for this study. In all cases a benign condition of the cervix was verified by preoperative cytological screening and postoperative histological examination.

III.3. Methods

After hysterectomy the cervices were amputated from the unfixed operation specimens.

The shrinkage caused by the subsequent steps in tissue processing, was measured in several different ways and directions. First the shrinkage caused by fixation was measured, then that caused by dehydration, clearing and paraffin wax embedding and finally that caused by section cutting and mounting.

III.3.1. Shrinkage caused by fixation

The dimensions of the fresh and fixed specimens were compared. We measured the following four dimensions of the amputated specimens before and after fixation for 24 hours in 8% formalin (see Fig. 1).

1. The length of the amputated part of the cervix (the longitudinal diameter = A).
2. The antero-posterior diameter of the portio (the distal antero-posterior diameter = B).

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3. The transverse diameter of the portio, perpendicular to B (the transverse diameter = C).
4. The antero-posterior diameter at the level of amputation (the proximal antero-posterior diameter = D).

In this way 25 cervices were examined and the results before and after fixation were compared to give the percentage of shrinkage by 8% formalin in these different directions. Other types of fixative, such as Methacarn (methanol carnoy mixture), Bouin's fluid (picric acid), sulphosalicylic acid and mercuric chloride solution have also been tested.

III.3.2. Shrinkage caused by dehydration, clearing and paraffin wax embedding

The size of the tissue blocks before and after embedding was compared. For this purpose 25 amputated formalin fixed cervices were sectioned in two ways: (see Fig. 2).

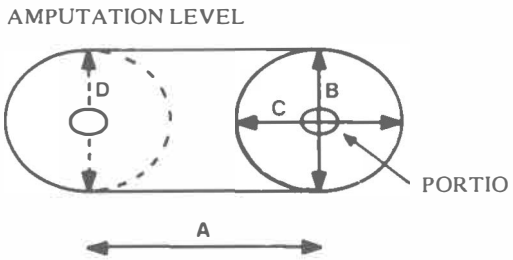


Figure 1: Schematic representation of the measurement directions: longitudinal (A), distal antero-posterior (B), transverse (C) and proximal antero-posterior (D) diameter of the amputated cervix uteri.

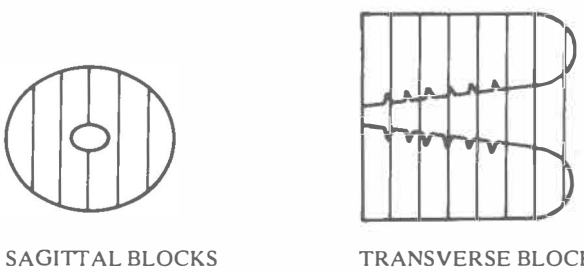


Figure 2: The section direction of the fixed amputated cervix in sagittal and transverse blocks.

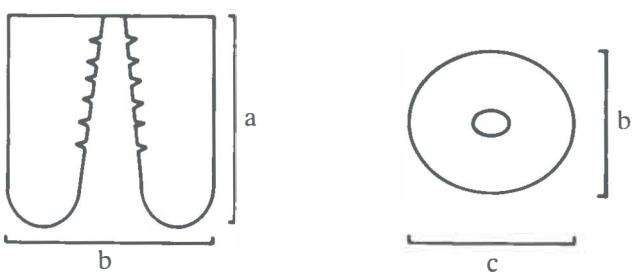


Figure 3: Schematic representation of the measurement directions: longitudinal (a), antero-posterior (b) and transverse (c) diameters of the sagittal and transverse blocks.

1. In sagittal blocks perpendicular to the external os (in the antero-posterior planes) with a thickness of 2.5 mm.
2. In transverse blocks with a thickness of 2.5 mm each.

Life-size photographs of all the slices were made and the greatest diameters were measured in two directions, namely: in the sagittaly cut blocks in the longitudinal and antero-posterior directions, respectively a and b in Fig. 3; and in the transversely cut blocks in the antero-posterior and transverse directions, respectively called b and c (see Fig. 3).

Next, the processes of dehydration, clearing and embedding of the blocks were performed automatically in a histokinette.

Dehydration was carried out with alcohol of increasing strength, beginning with 70% alcohol, then 96% and finally with absolute alcohol. Xylene was used as clearing agent. Xylene fulfils the essential requirement that a clearing agent is miscible with both the dehydration agent and the embedding agent (Gordon 1982). Because of the possible carcinogenic properties of methylbenzoate and benzol, recommended by Burghardt (1972), we prefer to use xylene.

Finally the tissues were embedded in paraffin and blocked out, using the so-called Leuckhardt's 'L' pieces (Gordon 1982). From each tissue block embedded in paraffin the above described dimensions (a, b, c) were measured and compared with those obtained from the photographs. In this way the percentage shrinkage caused by dehydration, clearing and embedding could be determined.

III.3.3. Shrinkage caused by section cutting and mounting

The size of embedded tissue blocks and sections of them were compared. In tissue blocks prepared as described, in longitudinal, antero-posterior and transverse direction, three pairs of recognizable points, 'landmarks', on the circumference of the tissue blocks were pinpointed, allowing measurement in these three directions. After this the sagittal blocks were sectioned in longitudinal direction (from proximal to distal) and the transverse blocks in antero-posterior direction. After cutting and mounting the 'landmarks' could be traced in the slides. Again the distances were measured and the differences with the measurements in the blocks recorded.

III.4. Results

III.4.1. Shrinkage by fixation

In 25 amputated cervices, the measurements described above were made. The median shrinkage by 8% formalin fixation, in both, distal and proximal antero-posterior diameter (B and D, Fig. 1) was found to be zero.

In the longitudinal direction (A, Fig. 1) the median shrinkage was 3.0%, and in the transverse direction (C, Fig. 1) it was 2.4% (see Table 1).

III.4.2. Shrinkage by dehydration, clearing and paraffin wax embedding

Sagittal blocks. To evaluate the influence of columnar epithelium upon the shrinkage, measurements were performed separately in two different groups of sagittal blocks. One group consisted of 51 sagittal blocks, without any columnar epithelium due to the fact that the cutting level was outside the endocervical canal. The other group was composed of 78 sagittal blocks, which were cut through the endocervical canal and, therefore, included columnar epithelium. In each group the percentage of shrinkage was determined in the two directions a and b (longitudinal and antero-posterior directions, Fig. 3).

In the first group the mean shrinkage for a was 12.8 ± 1.2% and for b 13.9 ± 2.4% of the fixed specimen dimensions. In the second group 11.0 ± 1.3% and 15.1 ± 1.3%, respectively (see Table 2).

Transverse blocks. In 113 transverse blocks the shrinkage was measured both in the antero-posterior and transverse directions (b and c respectively in Fig. 3). For b the mean shrinkage proved to be 13.9 ± 2.4%, for c it turned out to be 12.6 ± 2.8% (see Table 2).

III.4.3. Shrinkage by section cutting and mounting

Two hundred and sixty determinations have been performed in sagittal paraffin blocks. In longitudinal direction (= a, see Fig. 3), the cutting direction, there was a mean decrease of the distance between the two points of 2.2 ± 1.8%. In antero-posterior direction (= b, see Fig. 3), perpendicular on the cutting direction a mean increase of 0.4 ± 1.2%. In transverse blocks 32 measurements have been made. This resulted in a mean decrease of 4.3 ± 2.2% in antero-posterior direction (= b, see Fig. 3), the cutting direction, and a mean increase of 3.0 ± 1.5% in transverse direction (= c, see Fig. 3), perpendicular on the cutting direction (see Table 3).

Table 1: Cervical shrinkage by 8% formalin fixation. Median shrinkage and range in different directions in percent of original dimensions

Longitudinal	Distal antero-posterior	Transverse	Proximal antero-posterior
A	B	C	D
3.0% (0-7.5) N = 25	0% (0-3.6) N = 25	2.4% (0-5.3) N = 25	0% (0-6.7) N = 25

N = number of examined specimens

Table 2: Cervical shrinkage by dehydration, clearing and paraffin wax embedding

	Mean shrinkage + standard deviation in different directions in percent of the fixed specimen dimensions					
	Longitudinal	N	Antero-posterior	N	Transverse	N
	a		b		c	
Sagittal						
without columnar epith.	12.8 ± 1.2%	51	13.9 ± 2.4%	51		
with columnar epith.	11.0 ± 1.3%	78	15.1 ± 1.3%	78		
Transverse blocks			13.9 ± 2.4%	113	12.6 ± 2.8%	113
Mean value	11.9%	129	14.3%	242	12.6%	113

N = number of determinations

Table 3: Cervical tissue deformation caused by sectioning, cutting and mounting

	Mean decrease (+) and increase (−) + standard deviation in different directions in percent of the paraffin wax embedded tissue dimensions					
	Longitudinal a	N	Antero-posterior b	N	Transverse c	N
Sagittal blocks	+ 2.2 ± 1.8%	260	− 0.4 ± 1.2%	260		
Transverse blocks			+ 4.3 ± 2.2%	32	− 3.0 ± 1.5%	32

N = number of determinations

III.5. Discussion

From the literature little information is available about the shrinkage of tissues, especially cervical tissue, caused by fixation, processing, embedding, cutting and mounting. Berg (1908) measured the volume of a liver before and after fixation in formaldehyde 4% aq., dehydration with absolute ethanol and embedding in melted paraffin wax. The mean volume after this procedure was 68% of the volume of the fresh organ, that means a shrinkage of 32%. The amount of shrinkage due to fixation alone was only 1%. Przybora and Plutowa (1959) performing a histological study of the topography of carcinoma in situ of the uterine cervix, calculated this shrinkage coefficient by measuring the length of the cervical canal in 15 excised cervices before and after fixation in 10% formalin and paraffin wax embedding. They found a shrinkage of about 20%.

More recently, Anderson and Hartley (1980), who investigated the depth of the crypts and the depth of crypt involvement by CIN, stated that the shrinkage was less than 5% and could be neglected. It is remarkable that they used Bouin's fixation fluid, which contains picric acid, an agent which is generally well known to cause considerable shrinkage of tissue (Hopwood 1982, Berg 1908, Baker 1960). Puchtler et al. (1970) found that Methacarn caused little or no shrinkage. Our experience is that it is difficult to section Methacarn fixed specimens, because of wrinkling of the tissue. This effect may be the result of exposure of tissue to water which is known to cause disastrous effects, such as severe shrinkage artifacts (Puchtler et al. 1970). Also other fixatives tested (Bouin's fluid, sulphosalicylic acid and mercuric chloride solution) caused difficulties in section cutting. As described in the literature (Hopwood 1982) mercuric chloride solution caused excessive shrinkage. Four percent formaldehyde in saline, which is equivalent to 8% formalin, gave the best results.

Specimens fixed for 24 hours in this solution suffered less shrinkage and had a perfect consistency for good sectioning. After 24 hours most of the formalin could be washed out. Prolonged fixation in formaldehyde, however, causes more shrinkage and hardening of tissue (Hopwood 1982).

In our series we did not find an essential difference in the shrinkage caused by formalin fixation in longitudinal and transverse directions (3.0% and 2.4% respectively, see Table 1). It is interesting that the shrinkage by formalin fixation in the antero-posterior direction, both in distal and proximal parts of the cervix was nil, whilst there was undoubtedly shrinkage of the whole cervix. This might be explained by the fact that the shrinkage is associated with a change in shape of the cervix from oval to round so that the resulting increase in antero-posterior diameter cancels out the real decrease produced by shrinkage.

In longitudinal direction, the shrinkage produced by processing and paraffin embedding was 1.8% more in cervical tissue without columnar epithelium as compared to tissue with columnar epithelium. In antero-posterior direction it was 1.2% more in tissue with columnar epithelium (see Table 2). These differences, although statistically

significant, were too small to take into consideration in our morphometric study. Moreover the differences are not consistent in both directions. We concluded that the presence of columnar epithelium does not essentially influence the amount of shrinkage. It follows that the mean antero-posterior shrinkage in sagittally cut blocks is 14.5%. In transversely cut blocks it amounts to 13.9%. The difference of 0.6%, although also significant, is small and neglectable for practical purposes. This implies that the various methods we used, by making measurements of sagittal and transverse blocks, have not altered the results essentially. Based on these observations we feel justified in pooling these results of both groups. Thus the mean shrinkage caused by dehydration, clearing and embedding in the longitudinal direction is 11.9% in 129 determinations, in the antero-posterior direction is 14.3% (N = 242) and in transverse direction is 12.6% (N = 113). Based on the observations that the difference in shrinkage, caused by formalin fixation and paraffin embedding, is small in the various directions, it seems permissible for all practical purposes to use the mean of the alues in the different directions, which results in the following figures:

- the overall mean shrinkage caused by formalin fixation is 2.7% of the fresh specimens;
- the overall mean shrinkage caused by dehydration, clearing and paraffin embedding is 12.9% of the fixed specimen, that is 97,3/100 × 12.9% = 12.6% of the fresh specimen;
- fixation and processing together account for 15.3%.

From 260 determinations in sagittal blocks it was evident that the dimensional alterations of the cervical tissue caused by sectioning and mounting was variable, sometimes an increase and sometimes a decrease in size was found. The decrease in longitudinal dimension corresponded to the cutting direction and the increase in the antero-posterior dimension was perpendicular to this. From this it can be concluded that the alterations are not a process of shrinkage, but actually a deformation of the tissue, caused by the pressure on the tissue during sectioning. To prove this statement we did another 32 determinations in transverse blocks with an antero-posterior cutting direction, the opposite of the procedure in sagittal blocks. Now it was striking that there was a decrease in antero-posterior direction (see Table 3). In sagittal blocks, which will be relied on most in the morphometric analyses, the increase and the concomitant decrease were small and moreover variable, depending on the cutting direction and pressure. Therefore it is reasonable to neglect the influence of sectioning and mounting in the calculation of the total shrinkage percentage of cervical tissue.

We can conclude from our study that if cervical tissue for morphometric studies has been prepared by formalin fixation, dehydration, clearing, paraffin wax embedding, sectioning and mounting, one has to take into account a total shrinkage of about 15% of the fresh specimen.

III.6. Summary

To evaluate the efficacy of cryocoagulation as a treatment for cervical intraepithelial neoplasia (CIN), it is necessary to know the maximum depth of the glandular crypts, the maximum crypt involvement by CIN and the extension of the cryolesion, obtained under standardized conditions. In a morphometric study on this subject, one has to take into account the shrinkage of the cervical tissue, caused by processing the tissue for histological examination.

In the present study, tissue shrinkage of the cervix in different directions was measured in three separate steps. First shrinkage caused by formalin fixation was determined, second shrinkage caused by dehydration, clearing and paraffin wax embedding and finally that caused by section cutting and mounting.

Shrinkage caused by formalin fixation, and by dehydration, clearing and paraffin wax embedding did not differ significantly in the different directions and resulted in

an average shrinkage of respectively 2.7% and 12.6% of the original dimensions. The alterations of the dimensions by section cutting and mounting are not a process of shrinkage, but actually a deformation caused by pressure on the tissue during sectioning. Generally the dimension decreases in the cutting direction and increases in the direction perpendicular to it. In the calculation of the total shrinkage these alterations can be neglected, since the changes, although not consistent, are small.

It follows that in morphometric studies a total shrinkage of about 15% of the original dimensions has to be taken into consideration.

CHAPTER IV

Depth and topography of crypts in the uterine cervix

IV.1. Introduction

Cervical intraepithelial neoplasia involves the cervical crypts in about 85% of patients (Przybora and Plutowa 1959: 83.5% and Anderson and Hartley 1980: 88.6%). Therefore, data concerning the depth and localisation of the cervical crypts are important when selecting patients with CIN, who can be treated safely by cryosurgery.

In this study the depth of the cervical crypts, and the distribution of the deepest crypts in relation to the ectocervix have been investigated. These factors have also been evaluated in relation with a number of variables: age, period of life, parity, portio diameter and size of external os.

IV.2. Material and methods

The material consisted of 172 cervices of patients whose uteri were extirpated for a benign disease. The age of the women ranged from 21 to 80 years; the mean age was 46 years. After extirpation of the uterus, the cervix was amputated and fixed in 8% formalin for 24 hours. From the fixed cervices, tissue blocks were cut at the 3, 6, 9 and 12 o'clock positions. After processing the tissue using standard methods, from each block a single section of 5 μm was cut in sagittal direction, perpendicularly to the mucosal surface.

Sections in which the mucosal lining was absent, were discarded. That was seen exclusively at the 3 and 9 o'clock positions, because at these positions it was not always possible to cut the blocks exactly perpendicularly to the mucosal surface. This resulted in 153 and 138 sections at the 3 and 9 o'clock positions respectively, and 172 sections for both the 6 and 12 o'clock positions (in total: 635 sections). The slides were stained with hematoxylin-eosin.

For topographic studies in which the distribution and localization of the deepest crypts in the cervix are evaluated, an anatomical point of reference is needed (see Chapter II.2.3.1.). Because the most caudal point of the ectocervix can also be recorded clinically, it was taken as a reference point. In the slides this reference point can be traced by drawing a line along the endocervical surface, and perpendicular to it, the tangent of the ectocervix. The point of contact with the ectocervix is the reference point R (see figure IV.1).

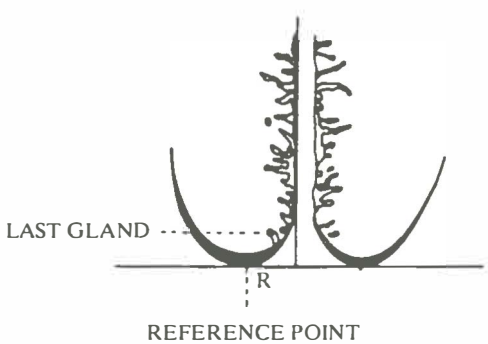


Figure IV.1: Anatomical point of reference (R).

A system was developed to allow quantitative assessment of:

I. The depth of the deepest crypts measured at regular intervals, perpendicular to the ecto- and endocervical surface.
II. The distance of the deepest crypt to the reference point R.
III. The distance of the most distal crypt, the so-called 'last gland' of Burghardt (1972), to the reference point R (see figure IV.2a).

The system for measuring these parameters is shown in figure IV.2b.

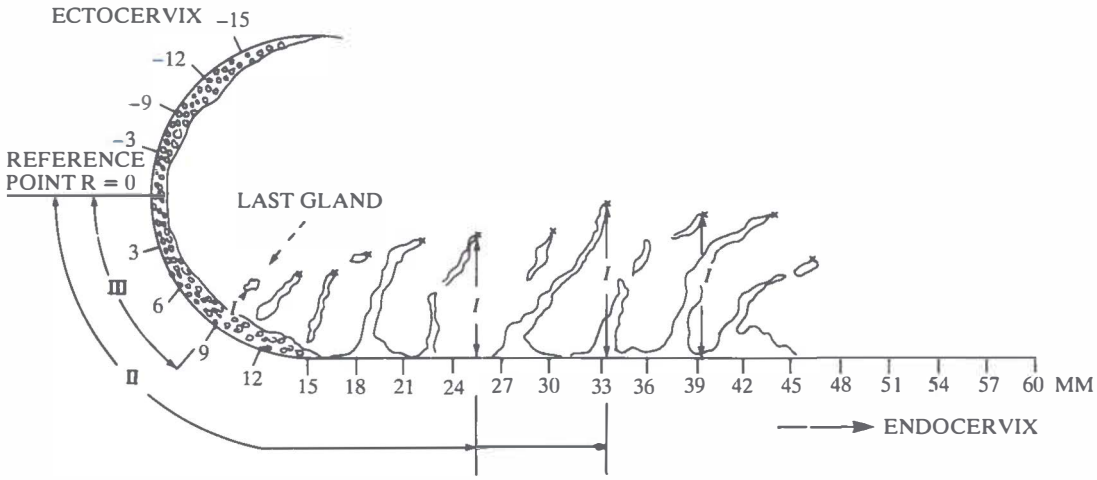


Figure IV.2a: Measurements of depth of crypts in a sagittal slide of the uterine cervix. R = reference point, I = maximum depth of the crypts in different areas, II = distance of deepest crypts to reference point R, III = distance of last gland to reference point R.

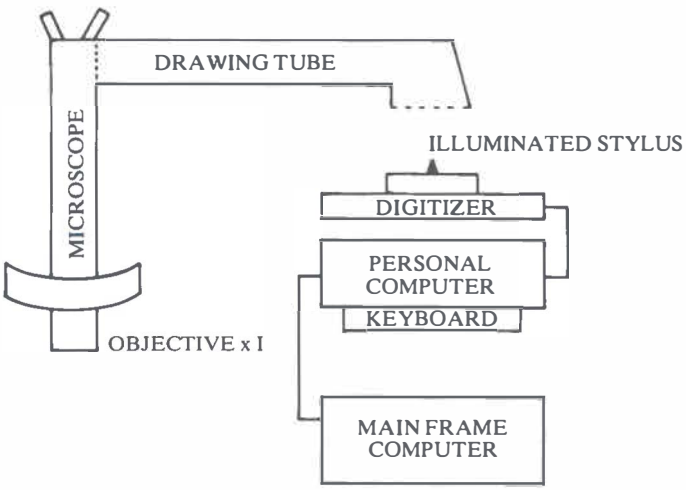


Figure IV.2b: Schematic outline of the measuring-system.

The image of a slide is projected via the drawing tube onto the image of the digitizer at a final magnification of 20 (see figure IV.2b). With a stylus, a line is drawn from the reference point along the ecto- and endocervical surface. The part of the line distal to the reference point is drawn as a line in a negative direction. The x, y-coordinates of the line are stored in the computer memory. For practical purposes the line is arbitrarily divided into equal parts of 3 mm, forming the baselines of 24 consecutive areas in which the deepest crypts have to be measured. Point 0 corresponds to the reference point and four areas are located distally and 20 areas proximally of the reference point, ranging from – 12 mm till + 60 mm. Next the x, y-coordinates of the deepest crypts and the 'last gland' have been obtained by pressing the stylus. The shortest distance between the deepest crypts and the baseline of one of the 24 consecutive areas is established via a computer algorithm and represents the depth of the deepest crypt in each area. The distance to the reference point is measured from the centre of the corresponding 3 mm area (II and III in figure IV.2a). In consequence, the distance of the point where the deepest crypt in a certain area has been measured is a multiple of 3 mm. Clinical date are added via the keyboard. All data are transmitted to a main frame computer where for the entire population or for a selected group of patients the mean maximum depth of the crypts in each area has been calculated. Statistical analysis has been performed using SPSS (Statistical Package for the Social Sciences, Nie 1975). Differences were considered significant when p-value was < 0.05.

If crypts were absent in certain areas they were recorded as missing values. This was most frequently seen in the first and last areas. Therefore, in those areas, especially in populations with small numbers of patients no statistical evaluation could be performed. As already mentioned, if the deepest crypt and the last gland were located distally to the reference point R the values of II and III (fig. IV.2a) were indicated negatively.

Although Nabothian cysts are essentially obstructed endocervical crypts, filled with mucus, they were excluded from the systematic measurements. That is because of the fact that by dilatation these cysts have a large diameter and generally penetrate deep into the cervical stroma. Such extreme depth will influence the mean values of the deepest crypts. However, in order not to neglect those cysts, from each slide with Nabothian cysts the deepest cyst was measured separately.

All results of measurements have been corrected for 15% shrinkage due to processing tissue for histological examination (Boonstra et al. 1983). In order to select groups of patients with CIN grade III, which can be safely treated with cryosurgery, the depth of crypts was measured in relation to variables from which it is conceivable that they may influence the depth of crypts (age, period of life, parity, portio diameter and shape of external os).

IV.3. Results

IV.3.1. Depth of cervical crypts in entire population

Firstly the depth of the deepest crypt in each slide has been classified according to groups with advancing depth, as indicated in table IV.1. The difference in depth in each group is 1 mm. Most frequently (178 slides = 28.0%) the depth of the deepest crypt was between 3 and 4 mm and in 61.7% of the slides the depth was less than 4 mm. The deepest crypt was in 38.3% of the slides deeper than 4 mm and in 16.9% deeper than 5 mm. In three slides no crypts could be observed (Table IV.1.), resulting in a total of 632 evaluable slides (153 at 3 o'clock, 171 at 6 o'clock, 137 at 9 o'clock and 171 at 12 o'clock).

The mean depth of the deepest crypts of the entire population (172 patients and 632 slides), which will be referred to as the overall Mean Maximum Depth (MMD) of all the crypts, was 3.8 ± 1.5 mm. The deepest crypt was 12.4 mm and it was found at the 6 o'clock position. The mean depth of the most distal crypt, the 'last gland' was 1.8 ± 1.1 mm and the deepest observed 'last gland' 7.2 mm (Table IV.2).

Table IV.1: The distribution of the maximum depth of the crypts in the entire population: Absolute (N) and relative (%) frequency

Maximum depth of cryps	N	%	Cumulative %
0 mm (no crypts observed)	3	0.5	0.5
> 0 mm and < 1 mm	5	0.8	1.3
⩾ 1 mm and < 2 mm	51	8.0	9.3
⩾ 2 mm and < 3 mm	155	24.4	33.7
⩾ 3 mm and < 4 mm	178	28.0	61.7
⩾ 4 mm and < 5 mm	136	21.4	83.1
⩾ 5 mm and < 6 mm	54	8.5	91.6
⩾ 6 mm and < 7 mm	35	5.6	97.2
⩾ 7 mm	18	2.8	100.0
	635	100	

Table IV.2: Depth of crypts and 'last gland' (mm) in entire population and at different clock positions (N = number of slides)

clock positions	N	crypts		'last gland'	
		Mean Max Depth ± SD	Max	Mean Depth ± SD	Max
– 3 o'clock	153	3.7 ± 1.4	8.2	2.1 ± 1.2	7.2
– 6 o'clock	171	4.0 ± 1.6	12.4	1.8 ± 1.0	4.8
– 9 o'clock	137	3.6 ± 1.4	10.8	2.0 ± 1.1	6.2
– 12 o'clock	171	3.8 ± 1.5	8.3	1.6 ± 1.0	7.1
total	632	3.8 ± 1.5 mm	12.4	1.8 ± 1.1 mm	7.2

IV.3.2 Depth and distribution of cervical crypts at the 3, 6, 9 and 12 o'clock positions

The overall MMD of the crypts ± SD, with its maximum and the mean depth of the 'last gland' at the different clock positions are shown in table IV.2. For the 3, 6, 9 and 12 o'clock positions the overall MMD ± SD are 3.7 ± 1.4 mm, 4.0 ± 1.6 mm, 3.6 ± 1.4 mm and 3.8 ± 1.5 mm and the maximum depth at each clock position was 8.2 mm, 12.4 mm, 10.8 mm and 8.3 mm respectively.

The mean depth of the most distal crypt at the different clock positions varies between 1.6 mm and 2.1 mm.

In figure IV.3, a diagram shows the MMD of the crypts at various distances from R at different clock positions.

The reference point R corresponds to point zero. The diagram reproduces the values from 3 mm distal up to and including 36 mm proximal from the reference point, as only in those areas a sufficient number of observations at each clock position have been registered.

From these results is can be concluded that the depth of crypts did not differ significantly at the four anatomical clock positions. For that reason, and because of the fact that from the 6 as well as from the 12 o'clock position from each patient one slide is available, it was decided to use the mean results at the 12 o'clock position as being representative for the entire population. At this clock-position, the MMD of the crypts at various distances from R did not exceed 2.7 ± 1.5 mm. The distribution of age, period of life and parity of this 12 o'clock population is summarized in Table IV.3.

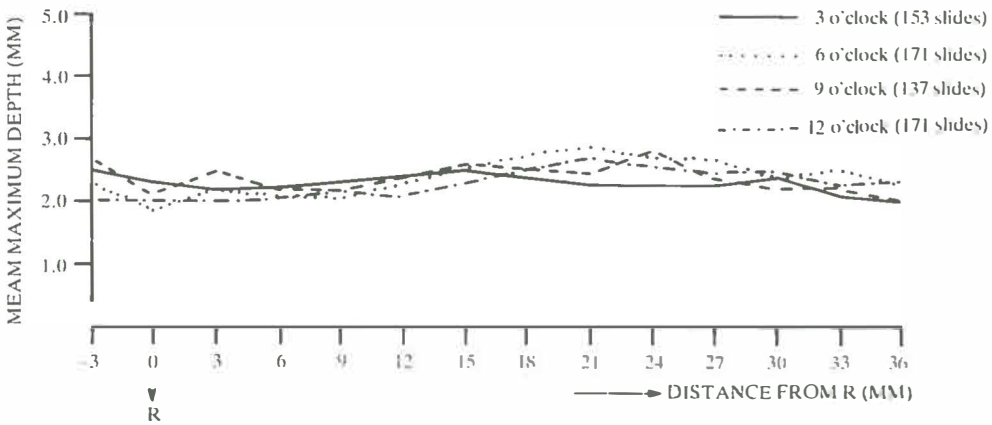


Figure IV.3: Mean Maximum Depth (MMD) of cervical crypts at various distances from the reference point R at 3, 6, 9 and 12 o'clock positions (for documentation: see appendix, table 1).

Table IV.3: Distribution of age, period of life and parity in the investigated women

age	N	%	period of life	N	%	parity	N	%
20-29 yrs	10	5.8	premenopause	135	78.9	0	14	8.2
30-39 yrs	47	27.5				1	11	6.4
40-49 yrs	66	38.6	postmenopause	36	21.1	2	85	49.7
50-59 yrs	26	15.2				3	39	22.8
60-69 yrs	13	7.6				≥4	22	12.9
≥ 70 yrs	9	5.3						
total	171	100	total	171	100	total	171	100

IV.3.3. Age and depth of crypts

To investigate the relation between age and depth of the crypts, the patients were subdivided in age-groups (table IV.3). The reults are summarized in figure IV.4 and IV.5. It appears that in the age-groups 20-29 years, 30-39 years and 40-49 years the mean maximum depth of the crypts does not vary with age and is about 3.8 mm. In the 50-59 years age-group there is an apparent increase to 4.60 mm which is not significant. In contrast a sudden decrease was found after 59 years with a mean maximum depth of 2.46 mm in the age-group over 70 years, being a highly significant difference.

As can be seen (figure IV.4) the mean depth of the last gland is almost the same in the different age-groups.

The results of the MMD at the various distances from R for different age-groups, are not quite reliable, because the number of observations was insufficient for each age-group in most areas. For that reason and also to stress the differences in overall MMD at different age, a comparison was made between age groups under and over 60 years. Under 60 years the overall MMD was 3.95 ± 1.49 mm, while over 60 years 2.65 ± 1.07 mm was found (figure IV.4). This difference is significant ($p < 0.001$).

Figure IV.5 shows the results of the MMD at the various distances from R between these two groups. The graph clearly demonstrates that mainly between 12 and 27 mm from the reference point R, the mean maximum depth of the crypts is less in the age-group over 60 years.

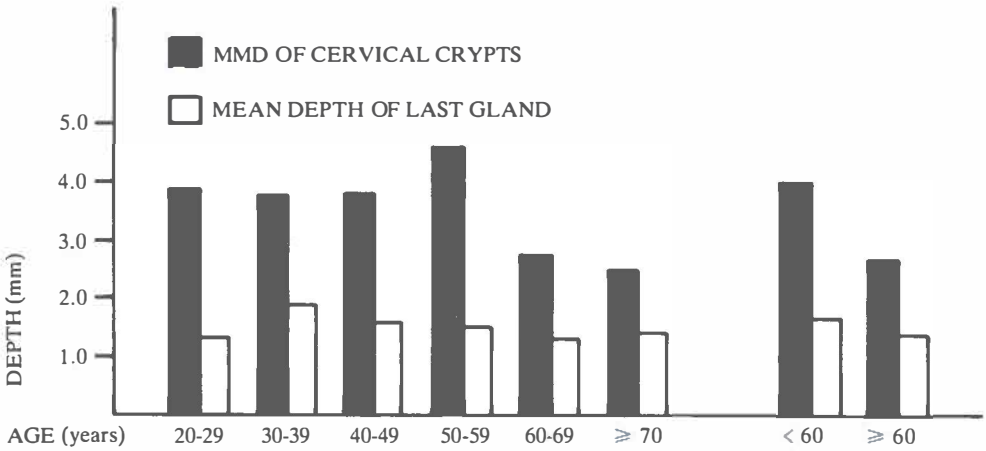


Figure IV.4: Mean Maximum Depth (MMD) of cervical crypts and mean depth of 'last gland' in relation to age (at 12 o'clock position) (for documentation: see appendix table 2).

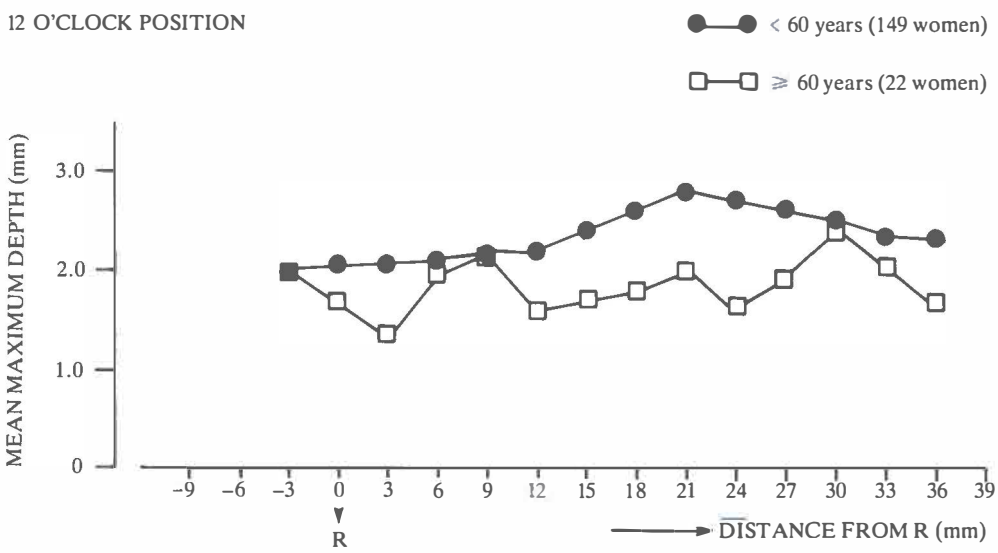


Figure IV.5: Mean Maximum Depth (MMD) of cervical crypts at various distances from the reference point R in relation to age (for documentation: see appendix table 3).

IV.3.4. Period of life and depth of crypts

From the entire population 135 (78.9%) of the patients were premenopausal and 36 (21.1%) postmenopausal at the time of hysterectomy (table IV.3).

Comparing the results of depth of cervical crypts in pre- and postmenopausal women, shown in figure IV.6 and figure IV.7 the same trend as for the different age groups can be observed. The overall MMD in the postmenopausal (older) women is also significantly less than in the premenopausal (younger) ones (3.0 ± 1.1 mm and 4.0 ± 1.5 mm respectively, $p < 0.001$). The mean depth of the last gland for both groups was almost the same (1.4 and 1.7 mm respectively; figure IV.6).

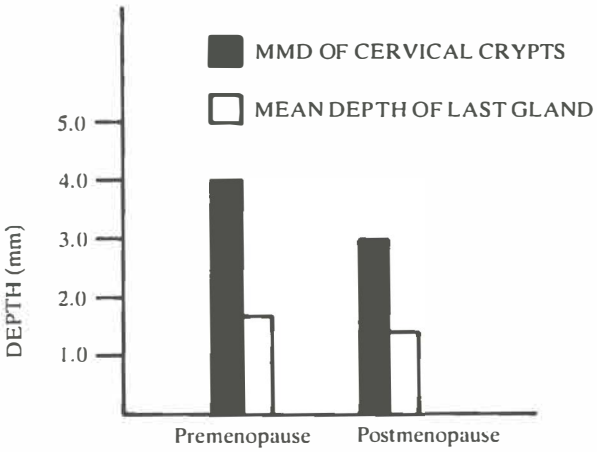


Figure IV.6: Mean Maximum Depth (MMD) of cervical crypts and mean depth of 'last gland' in relation to period of life (12 o'clock) (for documentation: see appendix table 4).

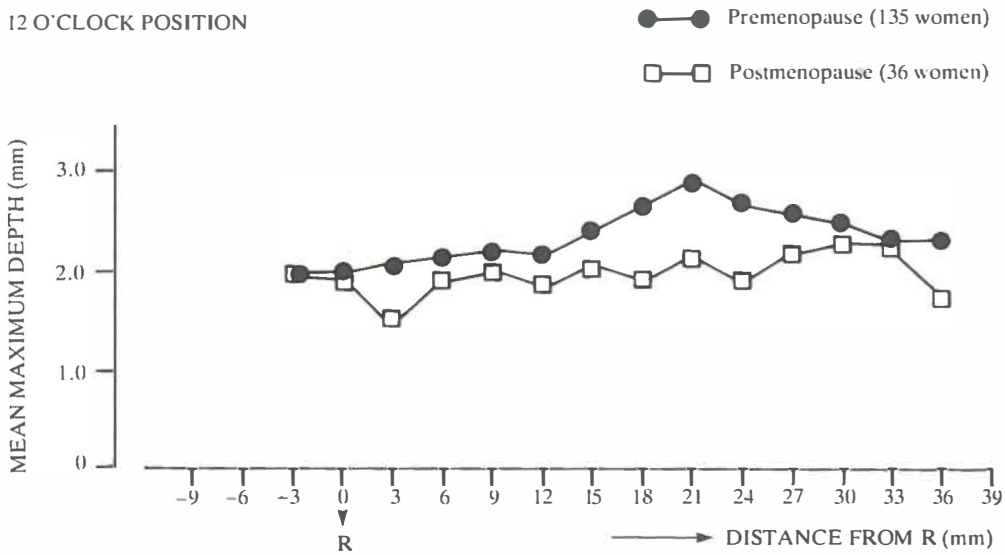


Figure IV.7: Mean Maximum Depth (MMD) of cervical crypts at various distances from the reference point R in relation to period of life (for documentation: see appendix table 5).

IV.3.5. Parity and depth of crypts

Fourteen (8.2%) of the examined women were nulliparous, 11 (6.4%) women had one delivery. The majority of women belonged to the group with two deliveries 85 (49.7%), followed by those with three 39 (22.8%), and 22 (12.9%) with four or more deliveries (table IV.3).

To analyze the relation between parity and depth of crypts, the results in the above

quoted groups are demonstrated in figure IV.8. The overall MMD in the nulliparous group is 3.0 ± 1.0 mm.

With the supposition that the depth of the crypts may be influenced by having a full term pregnancy and subsequent delivery, and that the number of pregnancies and deliveries is not an important factor, the nulliparous group has also been compared with the group of women having one or more deliveries. In that last group the overall MMD is 3.9 ± 1.5 mm (figure IV.8), being 0.9 mm deeper as compared to nulliparous women. Also the last gland is deeper in women with one or more deliveries. Both differences are statistically significant.

From figure IV.9 it can be seen that the MMD of the crypts at the various distances from R also differ in both groups. This figure shows that difference in a diagram, representing the graph of the nulliparous group and the group of one or more deliveries.

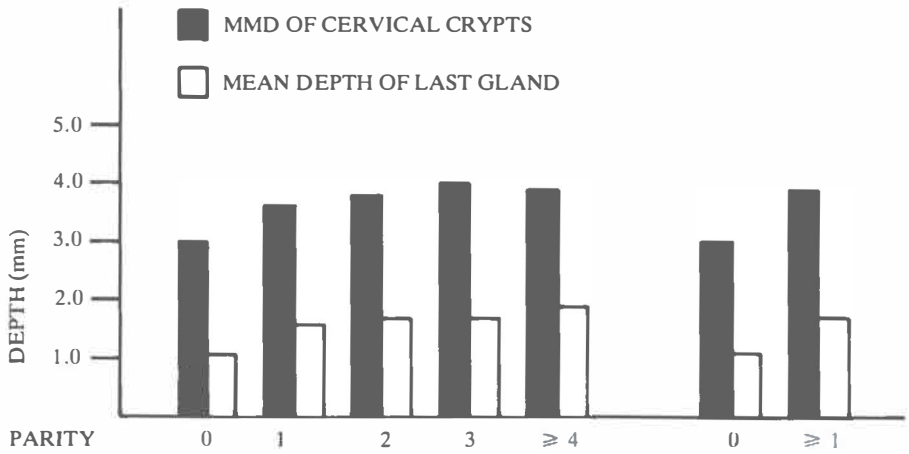


Figure IV.8: Mean Maximum Depth (MMD) of cervical crypts and mean depth of 'last gland' in relation to parity (12 o'clock position) (for documentation: see appendix table 6).

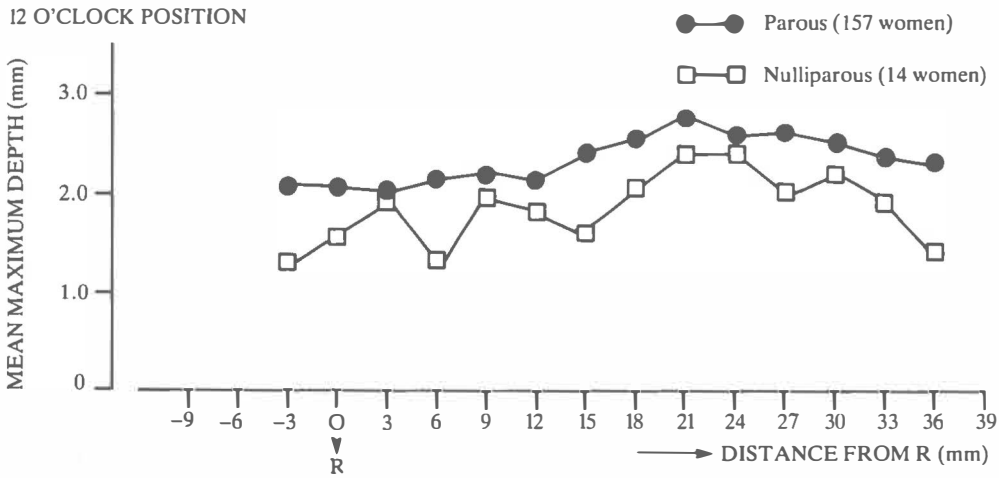


Figure IV.9: Mean Maximum Depth (MMD) of cervical crypts at various distances from the reference point R in relation to parity (for documentation: see appendix table 7).

It is possible that the lesser depth of the crypts in the nulliparous women is due to the older age of women in that group. To investigate that possibility the mean age in both groups has been calculated. The mean age of the nulliparous group was 43 years against 46 years in the group of one or more deliveries. It follows that in that respect the two groups are comparable.

In addition to exclude further that the difference in the depth of crypts between nulliparous and parous women might be explained by an age-factor, the overall MMD of the crypts in these two groups were compared in women younger and older than 60 years. In both age-groups a similar difference of 0.8 mm was found between the MMD of the crypts in nulliparous and parous women (table IV.4). Moreover, this difference appeared to be almost the same as in the entire population (0.9 mm, see previously).

Table IV.4: Depth (mm) of cervical crypts and 'last gland' in relation to age and parity (12 o'clock position, N = number of women)

age and parity	N	%	crypts Mean Max Depth ± SD	'last gland' mean depth ± SD
< 60 years				
parity: 0	12	7.0	3.2 ± 1.0	1.2 ± 0.5
⩾ 1	137	80.1	4.0 ± 1.5	1.7 ± 1.1
⩾ 60 years				
parity: 0	2	1.2	1.9 ± 0.3	1.4 ± 1.0
⩾ 1	20	11.7	2.7 ± 1.1	1.4 ± 0.9
total	171	100.0		

A graph in figure IV.10 shows these differences of MMD of the crypts between nulliparous and parous at the various distances from R in the two age-groups (< 60 and ⩾ 60 years).

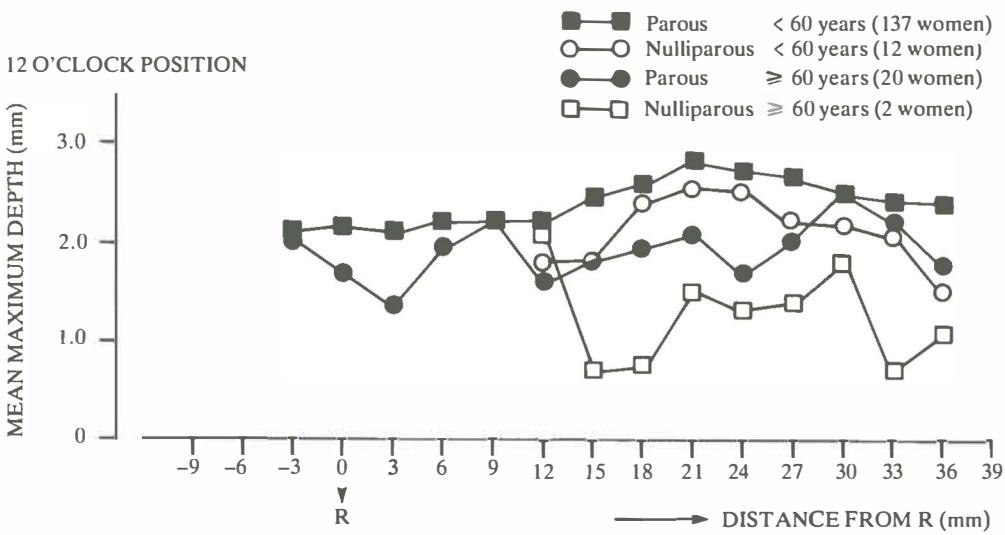


Figure IV. 10: Mean Maximum Depth (MMD) of cervical crypts at various distances from the reference point R, in relation to parity and age (for documentation: see appendix table 8).

IV.3.6. Shape of external os and depth of crypts

The overall MMD and the MMD of the crypts in the 24 areas in cervices with slit-shaped and round external ostia have been compared. One hundred and fifty-one slides at the 12 o'clock position (88.3%) belonged to women with a slit shaped external os and eighteen (10.5%) to women with a round one. From 2 cervices the shape of external os was unknown. Since it is more likely that at the 3 or 9 o'clock positions the MMD of the crypts and the depth of the last gland will vary with the shape of the external os, the variables were tested in all four clock positions.

Concerning the overall MMD of the crypts, the MMD of the crypts at various distances from R and the mean depth of the last gland, no conspicuous differences could be observed between slit-shaped and round external os at the 3 and 9 o'clock position. Only at the 12 o'clock position the overall MMD and the MMD at the various distances from R did prove to be significantly less in cervices with a round external os, compared with a slit shaped os (table IV.5, figure IV.11).

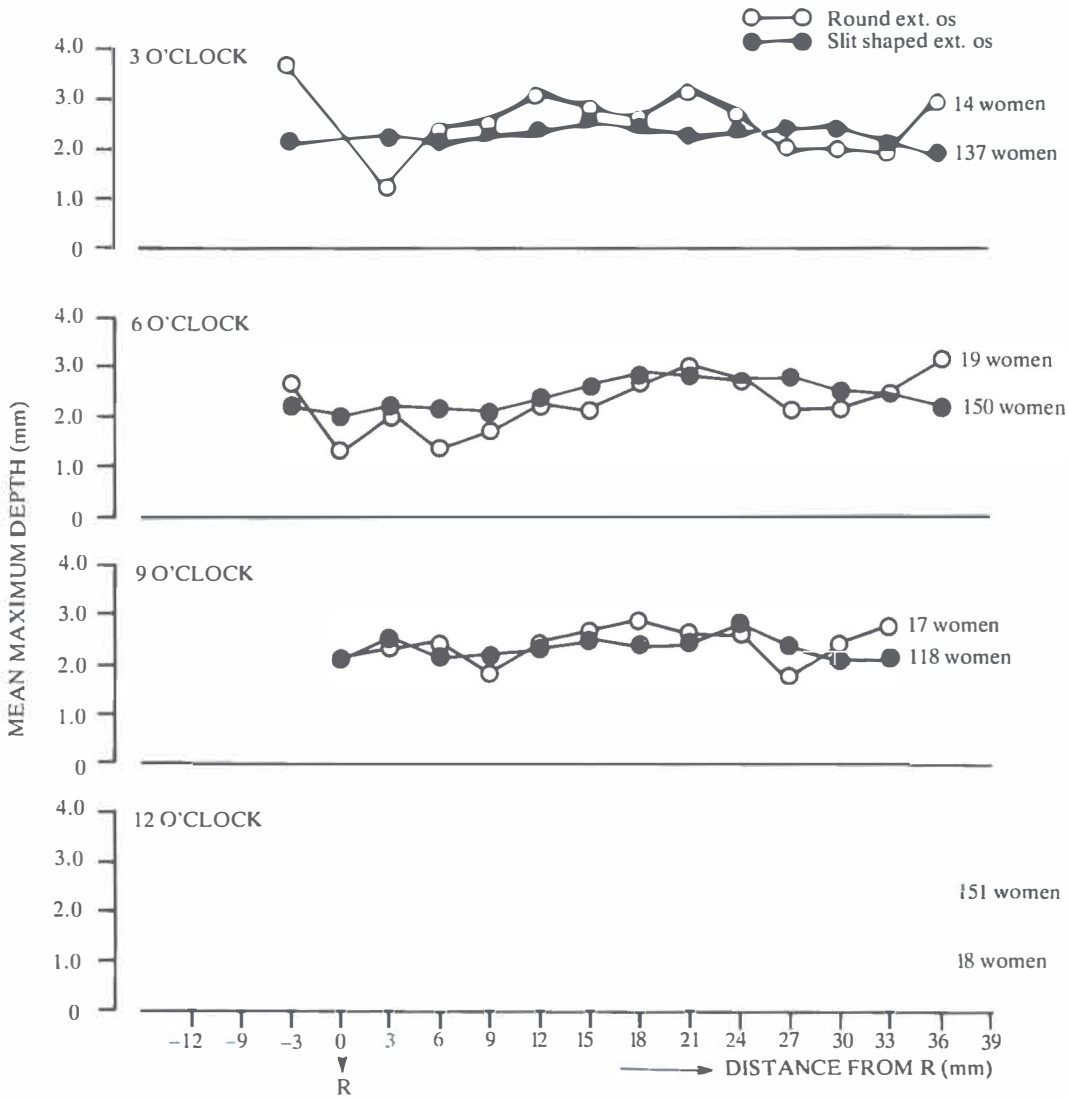


Figure IV.11: Mean Maximum Depth (MMD) of cervical crypts at various distances from the reference point R in relation to shape of external os at 3, 6, 9 and 12 o'clock position (for documentation: see appendix table 9).

Table IV.5: Depth (mm) of cervical crypts and last gland in relation to shape of external os at different clock positions (N = number of women)

shape of external os	N	%	crypts Mean Max Depth ± SD	'last gland' Mean Depth ± SD
3 o'clock:				
slit shaped	137	89.5	3.7 ± 1.4	2.1 ± 1.2
round	14	9.2	3.6 ± 1.4	2.1 ± 1.7
unknown	2	1.3		
6 o'clock:				
slit shaped	150	87.7	4.1 ± 1.6	1.8 ± 1.1
round	19	11.1	3.7 ± 1.4	1.5 ± 0.9
unknown	2	1.2		
9 o'clock:				
slit shaped	118	86.1	3.6 ± 1.4	2.0 ± 1.1
round	17	12.4	3.8 ± 1.3	1.5 ± 1.3
unknown	2	1.5		
12 o'clock:				
slit shaped	151	88.3	3.9 ± 1.5	1.7 ± 1.0
round	18	10.5	3.0 ± 1.0	1.4 ± 0.9
unknown	2	1.2		
total	635	100		

IV.3.7. Portio diameter and depth of crypts

To investigate a possible relation between depth of crypts and portio diameter the antero-posterior and transverse diameter were measured. The average of both diameters has been used as a measure for the size of the cervix. The mean portio diameter for the entire population was 29.7 mm. Most women (62.2%) had a portio diameter between 25 and 35 mm. Therefore in that subgroup the depth of crypts has been compared with those in the group > 35 mm and in the group < 25 mm i.e. 19.2% and 18.0% respectively of the population.
From 1 woman the portio diameter was unknown (table IV.6).

Table IV.6: Distribution of portio diameter in 172 women

Portio diameter	N	%
< 25 mm	31	18.0
25-35 mm	107	62.2
> 35 mm	33	19.2
unknown	1	0.6
total	172	100.0

A histogram in figure IV.12 shows the results. The overall MMD in the group with a small portio diameter (< 25 mm) is 2.8 ± 0.9 mm, which is significantly less than in portio's with larger diameters. In portio's with a diameter of 25-35 mm and more than 35 mm almost equal overall MMD's were found, 4.0 ± 1.6 mm and 4.1 ± 1.4 mm respectively. The same trend could be observed in the MMD of the crypts at various distances from R (Figure IV.13).

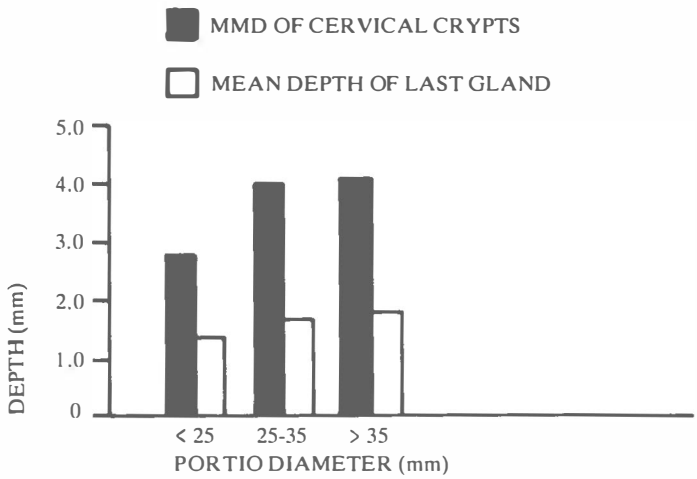


Figure IV.12: Mean Maximum Depth (MMD) of cervical crypts and mean depth of 'last gland' in relation to portio diameter at 12 o'clock position (for documentation: see appendix table 10).

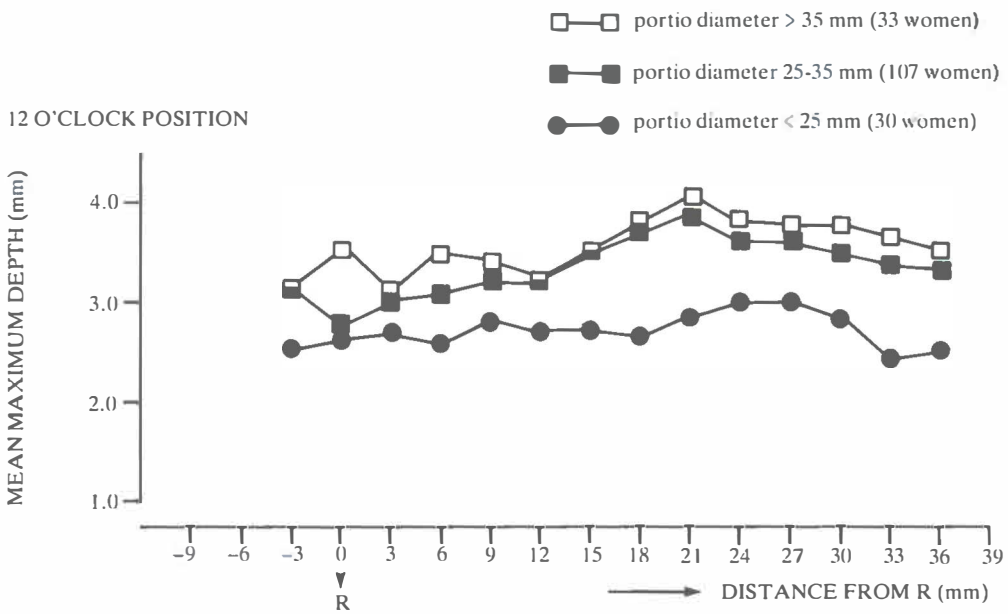


Figure IV.13: Mean Maximum Depth (MMD) of cervical crypts at various distances from the reference point R, in relation to portio diameter (for documentation: see appendix table 11).

Because the size of the cervix might be of importance in selecting patients suitable for cryosurgical treatment of CIN III, it became of interest to define patients with a large and those with a small cervix.

The data were therefore examined to see whether any relation existed between portio diameter and patient's age, parity, period of life, weight and height.

From 164 patients the height and weight was known. The height of 98 patients varied between 1.61-1.70 m. The mean portio diameter of these women was compared with that of women whose height was above 1.70 m (34 women) and of women with

a height below 1.61 m (32 women). The results are summarized in table IV.7 and as can be seen, no significant difference could be observed in portio diameter between tall and short women.

To evaluate whether obese women will have a more plump portio, the patients were, according to the Quetelet-index (weight/height^2) divided in three groups: a group of women with a Quetelet-index < 25, a group who were overweight (Quetelet-index 25-29.9) and a group of women with extreme obesity (Quetelet-index ⩾ 30). It appears that the portio diameter does not vary with the weight of the women (table IV.7).

Table IV.7: Portio diameter (mm) in relation to age, period of life, parity, height and weight (N = number of women)

Subgroups		N	Mean portio diameter ± SD	Range
Entire population		171	29.7	10.5 – 45.0
Age	< 60 years	148	30.5 ± 6.2	10.5 – 45.0
	⩾ 60 years	23	25.0 ± 7.4	14.5 – 38.5
Period of life	premenopause	135	30.8 ± 6.1	10.5 – 45.0
	postmenopause	36	25.5 ± 7.0	14.5 – 38.5
Parity	nulliparous	15	25.0 ± 6.9	10.5 – 35.0
	parous	156	30.2 ± 6.4	12.5 – 45.0
Parity –age	< 60 years			
	– nulliparous	12	25.9 ± 7.2	10.5 – 35.0
	– parous	136	30.9 ± 6.0	12.5 – 45.0
	⩾ 60 years			
	– nulliparous	3	21.5 ± 5.6	18.0 – 18.5
	– parous	20	25.6 ± 7.6	14.5 – 38.5
Height	< 1.60 m	32	28.1 ± 7.3	10.5 – 40.0
	161 – 170 m	98	30.1 ± 6.4	12.5 – 42.5
	>1.70 m	34	30.2 ± 6.8	17.5 – 45.0
	unknown	7		
Weight	Quetelet index < 25	94	30.0 ± 7.2	12.5 – 45.0
	Quetelet index 25 – 29.9	56	29.3 ± 6.2	10.5 – 40.0
	Quetelet index ⩾ 30	14	29.4 ± 4.9	18.5 – 38.0
	unknown	7		

This was even valid in the extremely obese group, in which it was imaginable that increased oestrogen production would have led to an increase in portio diameter. As could be expected, the mean portio diameter decreases with increasing age and in the postmenopausal women the portio diameter is significantly smaller than in women before the menopause. Also the difference in portio diameter between nulliparous and parous women is significant (25.0 and 30.2 mm respectively).

IV.3.8. Depth of Nabothian cysts

In 270 slides Nabothian cysts have been found. From each slide the deepest Nabothian cyst was measured and recorded. In such a way the overall MMD of the cysts could be calculated. It amounted to 4.0 ± 2.0 mm and the deepest cyst was 11.2 mm.

IV.3.9. Topography of the cervical crypts

IV.3.9.1. Localization of deepest crypts, 'last glands' and Nabothian cysts

In table IV.8 the mean distances ± SD of the deepest crypts and the last glands to the reference point R have been summarized at different clock positions.

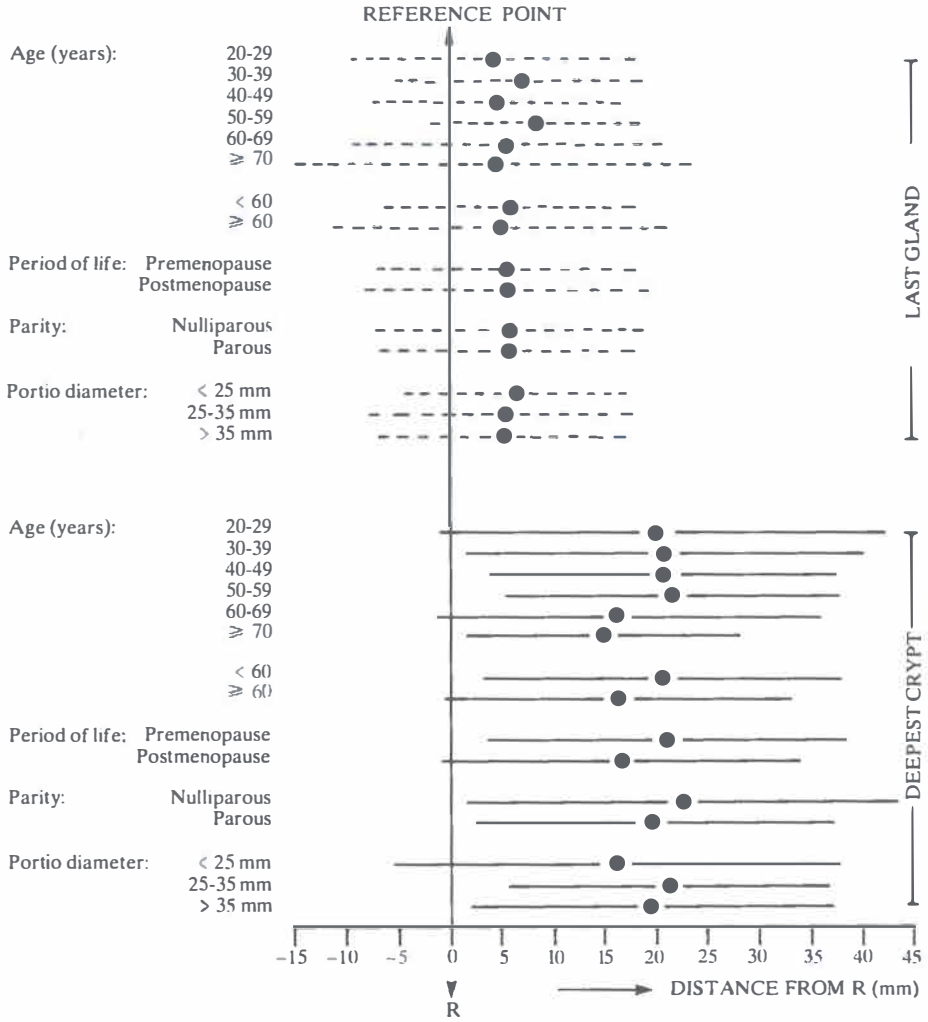


Figure IV.14: Distance (mean ± 2 SD) of last gland (2 SD ---- $\overset{mean}{\bullet}$ ---- 2 SD) and deepest crypt (2 SD —— $\overset{mean}{\bullet}$ —— 2 SD) to the reference point R in relation to age, period of life, parity and portio diameter at the 12 o'clock position (for documentation: see appendix table 12).

Table IV.8: Distance of deepest crypt and 'last gland' to reference point R at different clock positions (mm). (N = number of slides; prox. = proximal from R; dist. = distal from R)

		deepest crypt			'last gland'		
clock position	N	Mean ± SD	Maximum prox.	dist.	Mean ±SD	Maximum prox.	dist.
3 o'clock	153	16.8 ± 9.0	33	−9	4.2 ± 5.4	21	−9
6 o'clock	171	19.2 ± 9.0	39	−9	5.1 ± 5.7	27	−9
9 o'clock	137	16.8 ± 7.8	36	−3	5.4 ± 5.1	27	−9
12 o'clock	171	19.8 ± 8.7	39	−3	5.1 ± 6.3	21	−9

At the 6 and 12 o'clock position the deepest crypts are localized at a mean distance of 19.2 mm and 19.8 mm, respectively from the reference point R. That is at a longer distance than at the 3 and 9 o'clock position (both 16.8 mm). The difference (about 3 mm) is statistically significant. The most proximal deepest crypt was located at a distance of 39 mm from the reference point. Both, at the 6 and 12 o'clock positions the deepest crypt was found at such a maximum distance. The deepest crypts, most distally located, were found 9 mm distally from point R (indicated as a negative value at the 3 and 6 o'clock positions; see table IV.8).

No significant difference could be observed in the mean distance of the last gland to the reference point. In general the last gland is located at a distance of about 5 mm from the reference point. At all the clock positions the most distally located last gland was found at a distance of 9 mm distally from R, also indicated as a negative value (see table IV.8). The maximum proximal distance of the last gland was 27 mm and found at the 6 and 9 o'clock positions.

In figure IV.14 these mean distances ± 2 SD are shown in relation to age, period of life, parity and portio diameter, at the 12 o'clock position. Concerning the mean distance of the deepest crypts to the reference point R, a significant difference was found between pre- and postmenopausal women, and between cervices with a small and large diameter. Namely, it appeared that in the postmenopausal women and in cervices with a diameter less than 25 mm, the deepest crypt is located about 4.5 mm nearer to the reference point as compared to premenopausal women and to cervices with a diameter more than 25 mm. These results are in agreement with the fact that postmenopausal women in general have a smaller, atrophic cervix (see IV.3.7). The same can be seen between women over and below the age of 60. No significant difference could be observed between nulliparous and parous women. The distance of the last gland to the reference point did not vary with age, period of life, parity and portio diameter.

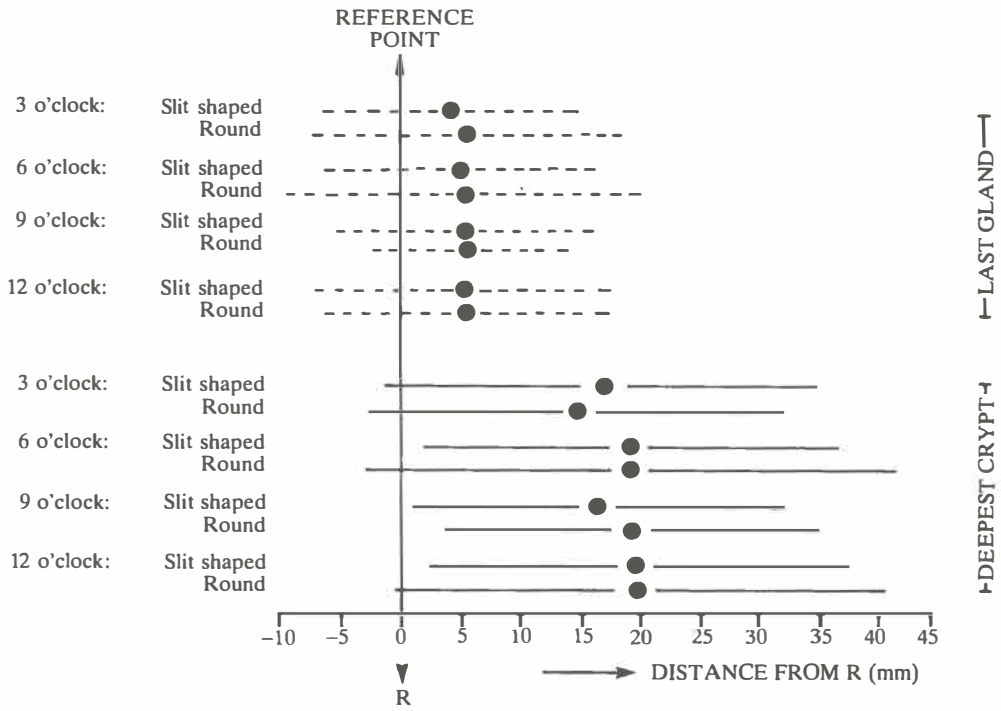


Figure IV.15: Distance (mean ± 2 SD) of last gland (2 SD ----$\overset{mean}{\bullet}$---- 2 SD) and deepest crypt (2 SD ——$\overset{mean}{\bullet}$—— 2 SD) to the reference point R in relation to shape of external os at different clock positions (for documentation: see appendix table 13).

From figure IV.15 it can be noticed that the mean distance of the deepest crypt as well as the mean distance of the last gland to the reference point R are almost the same in cervices with a slit shaped and round external os, at the different clock positions.

The Nabothian cysts appeared to be localized at a mean distance of 24.9 ± 8.7 mm from R.

IV.3.9.2. Distribution of deepest crypts, 'last glands' and Nabothian cysts in the uterine cervix

A histogram in figure IV.16 shows the distribution of the deepest crypts and of last glands per 'area' at the 12 o'clock position.

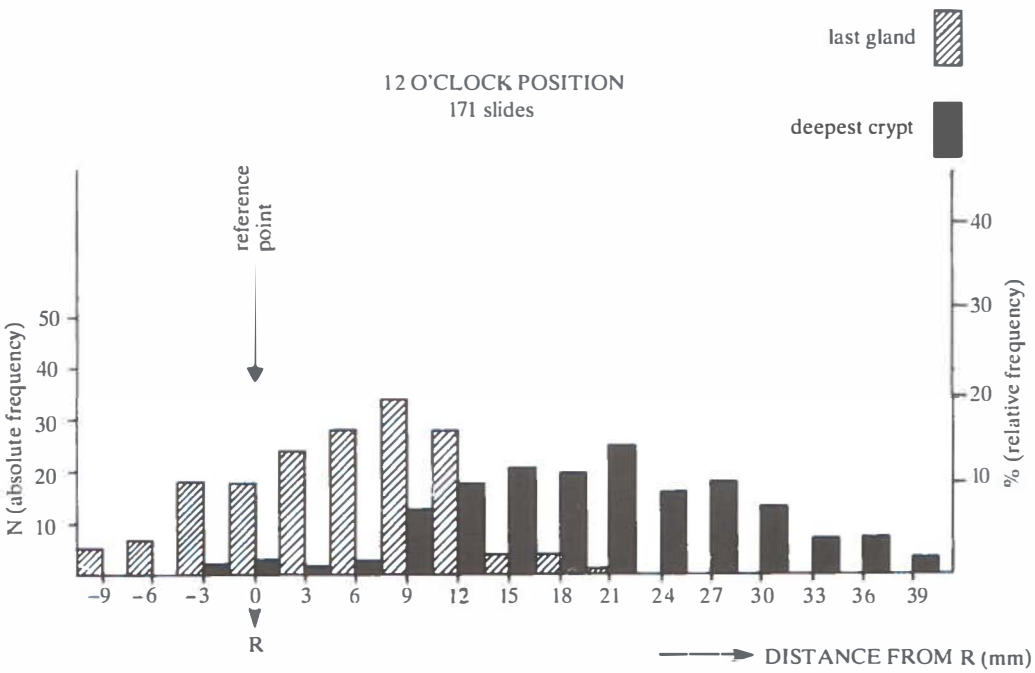


Figure IV.16: Frequency of topographical distribution of the last glands and the deepest crypts; absolute (N) and relative (%) frequency (for documentation: see appendix table 14).

In almost 70% of the 171 slides at the 12 o'clock position the deepest crypts are located and almost equally divided, from 12 mm up to and including 27 mm from R. The mode (14.6%) is at 21 mm from R. In 86.6% of the women the deepest crypt is located 12 mm or more from the most caudal part of the cervix (R). The last gland is most frequently found 9 mm from R. In 87.8% of the women the localization of the last gland is between 3 mm distally up to and including 12 mm proximally from the reference point. In the majority of women (72.0%) the last gland is located proximally of the reference point, in 17.5% distally of it and in 10.5% the last gland is situated at the reference point R, the most caudal part of the ectocervix. The majority of the Nabothian cysts (61.5%) is located between 18 and 30 mm from the reference point (see appendix table 14). This indicates that the deepest Nabothian cysts are located slightly more proximally than the deepest crypts.

IV.4. Discussion

No significant differences were observed between the overall MMD at the different clock positions. The supposition that in a slit shaped external os the depth of crypts at the 3 and 9 o'clock positions would be different from that at the 6 and 12 o'clock positions, was not found to be correct (Chapter IV.3.6.).

The overall MMD of the crypts, 3.8 ± 1.5 mm was slightly more than found by Anderson and Hartley (1980) and Abdul-Karim et al. (1982), 3.38 ± 0.97 mm and 2.94 ± 1.50 mm respectively (Table IV.9). The difference can be largely explained by the fact that the results in the present study have been corrected for shrinkage with a coefficient of 15% due to processing tissue for histological examination (Chapter III).

Another factor which might contribute to differences in the results is the fact that in both quoted studies the depth of crypts was measured in cone specimens. From the present study using hysterectomy specimens it was found that the mean distance of the deepest crypts to the reference point R, that is the most caudal point of the ectocervix was about 20 mm. Furthermore, in over 50% of the cases the deepest crypt of each cervix was located at a distance of 21 mm or more from that point R (Chapter IV.3.9.).

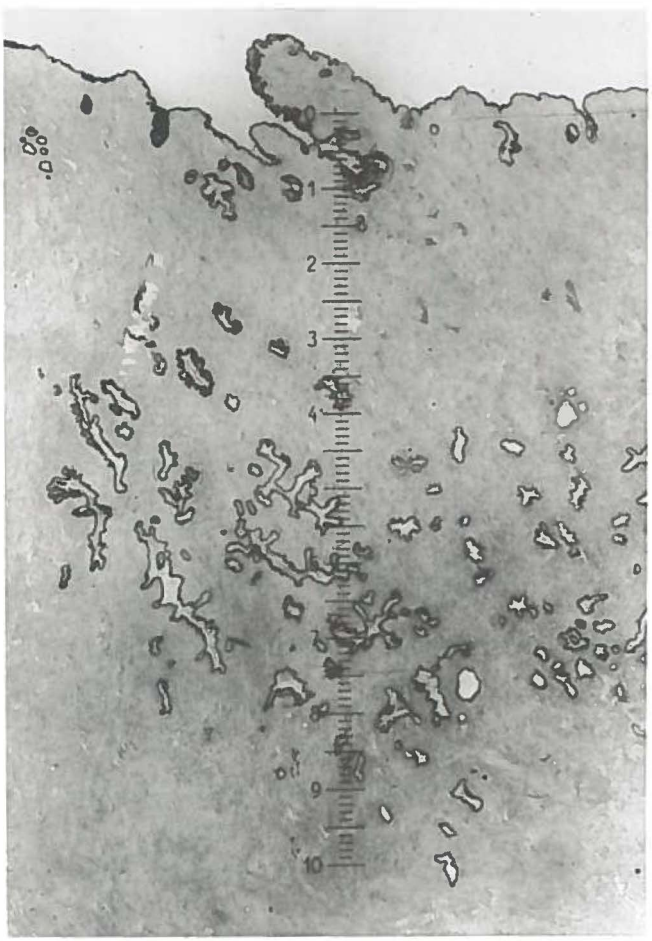


Figure IV.17: The deepest observed crypt: 10.5 mm; corrected for shrinkage: 12.4 mm (Alcian blue; × 10).

Table IV.9: Comparative studies on the depth of crypts (N = number of specimens)

Authors	Material	N	Mean Maximum Depth ± SD (mm)	Maximum (mm)
Anderson et al. 1980	Conization specimens	343	3.38 ± 0.97	7.83
Abdul-Karim et al. 1982	Conization specimens	319	2.94 ± 1.50	
Present study	Amputated cervices	145	3.8 ± 1.5	12.4

It is evident from the results of the present study, that the deepest crypts are located more endocervically, i.e. outside the proximal margin of the cone. That implies that probably many of the deepest crypts, measured by Anderson and Hartley (1980) and Abdul-Karim et al. (1982), actually were not the deepest crypts of the cervix.

The deepest crypt (12.4 mm, Figure IV.17) was found at the 6 o'clock position, strikingly deeper than previously reported by Anderson and Hartley 1980. Recently Wright stated that the crypts can reach a depth of 5 mm (Wright 1984). Because of the considerable difference, the slides, in which deep crypts were found, were reviewed.

The extreme values have been traced at a distance of 18 mm and more from the most caudal point of the ectocervix, the reference point R. These findings support the supposition that the deepest crypts are located beyond the proximal margin of the conization specimens. In other words, when a cervix is entirely and thoroughly investigated, crypts with a depth of about 10-12 mm can be found.

On the other hand, the part of the cervix present in a cone specimen, is the most important area concerning the local treatment of CIN. From several studies it is evident that the majority of the CIN III lesions involves the area in which the transformation zone and the neo-squamocolumnar junction is present (Przybora and Plutowa 1959, Johnson et al. 1964, Abdul-Karim et al. 1982. See chapter II.2.3.3.1.). Moreover, in the entire cervix, at the 12 o'clock position and therefore also especially in that specific area, the MMD of the crypts does not exceed 2.7 ± 1.5 mm.

The increase of the depth of crypts with increasing age, is in accordance with Anderson and Hartley (1980). In the present study a decrease was found over the age of 60 years instead of 50 years found by Anderson and Hartley (1980). The statistically significant difference in the depth of crypts between women under and over 60 years is more pronounced and was found to be 1.3 mm. The difference in depth of crypts between pre- and postmenopausal women is less (1.0 mm), but also statistically significant.

This decrease in depth of crypts at older age is explained by atrophy of the cervix, which is also reflected in the portio diameter. In the postmenopause, particularly after the age of 60, the cervices become smaller and at the same time also the depth of crypts, which are significantly smaller in cervices with a diameter < 25 mm than in more plump cervices (diameter ≥ 25 mm).

In conclusion, depths of crypts are less in small cervices of women older than 60 years and this is caused by atrophy of the cervix.

In addition to atrophy, the portio diameter is influenced by parity. In order to eliminate the age-factor, the difference in portio diameter between nulliparous and parous women has been compared in the age group below 60 years and still a significant difference of 5 mm is found (see table IV.7). Parallel to the significant increase in portio diameter between nulliparous and parous women, there is also a significant increase in the depth of the crypts. The influence of age was excluded by comparing the mean age in the nulliparous and parous group which was about the same and also by comparing the difference in overall MMD of those two populations in women over

and under 60 years. In both subgroups the differences are the same (0.8 mm).

As a result in parous women over 60 years, and nulliparous women under the age of 60, these influences abolish each other more or less. This is expressed in the overall MMD in those groups being 2.7 ± 1.1 mm and 3.2 ± 1.0 mm respectively (Table IV.4). On the other hand in women younger than 60 years with one or more deliveries these factors are consolidated. This is reflected in the relatively high overall MMD of 4.0 ± 1.5 mm in that group. In the nulliparous women over 60 years a very low value of the overall MMD would have been expected. Unfortunately only two evaluable patients belonged to that group. However, those two patients did have an extremely low overall MMD of 1.9 ± 0.3 mm (Table IV.4). The above mentioned issues, concerning the overall MMD in these various groups, can also be observed in the MMD of the crypts at the various distances to R. This is clearly demonstrated in the graphs of figure IV.10.

Of interest is the finding that the differences in MMD of the crypts both in the age groups (< 60 years and ≥ 60 years) as in the portio diameter groups (< 25 mm and ≥ 25 mm) are most striking at a distance of 12 mm and more proximal from R (Figure IV.5, 13). To a lesser extent this phenomenon could also be observed in the parity groups (Figure IV.9). This is particularly important, because crypt depth is relatively constant in the transformation zone.

Young patients who have not yet completed their family are the aim-group of our study, because they will benefit most from local destructive treatment of CIN. It is clear that in this population the depth of crypts does not exceed the depth in the entire population. Therefore it is justified and safe to take the graph of the MMD of the crypts of the entire population at 12 o'clock as a guide for further investigations into the applicability of cryosurgery (Figure IV.18). From this figure, in which also the graph of the mean + 2.SD value is drawn, it can be seen that in endocervical direction the depth of crypts is increasing.

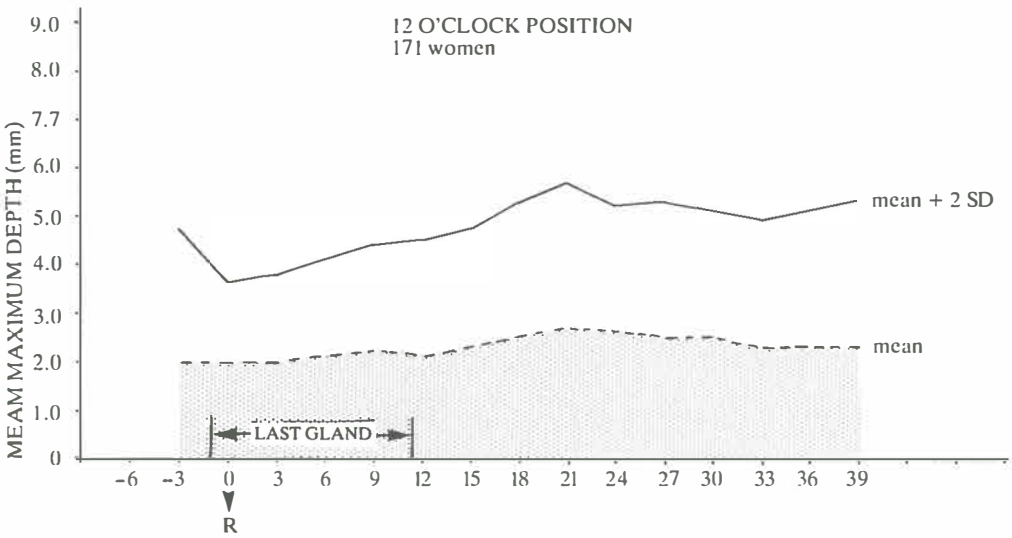


Figure IV.18: Mean Maximum Depth + 2.SD of cervical crypts at various distance from reference point R at the 12 o'clock position.

This is in accordance with the observation that in about 87% of the women the deepest crypt was located 12 mm or more from the reference point (Appendix Table 14 and Figure IV.16). By approaching the ectocervix, the depth of crypts decreases, while this distal part is most frequently involved with CIN (Gusberg and Moore 1953,

Przybora and Plutowa 1959, Reagan and Patten 1962, Johnson et al. 1964, Abdul-Karim et al. 1982). This is an auspicious coincidence, concerning the destructive treatment of CIN, because the proximal part of the cervix with the deepest crypt is most probably beyond the limitation of the cryolesions.

So far the depth of the Nabothian cysts has not been included in the discussion. From the literature (Anderson and Hartley 1980) there is evidence that the CIN rarely involves these cysts. However, large, often grossly visible cysts can penetrate deeply into the cervical stroma (Chapter IV.3.8). This should be taken into account, by selecting patients for cryosurgical treatment of CIN.

IV.5. Summary and conclusions

In this chapter the results of an analysis of the depth and topography of cervical crypts in relation with age, period of life, parity, size of external os and portio diameter are presented. In the topographic part of this study the most caudal point of the portio was used as a reference point (R). In addition an analysis was carried out as to whether depth of crypts varied at the different clock positions of the cervix. The investigations have been carried out in 172 cervices amputated from hysterectomy specimens. Sagittal slides were cut at the 3, 6, 9 and 12 o'clock positions. Computerized measurements were done by subdividing each slide in 24 consecutive areas. The following conclusions can be made:

1. No difference in depth of crypts was observed at different clock positions, not even at the 3 and 9 o'clock positions in cervices with slit-shaped external os.
2. The overall Mean Maximum Depth (MMD) of the crypts at the 12 o'clock position, proved to be representative for the whole cervix and was 3.8 ± 1.5 mm. A maximum value of 12.4 mm was found at the 6 o'clock position. Nabothian cysts have been excluded from these measurements.
3. The depth of crypts increases with increasing age until 60 years and decreases afterwards. The MMD of the crypts are significantly less in women over 60 years, being postmenopausal and in women with a portio diameter < 25 mm. However, age, period of life and portio diameter are related to each other.
4. Parallel to this, a decrease in portio diameter is found in postmenopausal women, especially those over 60 years and in the nulliparous women and corresponding with this a decrease of the overall MMD of the crypts.
5. By excluding the age factor parity proved to be a second independent variable influencing the depth of the crypts. In nulliparous women the MMD of the crypts is significantly less compared with parous women.
6. The MMD of the crypts at the various distances from R proved also to depend on age, period of life, parity and portio diameter in the same manner as for the overall MMD. At a distance of 12 mm or more from the most caudal point of the ectocervix, the MMD of the crypts at the various distances from R are slightly increasing.
7. The overall mean depth of the 'last gland' is 1.6 ± 1.0 mm with a maximum value of 7.2 mm found at the 3 o'clock position.
8. The depth of the 'last gland' also varies with age, period of life, parity and portio diameter but to a less extent than the depth of the deepest crypts does.
9. The overall MMD of the Nabothian cysts is 4.0 ± 2.0 mm and this is hardly deeper than the overall MMD of the crypts without cysts.
10. The majority (87.8%) of the 'last glands' are localized 3 mm distally as far as 12 mm proximally from the most caudal point of the ectocervix.
11. Only 17.5% of the 'last glands' are localized distally from the most caudal point of the ectocervix and the distance of the most distal 'last gland' to that point is 9 mm.
12. The mean distance from the deepest crypt to the reference point is 19.2 mm and 19.8 mm at the 6 and 12 o'clock positions and 16.8 mm at the 3 and 9 o'clock

positions, which are significant differences. This distance varies with period of life and portio diameter, with the understanding that in postmenopausal women with a small portio diameter the mean distance is significantly less compared to premenopausal women with more plump cervices.

13. The majority (86.6%) of the deepest crypts is localized 12 mm or more proximally from the most caudal point (R) of the ectocervix, outside the transformation zone, which is the prime target of destructive treatment of CIN.
14. In this threatened part of the cervix the MMD of the crypts, at various distances from R, does not exceed 2.7 + 1.5 mm.

CHAPTER V

Cervical intraepithelial neoplasia grade III; depth of crypt involvement, linear extent and topography

V.1. Introduction

To evaluate whether a cryolesion of the cervix extends widely enough to eradicate all the pathological epithelium one should know accurately the depth of crypt involvement, the linear extent and the localization of CIN-III. Only when the pathological epithelium is located within the frozen area it will be destroyed. Both the surface of the endo- and ectocervical epithelium and the underlying epithelium of the crypts can be involved with CIN-III.

Therefore, apart from knowledge about the depth and distribution of the cervical crypts (Chapter IV), it is important to be informed where, over what distance and how deep the epithelium is involved by CIN-III.

In the present study in cone specimens the maximum depth of the crypts, the maximum depth of CIN-III crypt involvement and the linear extent of the CIN-III lesion was measured. The topography of both the CIN-III in the crypts as well as the CIN-III on the surface was determined.

V.2. Material and methods

All the slides of cone specimens from patients treated for CIN-III in the last five years were reviewed. The specimens had been fixed in 8% formalin for 24 hours. After processing the tissue, using standard methods, slides were cut perpendicularly

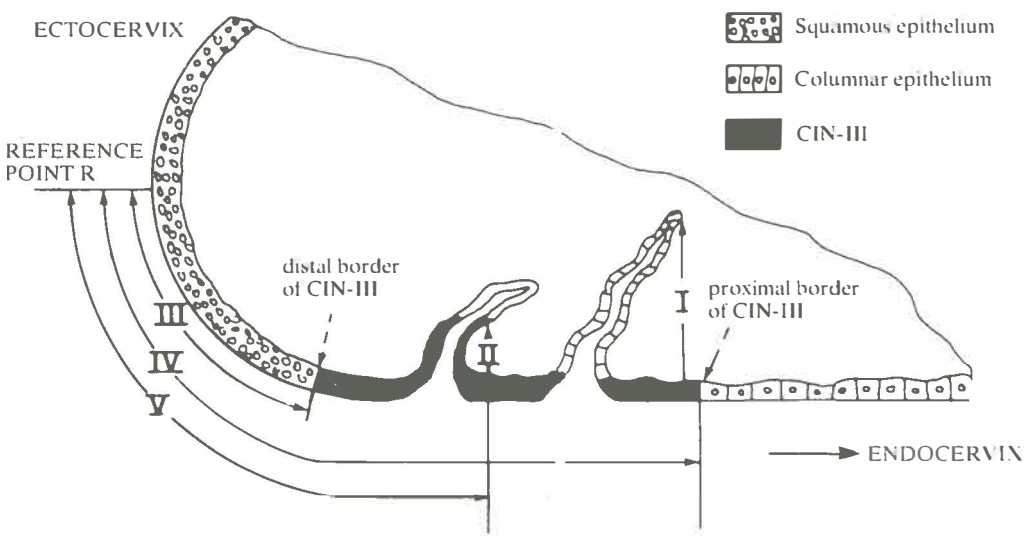


Figure V.1: Measurements of CIN-III, made in sagittal slides of cone specimens.
I The depth of the deepest crypt in the slide
II The depth of the deepest CIN-III crypt involvement
III The distance of the distal border of the CIN-III lesion to the reference point R
IV The distance of the proximal border of the CIN-III lesion to the reference point R
V The distance of the crypt with the deepest CIN-III involvement in the slide to the reference point R

to the mucosal surface in a plane parallel to the axis of the canal. After mounting the slides were stained hematoxylin-eosin. Only slides with CIN-III were selected for the study.

In a previous study the depth of the crypts was determined and their distance to a reference point R was measured. This reference point was the most caudal point of the ectocervix (Chapter IV.2). In the topographical part of this study, the same reference point was chosen. This point R was obtained by drawing a line along the endocervical surface and perpendicular to it the tangent of the ectocervix. The point of contact of the tangent with the ectocervix is point R (Chapter IV.2, Fig. IV.1). This means that only those slides in which the ectocervix and the entire transformation zone were visible, were eligible for the topographical part of the study.

Three groups of slides were selected in which the following measurements were carried out:

1. crypt involvement
2. linear extent
3. both crypt involvement and linear extent

Table V.1: Number of patients, and age and parity distribution in the investigated groups (see text).

	total	measurements of		
		crypt involvement only	linear extent only	crypt involvement and linear extent
no of patients	65	57	48	37
age (years)				
mean	37.5	37.8	37.9	38.5
range	24-85	24-85	24-85	24-85
parity				
nulliparous	20	19	14	11
parous	45	38	34	26

Group 1 Slides with measurable CIN-III crypt involvement.

This could be observed in 57 patients. From each patient the slide with the deepest involvement was taken, irrespective as to whether the ectocervix was present or not. In this group of slides no topographical measurements were carried out and they were only used for measuring the deepest crypt and CIN-III crypt involvement (I and II respectively in Figure V.1). The age of the patients in this group ranged from 24 to 85 years, with a mean age of 37.8 years. Of the patients, 19 were nulliparous and 38 were parous.

Group 2 Slides with CIN-III in which the ectocervix and the complete transformation zone were present whether crypt involvement was present or not.

In 48 of the cone specimens at least one slide was available with this condition. The mean age of these patients was 37.9 years and ranged also from 24 to 85 years, 14 were nulliparous and 34 parous. In the most representative slide of each cone specimen, the topographical position and the linear extent of the CIN-III lesion was measured. This was done by determining the distance from the distal and proximal border of the area with CIN-III to the reference point R (III and IV respectively in Figure V.1). A localization of the CIN-III distal of the reference point was indicated with a negative value.

Group 3 Slides in which not only the ectocervix and the transformation zone were present, but also CIN-III crypt involvement (although not being of necessity the deepest involvement of the whole cone specimen).

In these slides the distance of the crypt involvement to the reference point R, that means the topographical position of the involved crypt could be determined (V, see Figure V.1). This group includes the cone specimens from 37 patients, with a mean age of 38.5 years, ranging from 24 to 85 years; 11 were nulliparous and 26 parous.

All measurements were carried out with a computerized graphic tablet, as described in Chapter IV.2. The results were also corrected with a shrinkage factor of 15%, due to processing tissue for histological examination (Boonstra et al. 1983).

V.3. Results

V.3.1. CIN-III crypt involvement

The overall mean maximum depth (MMD) of the CIN-III crypt involvement in 57 slides was 1.6 ± 1.0 mm. The deepest involvement, measured from the endocervical surface was 4.5 mm (Table V.2). The deepest, not involved crypt, was 5.6 mm, while the overall MMD of these uninvolved crypts appeared to be 2.6 ± 1.1 mm. Rarely, CIN-III involvement of Nabothian cysts could be observed (Figure V.2). The ratio between depth of CIN-III involvement and depth of uninvolved crypts, calculated for each slide separately, was on an average 65%.

Table V.2: Mean maximum and maximum depth (mm) of CIN-III crypt involvement and uninvolved crypts and the mean percentage of crypt involvement (N = number of patients).

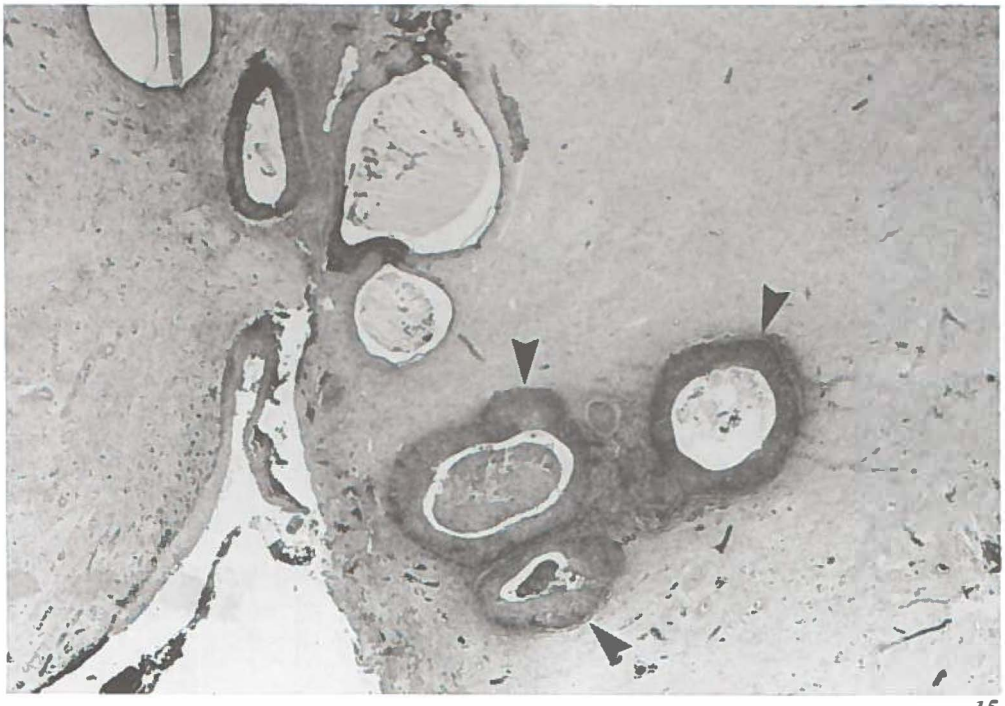
		CIN-III crypt involvement		uninvolved crypts		
	N	mean max. depth ± SD	max.	mean max. depth ± SD	max.	mean percentage of involvement
entire population	57	1.6 ± 1.0	4.5	2.6 ± 1.1	5.6	64.7 ± 26.6
age:						
20-40 yrs	44	1.6 ± 1.0	4.0	2.7 ± 1.1	5.6	61.5 ± 25.5
41-50 yrs	5	1.0 ± 0.6	2.0	1.9 ± 0.6	2.8	54.7 ± 27.6
>50 yrs	8	2.3 ± 1.1	4.5	2.6 ± 1.0	4.5	88.3 ± 21.5
parity:						
nulliparous	19	1.3 ± 0.8	3.4	2.5 ± 1.0	4.6	51.8 ± 21.9
parous	38	1.8 ± 1.1	4.5	2.7 ± 1.1	5.6	71.1 ± 26.7

In table V.3 it is shown how the depth of CIN-III crypt involvement varies with age. Over the age of 50 years there is an increase in depth of involvement to 2.3 ± 1.1 mm. In this age-group the maximum depth of involvement is 88.3% of the maximum depth of the crypts. This differs significantly with the percentage in the younger age-groups.

Both the mean maximum depth of the CIN-III involvement and the ratio between the maximum depth of CIN-III involvement and the maximum depth of the crypts are significantly greater in the parous group, as compared to the nulliparous group (see table V.2).

V.3.2. CIN-III linear extent

The overall mean linear extent of the CIN-III lesion of the cervical surface epithelium was 7.4 ± 3.7 mm. The length of the smallest lesion was 1.4 mm and that of the largest lesion 17.6 mm (Table V.3).



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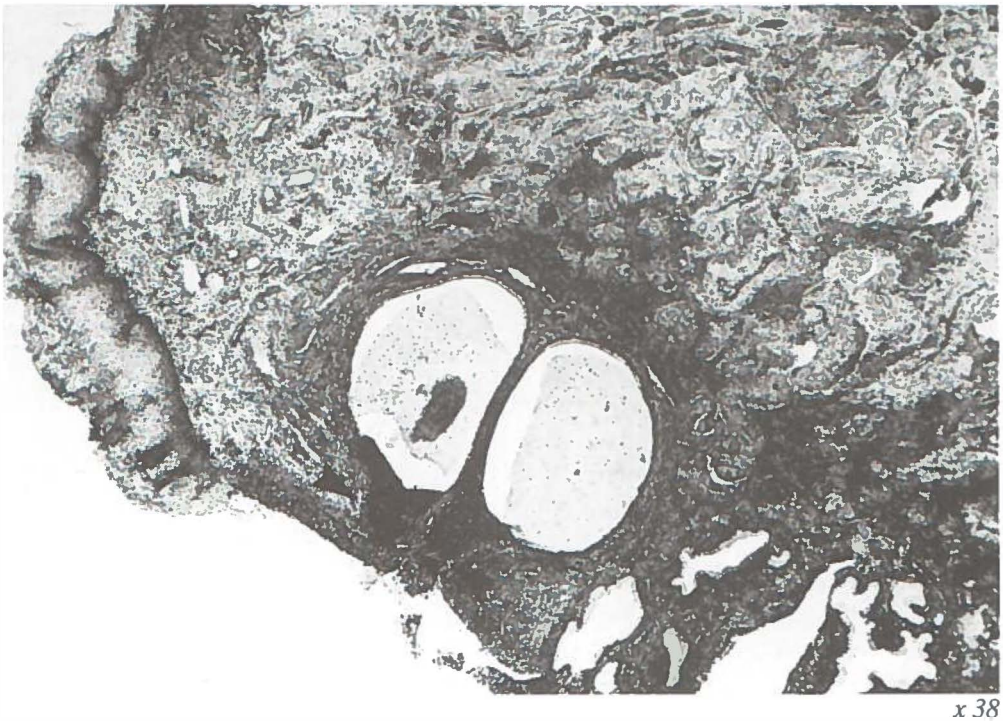


x 38

Figure V.2: CIN-III involvement of Nabothian cysts (see arrows). Hematoxylin-eosin.

Table V.3: Mean, minimum and maximum linear extent (mm) of CIN-III lesion in the entire population and in relation to age and parity (N = number of patients).

	N	linear extent		
		mean ± SD	min.	max.
entire population	48	7.4 ± 3.7	1.4	17.6
age:				
20-40 yrs	35	7.4 ± 3.5	2.4	17.6
41-50 yrs	7	5.8 ± 3.2	1.4	9.9
> 50 yrs	6	8.9 ± 4.9	2.2	15.7
parity:				
nulliparous	14	8.5 ± 4.2	3.4	17.6
parous	34	6.9 ± 3.4	1.4	15.7

There is an increase in linear extension of the CIN-III lesion after age 50 to 8.9 ± 4.9 mm. However, the difference in comparison with the younger age groups is not significant. The same applies to the more extensive lesions found in the nulliparous group.

Table V.4: Mean, minimum and maximum distance (mm) of distal and proximal border of the CIN-III lesion and mean, minimum and maximum distance (mm) of deepest CIN-III crypt involvement to the reference point R in relation to age and parity (N = number of patients).

	distance of CIN-III lesion to R							distance of crypts with deepest CIN-III involvement to R			
	distal border				proximal border						
	N	mean ± SD	min	max	mean ± SD	min	max	N	mean ± SD	min	max
entire population	48	8.2 ± 4.4	−5.2	16.3	13.3 ± 3.7	5.7	19.3	37	10.2 ± 4.2	−3.6	17.3
age:											
20-40 yrs	35	7.2 ± 4.4	−5.2	16.1	12.5 ± 3.7	5.7	19.3	27	9.3 ± 4.3	−3.6	17.3
41-50 yrs	7	11.2 ± 2.7	6.8	15.2	14.5 ± 3.5	10.0	18.9	4	13.1 ± 2.6	10.2	15.4
> 50 yrs	6	10.5 ± 4.3	5.9	16.3	16.4 ± 2.1	13.8	18.6	6	12.3 ± 2.8	6.8	14.4
parity:											
nulliparous	14	6.3 ± 5.6	−5.2	15.2	12.6 ± 3.7	5.7	17.6	11	7.8 ± 4.6	−3.6	12.8
parous	34	9.0 ± 3.7	1.5	16.3	13.5 ± 3.8	7.0	19.3	26	11.2 ± 3.6	5.0	17.3

V.3.3. Topography of the CIN-III lesion

V.3.3.1. Localization of CIN-III of the surface epithelium

In table V.4 the results of the topographical study of the CIN-III lesions are summarized. The localization of the CIN-III of the surface epithelium in the uterine cervix has been determined by measuring the distance of both, its distal and proximal border to the reference point R. Also the distance of the crypt with the deepest CIN-III involvement to the reference point was measured.

The mean distance of the distal border of the CIN-III area to the reference point R is 8.2 ± 4.4 mm. The proximal border was located at a mean distance of 13.3 ± 3.7 mm from R. In only 2 cases the CIN-III was located distally from the reference point, of which the most distal one was 5.2 mm from R. The most proximal lesion was 19.3 mm extended from R.

It appeared that the distance from the distal border of the CIN-III lesion to the

reference point in the 20-40 yrs age-group (7.2 ± 4.4 mm) is significantly less compared to that in the 41-50 yrs age-group (11.2 ± 2.7 mm). Similar results can be seen by comparing the distance of the proximal border in the age-group 20-40 yrs and the group older than 50 yrs (12.5 ± 3.7 mm and 16.4 ± 2.1 respectively). That suggests that in young patients, the CIN-III tends to be more ectocervical than in older patients.

V.3.3.2. Localization of the CIN-III involved crypts

The mean distance of the most deeply involved crypt to the reference point R is 10.2 ± 4.2 mm. In 1 case the involved crypt was located 3.6 mm distally from R. The farthest removed involved crypt was located at a distance of 17.3 mm from R (Table V.4).

In conformity with the CIN-III of the surface epithelium, it looks like that in young nulliparous patients the CIN-III crypt involvement is also located nearer to the ectocervix. However, only the difference in distance of the deepest involvement to R between nulliparous and parous patients is significant.

V.4. Discussion

The overall Mean Maximum Depth (MMD) of the crypts in this study found in cone specimens was less than that found in the whole cervix (Chapter IV.3.1). This is probably due to the fact that the deepest crypts are located more proximally in the endocervical canal, beyond the border of a cone specimen (Chapter IV.4).

The mean maximum depth of the CIN-III crypt involvement found in this study (1.6 mm) was deeper than found by Anderson and Hartley (1980) (1.2 mm). This may be caused by the fact that 11.4% of the patients in the study of Anderson did not have crypt involvement at all.

Similar to the results of Reagan and Hicks (1953) and Anderson and Hartley (1980) an increase of depth of CIN-III crypt involvement with increasing age was found. Also an increase in depth of involvement was found in parous patients compared to nulliparous patients. However, the mean age of the nulliparous-group (30.8 ± 4.4 yrs) was significantly lower than the mean age of the group of patients with one or more deliveries (41.3 ± 14.2 yrs). Therefore the difference of depth of CIN-III involvement in these two groups may be caused by age.

Reagan and Hicks (1953) and Reagan and Patten (1962) reported for dysplasia and carcinoma in situ a mean linear extent of 9.02 ± 4.37 mm and 6.30 ± 3.21 mm, respectively. Abdul-Karim et al. (1982) found a mean linear extent of 7.60 ± 4.32 mm in CIN-III lesions. The mean linear extent of CIN-III lesions in the present study is completely in keeping with these results and appeared to be 7.4 ± 3.7 mm. That means that in about 95% of the patients, the linear extent of the lesions is within 14.8 mm (mean ± 2SD). Therefore to eradicate the CIN lesion completely with cryosurgery, the cryolesion must have a sufficient depth over a distance of a least 15 mm.

The experience, published by others (Reagan and Hicks 1953, Reagan and Patten 1962, Abdul-Karim et al. 1982), that in young patients the CIN-III lesions are smaller than in older women, could not be confirmed significantly in the present study.

Virtually all CIN-III lesions are related to the transitional zone and are located near to the Neo-Squamo Columnar Junction (N-SCJ) and the external cervical os (Reagan and Hicks 1953, Gusberg and Moore 1953, Przybora and Plutowa 1959, Reagan and Patten 1962, Johnson et al. 1964, Richart 1968, Patten 1969, Burghardt and Holzer 1972, Abdul-Karim et al. 1982, see Chapter II.2.3.3.). In all these topographical studies, the localization of CIN-III was recorded on the basis of an anatomical point of reference. However, most investigators used a different reference point and nobody used the most caudal point of the ectocervix, which was used throughout the present study because of the fact that it can be identified clinically. To

compare the topography of CIN-III found in this study with that in, for example, the study of Reagan and Patten (1962), it is necessary to know the relation between the different reference points used. In this study, Chapter IV.3.9, it was found that the distance of the last gland to the reference point was proximally about 5 mm. In the study of Reagan and Patten (1962) the distal border of the transformation zone, that is the last gland, was located 6 mm left, that is distally, of their reference point. That means that the estimated distance between the two reference points is about 11 mm (see Figure V.3).

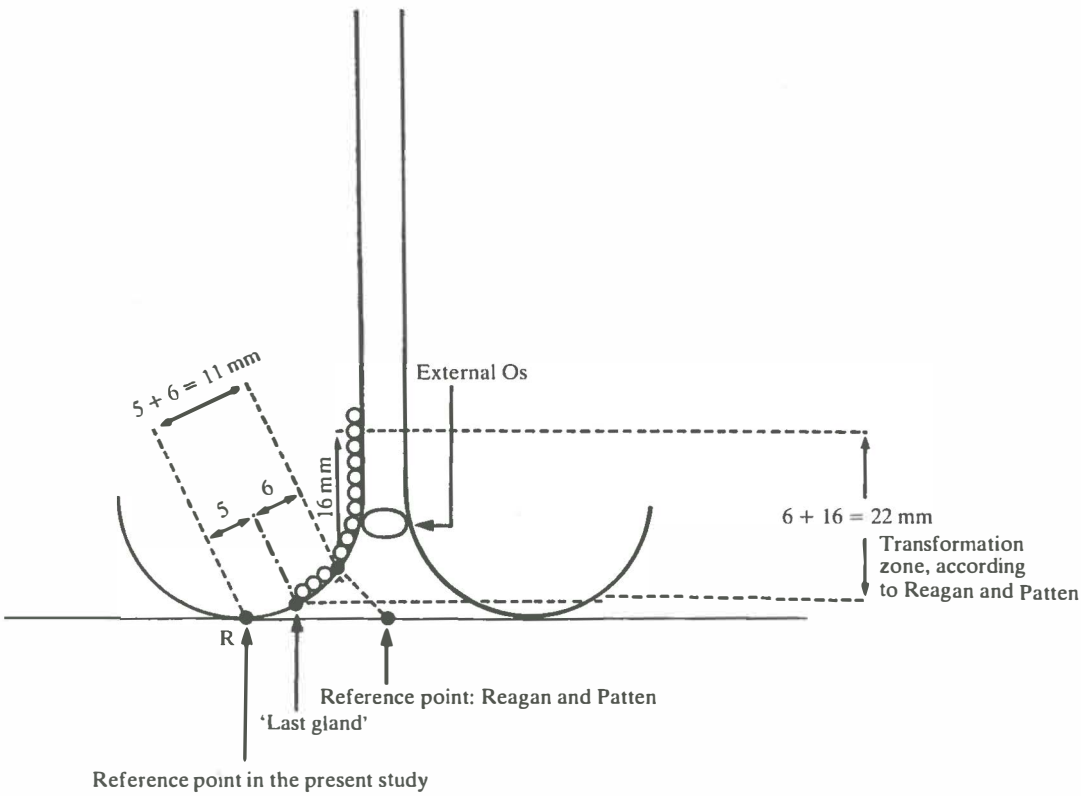


Figure V.3: Relation between the reference point in the study of Reagan and Patten (1962) and the reference point in the present study.

The maximum involvement of the cervix by lesions classified as dysplasia in the study of Reagan and Patten (1962) occurred at a site 5 mm to the right, that is 5 mm proximal, of their reference point, corresponding to the approximate location of the external cervical os. This point corresponds to 5 + 11 mm = 16 mm proximal from the reference point R in the present study. The maximum involvement of the carcinoma in situ in the study of Reagan and Patten (1962) was located a few millimeters more proximally than the dysplastic reactions, but all lesions occurred within the limits of the transitional zone. In Reagan's study this transitional zone extended in 95% of the junctions from 6 mm to the left (distally) of their reference point to a site 16 mm to the right (proximally) of their reference point. This corresponds to 5-27 mm proximally from the reference point R in the present study. In the present study it appeared that the CIN-III lesions extended at a mean distance of 8.2 ± 4.4 mm as far as 13.3 ± 3.7 mm from the reference point R and that implies that about 95% (= mean ± 2 SD) were located between 0.6 distally and 20.7 mm proximally from the reference point R. These figures correspond more or less to the

estimated results from the study of Reagan and Patten (1962).

The observation in the present study that in young patients the CIN-III was located more ectocervically and in older patients more endocervically, is in accordance with the literature (Einerth 1978, Abdul-Karim et al. 1982). This is an important result concerning the local destructive treatment of CIN and makes the young CIN patient more suitable for this type of treatment.

The crypt with the deepest involvement was located at a mean distance of 10.2 ± 4.2 mm from the reference point. That is according to the study of Reagan and Patten (1962) near the external os. On the basis of their measurements it appears that the involved crypt most remote from the reference point R (17.3 mm) is within the limits of the transitional zone.

Concerning the cryosurgical treatment of CIN, it has to be kept in mind that occasionally the CIN involves the more deeply situated and grossly visible Nabothian cysts (Chapter IV.3.8; Figure V.2).

V.5. Summary and conclusions

In this study the extent and depth of the CIN-III involvement of the cervical surface epithelium and the epithelium of the underlying crypts and its topographical distribution have been investigated.

The depth of CIN-III crypt involvement was studied in 57 cone specimens. The depth of involvement was measured from the cervical surface with the aid of a computerized graphic tablet. In 48 cone specimens both the linear extent of CIN-III and its localization in the cervix was determined. Finally in 37 cone specimens the localization of the crypt with the deepest CIN-III involvement also was measured.

The topography of the CIN-III was related to the most caudal point of the ectocervix, the reference point and the measurements were also performed with the computerized graphic tablet.

The following conclusions can be made:

- The overall mean maximum depth of crypt involvement is 1.6 ± 1.0 mm and the deepest involvement 4.5 mm.
- The ratio between the deepest involved and uninvolved crypts is about 65%.
- The depth of CIN-III involvement and the percentage involvement in relation to the deepest crypt is significantly more in patients over the age of 50 and in patients who have had one or more deliveries.
- The mean linear extent of the CIN-III lesions was 7.4 ± 3.7 mm. The smallest lesion was 1.4 mm, the largest one 17.6 mm.
- CIN-III lesions tend to be more extensive in patients over the age of 50.
- The mean distance from the distal and proximal border of the CIN-III lesion to the reference point is 8.2 ± 4.4 mm and 13.3 ± 3.7 mm respectively. That implies that on average those lesions occur between 8.2 mm and 13.3 mm proximally from the most caudal point of the ectocervix, the reference point R, and in about 95% of the patients between 0.6 mm distally from the reference point and 20.7 mm proximally from it. The crypt with the deepest CIN-III involvement was located at a mean distance of 10.2 ± 4.2 mm from R.
- The majority of CIN-III lesions are located proximally from the reference point. Only in 4.2% (2 patients) the lesion extended distally of the reference point. The maximum distal extension was 5.2 mm and the maximum proximal extension 19.3 mm.
- In younger patients not only a tendency was observed that the linear extent of the lesion is smaller, but also that the lesion is situated nearer to the ectocervix. These data make the younger patient with CIN more suitable for cryosurgical treatment.
- It appeared that Nabothian cysts can be involved with CIN-III. These cysts can penetrate deep into the cervical stroma, although the overall Mean Maximum Depth of it is similar to that of the crypts. These facts must be kept in mind when using cryosurgical treatment for CIN in patients with Nabothian cysts.

CHAPTER VI

A standard cryolesion of the uterine cervix; application techniques, extension and failures

VI.1. Introduction

Cryotherapy is one of the most commonly used methods of local destructive treatment of cervical intraepithelial neoplasia (CIN). Treatment results vary and its efficiacy in CIN-III is about 80-90% (Table II.4, Wright and Davies 1981, Trimbos and van Lent 1982, Townsend and Richart 1983, Creasman et al. 1984, Berget and Lenstrup 1985, Bryson et al. 1985, Kwikkel et al. 1985b). After several reports describing invasive carcinoma in cryosurgically treated patients and with the increasing trend to more conservative treatment, the question arises whether a guarantee can be given for the safety of local destructive treatment methods (Sevin et al. 1979, Townsend et al. 1981).

Regarding cryotherapy it can be questioned whether the cryolesion reaches deep enough to eradicate a CIN lesion especially when it extends into the endocervix and into the cervical crypts. No adequate data are available from the literature about the effective depth of tissue destruction produced by cryosurgery. Moreover, there is a lack of knowledge on how to standardize cryosurgical techniques. Therefore in the present study the extension of cryolesions, applied with standardized techniques, has been investigated, for different probes and at different clock positions. The extension of the cryolesion with various freeze times, has been studied in different age and parity groups and in cervices with slit shaped and round external os, particularly at the 3 and 9 o'clock positions. If insufficient extension of the cryolesion was obtained, the causes of failures have been analyzed. The results are useful in selecting patients and improving standardization of freeze technique.

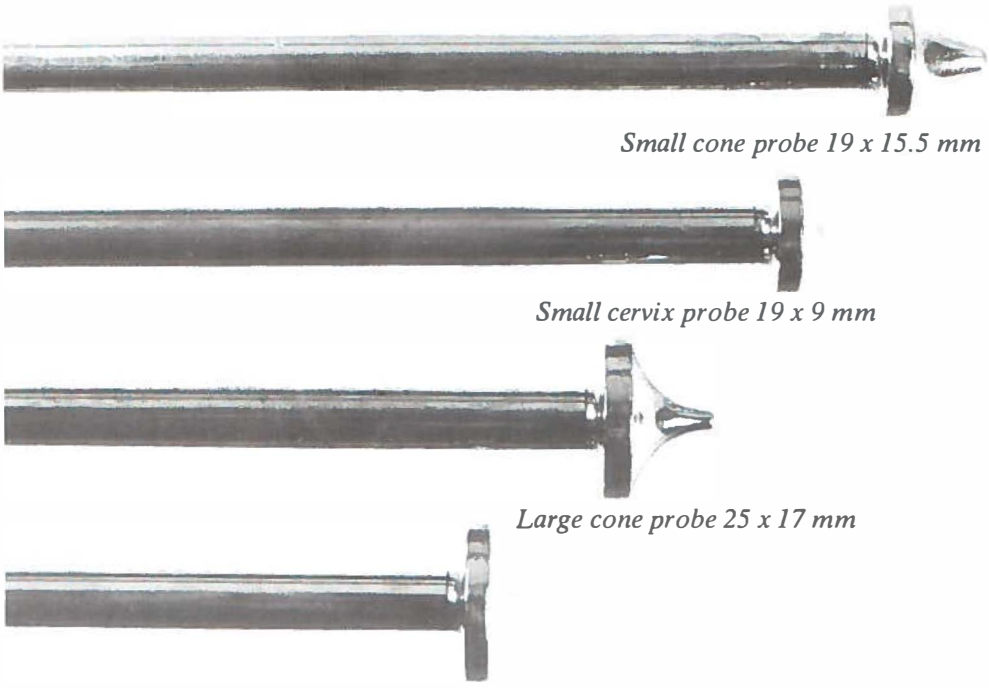
VI.2. Material and methods

This study has been performed in 54 women. Age, period of life and parity of the women and the shape of the external os of the uterine cervix are given in table VI.1. In the majority of women (61.1%) the age ranged between 40 and 49 years. Nine women were 50 years or older, 6 of the 54 women were postmenopausal; only two women were nulliparous.

With informed consent of the women, cryosurgery was applied to the uterine cervix, the day before they underwent a hysterectomy for a benign disease. A cryosurgical system, with nitrous oxide as a refrigerant was used in all cases. This equipment produces a probe tip temperature of about -70 to -80°C. Basically, two types of probes have been tested: a probe with a cone-shaped tip, the so-called cone probe, and a more flat one, the so-called cervix probe. From each type a small and a large one has been used, resulting in 4 different probes with the following tip diameters and lengths respectively: 19 × 15.5 mm, 19 × 9 mm, 25 × 17 mm and 25.4 × 7.87 mm (see figure VI.1). The large probe was only used in case both the antero-posterior and transverse diameter was at least 35 mm.

The small cone probe was tested in 14 women, the small cervix probe in 20 and the large cone and cervix probe in 12 and 8 women, respectively (Table VI.1).

All the probes had a tip temperature indicator. The unit was equipped with a monitor to register both the tip temperature and the tissue temperature by a thermocouple needle. The system also had a gas purifier to avoid inefficient and unreliable freezing, due to blockage of the tubes by pollution. To guarantee optimal freeze, a minimal pressure of 40 kg/cm² in the nitrous oxide tank was maintained before and after



Small cone probe 19 x 15.5 mm

Small cervix probe 19 x 9 mm

Large cone probe 25 x 17 mm

Large cervix probe 25.4 x 7.87 mm

Figure VI.1: The four probes used in this study.

Table VI.1: Type of probe with distribution of age, parity, period of life and shape of external os (N = number of women)

probe type	N	age			parity		period of life		shape of external os	
		30-39	40-49	50-59	0	1	pre-menopause	post-menopause	slit shaped	round
small cone probe 19 × 15.5	14	3	9	2		14	13	1	11	3
small cervix probe 19 × 9	20	6	9	5	2	18	15	5	12	8
large cone probe 25 × 17	12	2	9	1		12	12		9	3
large cervix probe 25.4 × 7.87	8	1	6	1		8	8		7	1
Total	54	12 22.2%	33 61.1%	9 16.7%	2 3.7%	52 96.3%	48 88.9%	6 11.1%	39 72.2%	15 27.8%

Figure VI.2: A pair of compasses with an externally readible scale, constructed for measuring of the frozen area on the portio (The width of the compasses can be fixed with a screw).

freezing. Before freezing a thin layer of water-soluble lubricant was applied on the probe, allowing a better heat transfer and resulting in a more uniform freezing. The freeze-thraw-freeze technique was used.

The required freeze time to reach a certain extension of the cryolesion varies and depends mainly on the blood circulation in the tissue. Therefore the size of the achieved iceball around the probe is more important than the length of freeze (Creasman et al. 1984). In this study the size of the frozen area was kept constant and as a consequence the freeze time varied. The diameter of the frozen area on the ectocervix in antero-posterior and transverse direction was measured with a pair of compasses with an externally readible scale, constructed for this purpose (see figure VI.2). Freezing was stopped when the antero-posterior diameter of the iceball was 10 mm larger than the diameter of the probe; that is 29 mm for the small probe and 35 mm for the large probe. Next, the cervix was allowed to thaw for 5 minutes, followed by a second freezing procedure with the same criteria.

After extirpation of the uterus, the cervix was amputated and fixed in 8% formalin for 24 hours. From the fixed cervices two tissue blocks were cut at the 6 and 12 o'clock positions and for technical reasons 1 block at the 3 and 9 o'clock positions. Thus 6 blocks were cut from each cervix. The tissue was processed using standard methods and from each tissue block single sections of 5 μm were cut in a sagittal direction, perpendicularly to the mucosal surface, resulting in 324 slides. After mounting the slides were stained with hematoxylin only and microscopically examined. The frozen area was pale due to fading of nuclei and edema. The mucosal lining of surface and crypts was necrotic and usually sloughed off. The veins and capillaries were dilated and congested and showed varying degrees of thrombosis. In the margin of the frozen area extensive margination and diapedesis of polymorph nuclear leucocytes was seen.

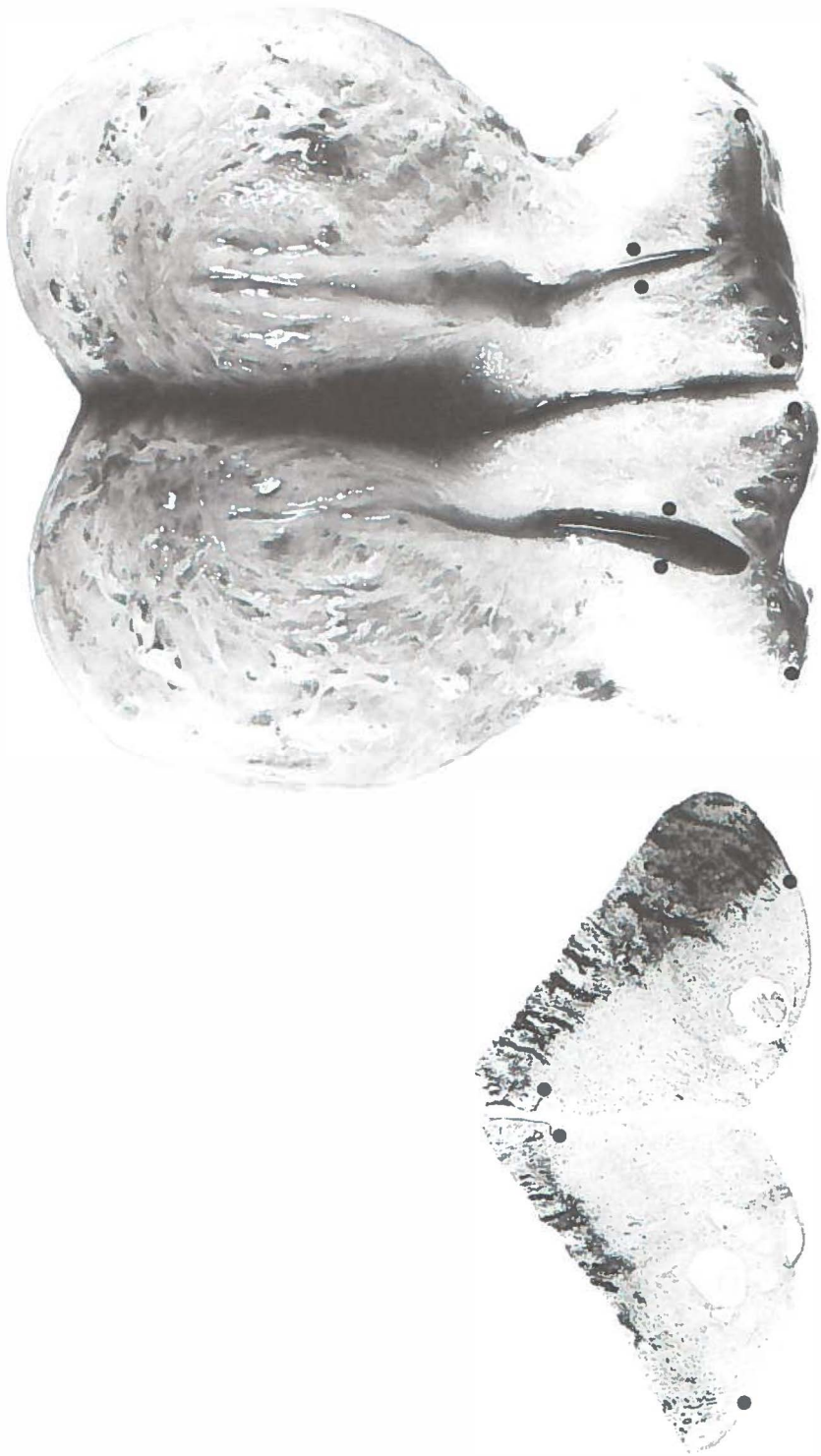


Figure VI.3: a. Uterus showing the cut surface of the cervix with the cryolesion. b. Sagittal Section of the cryolesion. The frozen area is negative in the enzymhistochemical staining for NADPH-reductase. x 2, (The localization of the cryolesion is marked by dots).

These histological features allowed reliable recognition of the frozen area. In figure V1. 3a the cryolesion is macroscopically shown in a cut section of the uterus. In a sagittal section of the cervix (figure V1.3b) the frozen area is negative in the enzymhistochemical staining for NADPH-reductase and therefore the border with the unfrozen area is clearly visible.
After a microscopic examination, all histological preparations were directly projected through a photographic magnifier onto white paper with magnification of 8×. Next the contour of the cervix and the border of the frozen area were drawn in pencil on the paper. The reference point R (the tangent of the ectocervix, see Chapter IV.2) was marked on the drawing.

From these drawings, in the same way as described for measuring the depth of the crypts (see Chapter IV.2), the following measurements have been performed with a digitizer:

I. The extension of the cryolesion, measured at regular intervals perpendicular to the ecto- and endocervical surface.
II. The distance of the maximum extension of the cryolesion to the reference point R.
III. The distance of the distal border of the cryolesion to the reference point R (see figure VI.4).

With the stylus a line is drawn from the reference point along the line, representing the surface of the cervix. The part of the cervical surface distal of the reference point is indicated by negative values. The line is divided in equal parts of 3 mm and the x, y coordinates stored in the memory. Next the x, y coordinates of the border of the cryolesion are obtained by pressing the stylus at regular points on the line representing this border. The depth of the cryolesion (the distance between the border of the cryolesion and the cervical surface) was established. These values are assigned to 3 mm areas, ranging from - 24 mm till + 60 mm from the reference point. The distance to the reference point is measured from the centre of a given 3 mm area. Clinical data were added via the keyboard.

All data where transmitted to a main frame computer, where statistical analyses were performed, using SPSS (Statistical Package for the Social Sciences, Nie et al. 1975).

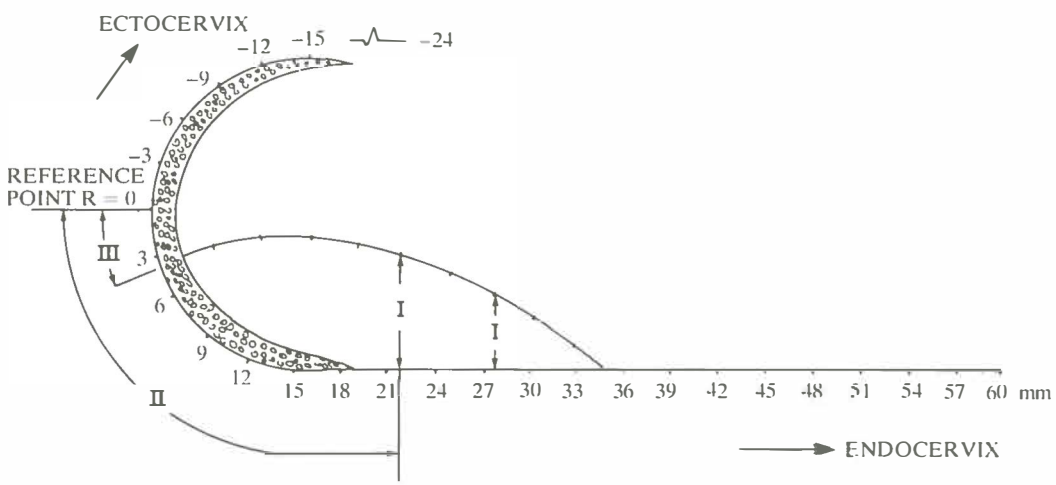


Figure VI.4: Measurements of the cryolesions, made in sagittal slides of the uterine cervix.
R = reference point
I = maximum extension of the cryolesion in the different areas
II = the distance of the maximum extension of the cryolesion to the reference point R
III = the distance of the distal border of the cryolesion to the reference point R

Table VI.2: Required first (t_1) and second (t_2) freeze time for different probes and relation to age, period of life and parity (N = number of women)

	entire population	type of probe				probe 19 × 9 (N = 19 evaluable women)							
						age			period of life		parity		
		small cone 19 × 15.5	small cervix 19 × 9	large cone 25 × 17	large cervix 25.4 × 7.87	30-39	40-49	50-59	pre-menopause	post-menopause	nulli parous	parous	
	N mean	N mean ± SD	N mean ± SD	N mean ± SD	N mean ± SD	N mean ± SD	N mean ± SD	N mean ± SD	N mean ± SD	N mean ± SD	N mean ± SD	N mean ± SD	
t_1	51 125.4	12 124.4±25.5	19 112.6±22.2	12 137.0±22.5	8 139.9±24.8	6 132.2±22.4	9 105.1±15.9	4 100.0±15.2	15 113.9±22.8	4 107.8±20.4	2 107.5±10.2	17 113.2±23.2	
t_2	52 97.9	13 89.5±21.7	19 88.8±16.6	12 117.3±28.8	8 103.9±14.9	5 101.2±18.4	9 87.6±13.9	5 78.8±12.4	14 89.7±16.5	5 86.4±17.7	2 78.5± 6.6	17 90.1±17.1	

If no cryolesion was present in certain areas, the extension of the cryolesion was recorded as zero. A localization of the cryolesion distal to the reference point was indicated by a negative value of II and III. All the results have been corrected for 15% shrinkage due to processing tissue for histological examination (Boonstra et al. 1983, Chapter III).

VI.3. Results

VI.3.1. Duration of freezing

The freeze time proved to depend on the probe-tip temperature. It was observed that due to too low nitrous oxide tank pressure, in a few cases the probe-tip temperature decreased insufficiently, resulting in a prolonged freeze time. These extremely prolonged freeze times were excluded from the evaluation of the mean freeze time, leaving 51 women in which the first freeze time (t_1) and 52 women in which the second freeze time (t_2) could be evaluated. The overall mean first and second freeze times were 125.4 and 97.9 sec respectively (table VI.2).

Also in table VI.2 the first and second freeze time required for the different probes and its variety with age, period of life and parity, are summarized. Five of the 6 postmenopausal women and both nulliparous women belong to the group of women whose cervices were frozen with the small cervix probe (19 × 9) and also in that group the most proportional age distribution was found. Therefore in that specific group the variation of freeze time with age, period of life and parity was evaluated. The required freeze times were significantly less when the small probes were used. Also a significant difference in freeze time was found between using the small cervix and the small cone probe, while it did not matter whether the large cervix or large cone probe was used. Compared with the older age groups, a significantly longer freeze time was necessary in cervices of younger women. The variation in duration of freeze in the pre- and postmenopausal and the nulliparous and parous women was not significantly different (see table VI.2).

VI.3.2. Extension of the cryolesion

In three women at the 3 o'clock position and in two women at the 9 o'clock position, a deeply scarred cervix was observed. After freezing it was noticed that the resulting iceball was asymmetric and did not extend into the scarred irregular surface. It was clear that in those areas suboptimal freezing would occur. These obviously insufficient lesions (n=5) have been excluded from the evaluation of the cryolesions. Consequently of the 54 women, 51 slides were examined at the 3 o'clock position, 52 at the 9 o'clock and 108 each at the 6 and 12 o'clock positions.

In table VI.3. the Maximum, Minimum and Mean Maximum Extension (MME) of the cryolesions at different clock positions and using the four types of probes are summarized. The MME at the 3 and 9 o'clock positions is significantly less as compared to the 6 and 12 o'clock positions.

The difference between the MME of the lesions obtained with the small and the large probes, is also evident especially at the 6 and 12 o'clock positions.

The MME of the cryolesions in relation to age, period of life and parity at the different clock positions were, for the same reason as previously mentioned in connection to the freeze time (Chapter VI.3.1) evaluated only in cryolesions obtained with the small cervix probe. No obvious differences could be observed between cryolesions in the different age-groups and between nulliparous and parous women. Only at the 6 o'clock position the MME of the cryolesion in cervices of postmenopausal women was significantly greater, compared to the premenopausal women. However, this was not observed at the other clock positions (For documentation: see appendix table 15).

The MME of the cryolesion in cervices with a slit shaped and round external os is compared in table VI.4. The majority of women with a round external os, 8 of the

15, were represented in the group frozen with the small cervix probe. Moreover, only in that group a sufficient number of women with round external os were comparable with those who had a slit shaped os (8 versus 12). For those reasons the variation of the MME with the shape of external os was studied in the small cervix probe population.

Table VI.3: Mean Maximum, Maximum and Minimum Extension (mm) of cryolesions achieved with different probes at 3, 6, 9 and 12 o'clock positions

probe and clock position	N (slides)	extension of cryolesions Mean Maximum Ext. MME ± SD	Max.	Min.
small cone probe (19 × 15,5)				
– 3 o'clock	13	4.7 ± 2.6	9.1	0
– 6 o'clock	28	7.0 ± 1.9	9.8	3.6
– 9 o'clock	13	5.4 ± 1.8	8.6	2.8
– 12 o'clock	28	6.7 ± 1.6	10.3	3.6
small cervix probe (19 × 9)				
– 3 o'clock	20	4.8 ± 2.0	9.1	0
– 6 o'clock	40	6.5 ± 1.3	9.1	3.5
– 9 o'clock	20	4.2 ± 2.1	7.3	0
– 12 o'clock	40	6.2 ± 1.6	9.6	2.6
large cone probe (25 × 17)				
– 3 o'clock	10	5.4 ± 1.9	8.6	1.7
– 6 o'clock	24	7.9 ± 1.2	10.8	6.2
– 9 o'clock	12	4.9 ± 1.9	7.5	2.0
– 12 o'clock	24	8.6 ± 1.5	12.0	6.7
large cervic probe (25.4 × 7.87)				
– 3 o'clock	8	5.0 ± 1.9	8.1	2.2
– 6 o'clock	16	7.8 ± 2.2	11.4	4.3
– 9 o'clock	7	4.9 ± 1.4	6.9	2.8
– 12 o'clock	16	8.7 ± 3.0	12.7	4.4

Table VI.4: Mean Maximum Extension (MME) and Maximum and Minimum Extension (mm) of cryolesions in relation to shape of external os at different clock positions (small cervix probe 19 × 9)

clock position and shape of external os		N women	N slides	Extension of cryolesions MME ± SD	Max.	Min.
– 3 o'clock	slit shaped ostium	12	12	4.2 ± 2.3	9.1	0
	round ostium	8	8	5.5 ± 1.5	7.9	3.1
– 6 o'clock	slit shaped ostium	12	24	6.2 ± 1.3	8.3	3.5
	round ostium	8	16	7.0 ± 1.2	9.1	5.6
– 9 o'clock	slit shaped ostium	12	12	3.9 ± 2.5	7.3	0
	round ostium	8	8	4.7 ± 1.5	6.5	3.0
– 12 o'clock	slit shaped ostium	12	24	6.0 ± 1.7	9.6	2.6
	round ostium	8	16	6.7 ± 1.5	8.8	4.2

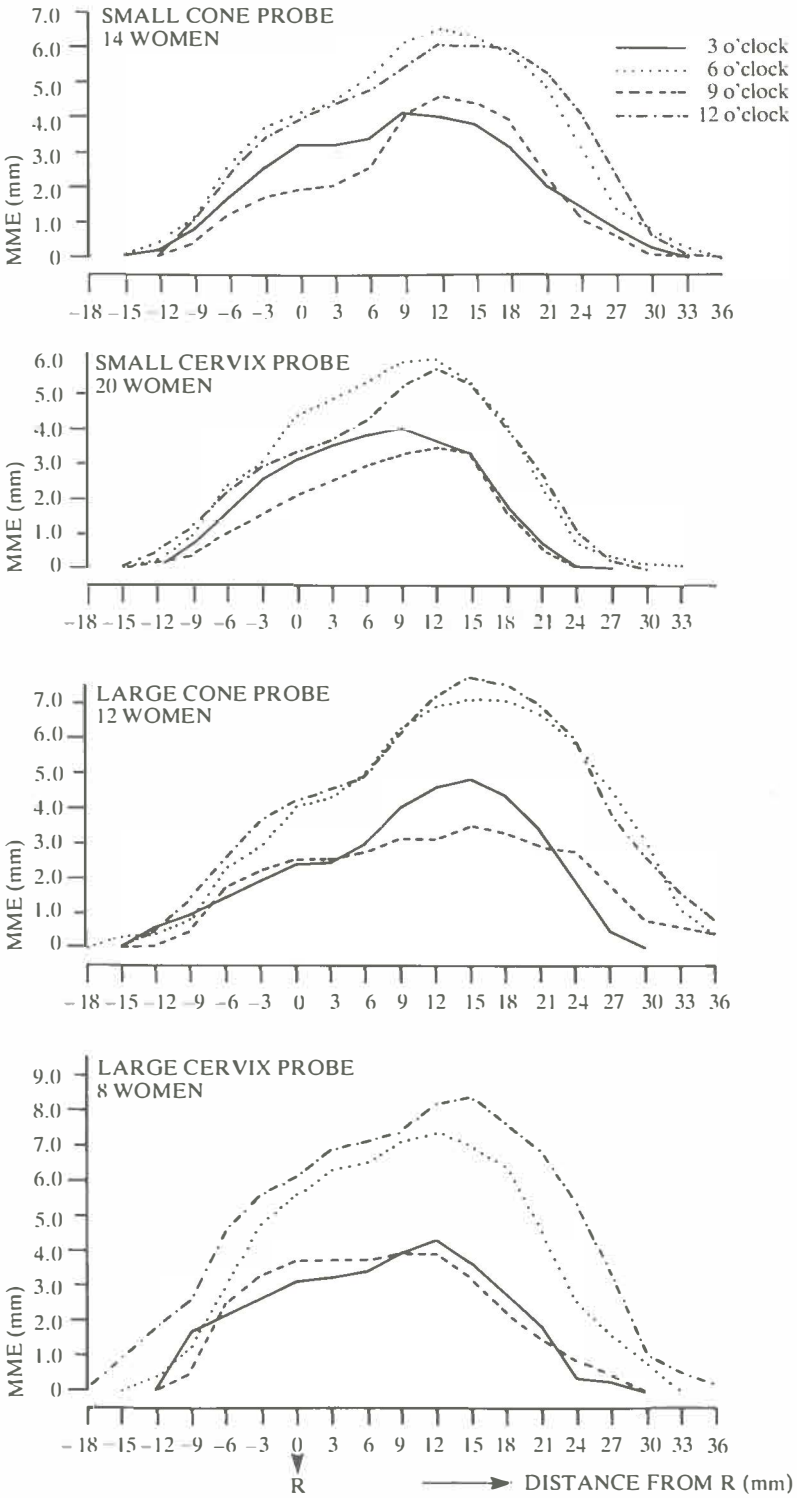


Figure VI.5: Mean Maximum Extension (mm) of the cryolesions at various distances from the reference point R, achieved with four types of probes at 3, 6, 9 and 12 o'clock positions (For documentation: see appendix table 16).

It is imaginable that a slit shaped external os will influence the extension especially at the 3 and 9 o'clock positions. Therefore the extension was investigated at all four clock positions. As was noticed previously, a difference in MME between on the one hand 3 and 9 o'clock and at the other hand 6 and 12 o'clock was observed. However, this difference does not seem to depend on the shape of the external os. In both, slit shaped and round external os, the MME of the cryolesion at the 3 and 9 o'clock positions is less than at the 6 or 12 o'clock positions.

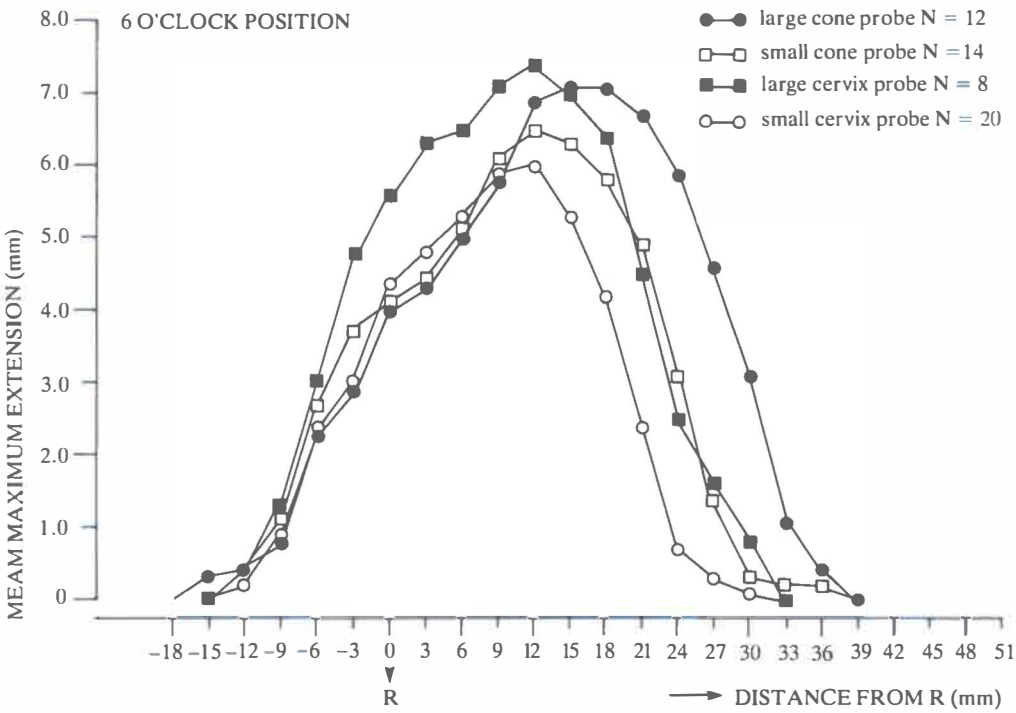


Figure VI.6: Mean Maximum Extension (mm) of the cryolesions at various distances from the reference point R, achieved with four types of probes at 6 o'clock position (For documentation: see appendix table 16).

In figure VI.5 the MME of the cryolesions at various distances from R are shown. Diagrams of the cryolesions at the 3, 6, 9 and 12 o'clock positions, achieved with the four types of probes, are demonstrated separately. The diagrams show that at various distances from R the cryolesions at the 6 and 12 o'clock positions are also more extensive than at the 3 and 9 o'clock positions (see figure VI.5). These differences can be observed in cryolesions obtained with all types of probes.

In figure VI.6 the differences between the cryolesions performed with the different probes at the same clock position can be compared. For this the result at the 6 o'clock position has been used. As can be seen, each probe produces its own characteristic shape of lesion, depending on the length and diameter of the probe. The differences between the cryolesions of the four types of probes can be summarized as follows:

- The maximum extension of the cryolesions obtained with the large cone and cervix probes is more extensive than those obtained with the small probes. This is in accordance with what previously has been demonstrated about the MME.
- The maximum value of the large flat cervix probe is 12 mm removed from the reference point R, which is nearer than the maximum of the large cone probe whose distance to the reference point is between 15 and 18 mm. This is less

pronounced when the maximum values of the small flat cervix and small cone probes were compared.
- The extension of the cryolesion in the endocervix is most extensive when the large cone probe has been used and least extensive with the small flat cervix probe. As far as the small cone and large flat cervix probe are concerned, the extension in the endocervix is almost the same and in between the two extremes.

VI.3.3. Analysis of failures

In four slides no cryolesion was found, in an additional five slides the cryolesion extended less than 2 mm in the stroma. These 9 slides were at the 3 or 9 o'clock positions, in cervices with a slit shaped external os.

In a previous study (Chapter V.3.3.1) it appeared that the CIN-III involves the cervical surface epithelium from 8.2 ± 4.4 mm to 13.3 ± 3.7 mm from the reference point. That means that in about 95% of the patients (Mean ±2 SD) the CIN-III occurred 0.6 mm distally from the reference point to 20.7 mm proximally to it. In that part of the cervix, at the 12 o'clock position, which was representative for the entire cervix, the deepest Mean Maximum Depth of the crypts was 2.7 ± 1.5 mm (Chapter IV.3.2: Figure IV.3). This implies that at least 95% of the crypts (Mean ± 2 SD) do not exceed a depth of 5.7 mm.

In the previous study about the depth of CIN-III crypt involvement (Chapter V.3.1) it was found that the ratio between the deepest involvement and the deepest crypt is 65%. It follows that the deepest involvement will not exceed a depth of 3.7 mm (65% from 5.7 mm).

The same conclusion can be drawn when the actual measured depth of crypt involvement is taken as a starting-point. In Chapter V.3.1. it was found that the Mean Maximum Depth of CIN-III crypt involvement was 1.6 ± 1.0 mm. That means that in at least 95% of the patients (Mean ± 2 SD) the involvement does not exceed 3.6 mm. On the basis of this, the depth of the cryolesion should be at least 4 mm.

The mean linear extent of CIN-III lesions varies in the literature between 6 and 11 mm (Chapter II) and in this study (Chapter V) a mean linear extent of 7.4 ± 3.7 mm was found. That implies that at least over a distance of approximately 15 mm (Mean ± 2 SD = 14.8 mm) the cryolesion should have a mean maximum depth of 4 mm. In this study the depth of the cryolesion is measured in areas of 3 mm each, which means that the distance of 15 mm corresponds to 5 areas. Therefore all cryolesions, having 5 or more areas in a row a MME of 4 mm or more, were considered to be sufficient. When 3-4 areas in a row showed a mean maximum extension of at least 4 mm it was estimated as a moderate lesion and lesions with less than 3 areas in a row as insufficient. Following these criteria 21.6% of all cryolesions proved to be insufficient, 11.0% moderate and 67.4% sufficient (table VI.5).

From table VI.5 it can be seen that in the different age-groups, in pre- and postmenopausal women and in nulliparous and parous women a comparable distribution between insufficient, moderate and sufficient lesions was found. These factors apparently do not influence the extension of the cryolesions. That does not hold for clock position, shape of external os and type of probe. At the 3 as well as at the 9 o'clock positions about half on the lesions were insufficient and only about 35-39% were sufficient, the rest was moderate. This is in contrast to the 6 and 12 o'clock positions at which the majority of the lesions were sufficient (82.4% and 81.5% respectively) and only 6.5% and 8.3% respectively, insufficient. In 54 of the 229 slides (23.6%) from cervices with a slit shaped external os an insufficient lesion was found as compared to 16.7% in case of round shaped external os. This difference was not significant. The frequency of sufficient lesions in cervices with slit and round shaped external os, i.e. 65.0% and 73.3% respectively, were also not significantly different. Cryolesions obtained with a small cone probe proved to be insufficient in 19.5% and sufficient in 67.1%. That is almost the same as found in the entire population. Cryolesions obtained with a large cone probe were less frequently insufficient compared to the entire population (17.1%), while a significantly higher percentage of

Table VI.5: Frequency of insufficient, moderate and sufficient cryolesions in the entire population, and in relation to clock position, age, period of life, parity, shape of external os and type of probe (N = number of slides)

extension of cryoplesion	entire population	clock position				age			period of life		parity		shape of external os		type of probe			
		3 o'clock	6 o'clock	9 o'clock	12 o'clock	10-39 yrs	40-49 yrs	50-59 yrs	pre-menop.	post-menop.	nulli-parous	parous	slit shaped	round	small cone	small cervix	large cone	large cervix
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
insufficient	69 (21.6)	28 (54.9)	7 (6.5)	25 (48.0)	9 (8.3)	10 (13.9)	50 (25.9)	9 (16.7)	60 (21.2)	9 (25.0)	1 (8.3)	68 (22.1)	54 (23.6)	15 (16.7)	16 (19.5)	31 (25.8)	12 (17.1)	10 (21.3)
moderate	35 (11.0)	3 (5.9)	12 (11.1)	9 (17.3)	11 (10.2)	9 (12.5)	17 (8.8)	9 (16.7)	30 (10.6)	5 (13.9)	4 (33.3)	31 (10.1)	26 (11.4)	9 (10.0)	11 (13.4)	19 (15.8)	2 (2.9)	3 (6.4)
sufficient	215 (67.4)	20 (39.2)	89 (82.4)	18 (34.6)	88 (81.5)	53 (73.6)	126 (65.3)	36 (66.6)	193 (68.2)	22 (61.1)	7 (58.4)	208 (67.8)	149 (65.0)	66 (73.3)	55 (67.1)	70 (58.4)	56 (80.0)	34 (72.3)
total	319 (100)	51 (100)	108 (100)	52 (100)	108 (100)	72 (100)	193 (100)	54 (100)	283 (100)	36 (100)	12 (100)	307 (100)	229 (100)	90 (100)	82 (100)	120 (100)	70 (100)	47 (100)

sufficient lesions were achieved with this large type of probe (80.0%). The incidence of insufficient and moderate lesions is the highest in the small cervix probe group (25.8% and 15.8% respectively) and 58.4% of the lesions in this group is sufficient, being a much lower percentage than in the large probe groups.

In summary, it can be concluded that the extension of the cryolesions can be influenced by the type of probe, the anatomical clock position and possibly by the shape of the external os of the cervix.

The question arises whether the high incidence of failures at the 3 and 9 o'clock positions is causality related to the shape of the external os.

From the figures (table VI.6) this is not apparent. Of the 69 insufficient lesions 53 were found at the 3 and 9 o'clock and 16 at the 6 and 12 o'clock positions. In case of a relation between a slit shaped external os and insufficient lesions at the 3 and 9 o'clock positions, it would be expected that a relatively high percentage of 3 and 9 o'clock failures were found in cervices with slit shaped external os. It appeared that at the 3 or 9 o'clock positions 73.6% of the insufficient lesions was found in cervices with a slit shaped external os. This percentage is only slightly more compared to the percentage slit shaped external os of the total number of slides at 3 and 9 o'clock positions (70.9%). Contrary to expectation, a relatively high percentage of the failures at 6 and 12 o'clock was found in cervices with a slit shaped external os (93.8%). In case of a moderate extension of the cryolesion, at 3 and 9 o'clock 83.3% were observed in cervices with a slit shaped external os, being 12.4% more than the percentage slit shaped external os in the entire 3 and 9 o'clock population. It can be concluded that the slit shaped external os is not the main factor causing an insufficient extension of the cryolesion at 3 or 9 o'clock positions, although it may contribute to it.

Two factors remain under consideration: type of probe and anatomical clock position. From the figures in table VI.7 it is very suggestive that these two factors

Table VI.6: Frequency of slit shaped and round external os in insufficient, moderate and sufficient cryolesions in relation to clock position (N = number of slides)

extension of cryolesion and clock position		total N	shape of external os: slit shaped N (%)	shape of external os: round N (%)
insufficient	3 + 9	53	39 (73.6)	14 (26.4)
	6 + 12	16	15 (93.8)	1 (6.2)
		69	54 (78.3)	15 (21.7)
moderate	3 + 9	12	10 (83.3)	2 (16.7)
	6 + 12	23	16 (69.6)	7 (30.4)
		35	26 (74.3)	9 (25.7)
sufficient	3 + 9	38	24 (63.2)	14 (36.8)
	6 + 12	177	125 (70.6)	52 (29.4)
		215	149 (69.3)	66 (30.7)
total	3 + 9	103	73 (70.9)	30 (29.1)
	6 + 12	216	156 (72.2)	60 (27.8)
		319	229 (71.8)	90 (28.2)

Table VI.7: Success and failure rate for each probe at different clock positions (N = number of slides)

type of probe and clock position		N	cryolesion insufficient N(%)	moderate N(%)	sufficient N(%)
small cone probe	3 + 9	26	10 (38.5)	7 (26.9)	9 (34.6)
	6 + 12	56	6 (10.6)	4 (7.2)	46 (82.2)
small cervix probe	3 + 9	40	24 (60.0)	2 (5.0)	14 (35.0)
	6 + 12	80	7 (8.8)	17 (21.2)	56 (70.0)
large cone probe	3 + 9	22	12 (54.5)	2 (9.1)	8 (36.4)
	6 + 12	48	0 (0)	0 (0)	48 (100.0)
large cervix probe	3 + 9	15	7 (46.7)	1 (6.6)	7 (46.7)
	6 + 12	32	3 (9.4)	2 (6.2)	27 (84.4)
entire population	3 + 9	103	53 (51.5)	12 (11.6)	38 (36.9)
	6 + 12	216	16 (7.4)	23 (10.7)	177 (81.9)
total		319	69 (21.6)	35 (11.0)	215 (67.4)

independently influence the extension of the cryolesion. Considering the entire population 53 (=51.5%) of all the cryolesions at 3 and 9 o'clock were insufficient and only 7.4% of all cryolesions at 6 and 12 o'clock.

By tracing this for the four different probes, the influence of the probe-type can be excluded. It appears that for each probe-type the failures at 3 and 9 o'clock are much higher than at 6 and 12 o'clock. Thus, independent of what type of probe has been used, the failure rate is always significantly higher in the 3 and 9 o'clock group. However, the failure rate is not the same for each type of probe. At the 3 and 9 o'clock positions the lowest failure rate is found with the small cone probe (38.5%) and the highest failure rate occurs with the small flat cervix probe (60.0%). It is striking, that the large cone probe is not ineffective at all at the 6 and 12 o'clock positions, it does not even produce moderate lesions, all lesions were sufficient, in other words, a 100% success-rate. However, the lesions obtained with the small cone and large cervix probe at the 6 and 12 o'clock positions were only in about 80% of the cases successful. Only the small flat cervix probe showed a lower success-rate at the 6 and 12 o'clock positions of 70%. At the 3 and 9 o'clock positions the success-rate is significantly lower for all types of probes and varies between 34.6 and 46.7%.

Thus by considering the results only at the 6 and 12 o'clock positions, in other words, by eliminating the factor which negatively influences the extension of the cryolesions at 3 and 9 o'clock, it is evident that the large cone probe gave the best results, followed by the small cone and large cervix probe. The small cervix probe emerges as the probe with the worst results.

It was evident that for every probe the anatomical clock position was the main factor determining the extension of the cryolesion and thus the sufficiency of the lesion.

Finally the conclusion can be drawn, that in one way or another at the 3 and 9 o'clock positions there are circumstances inhibiting the extension of the cryolesion. Therefore these positions are at risk concerning the cryosurgical treatment of CIN!

VI.3.4. Topography of the cryolesion

VI.3.4.1. Localization of the maximum extension and the distal border of the cryolesion

In table VI.8 the mean distance from the maximum extension and the distal border

of the cryolesion to the reference point is shown in mm at the different clock positions and for all types of probes. In general the cryolesion starts about 2-3 mm distally (negative value) from the reference point R. This distance did not differ significantly between cryolesions achieved with different probes and at the different clock positions. The distance from the distal border of the cryolesion to the reference point ranges between 15 mm proximally and 21 mm distally.

Table VI.8: Mean, maximum and minimum distance (mm) from maximum extension and distal border of the cryolesions to reference point R. Different probes, at different clock positions (N = number of slides)

type of probe and clock position	N	distance from maximum extension of the cryolesion to reference point R			distance from distal border of the cryolesion to reference point R		
		mean ± SD	max.	min.	mean ± SD	max.	min.
small cone probe (19 × 15.5)							
– 3 o'clock	12	9.0 ± 5.9	18	0	−3.9 ± 8.2	9	−21
– 6 o'clock	28	11.9 ± 4.5	18	0	−1.7 ± 7.2	12	−12
– 9 o'clock	13	11.3 ± 7.1	24	0	2.1 ± 7.7	15	− 9
– 12 o'clock	28	13.4 ± 8.1	30	0	−2.8 ± 6.5	9	−12
small cervix probe (19 × 9)							
– 3 o'clock	19	9.6 ± 5.5	21	0	−2.0 ± 8.8	15	−21
– 6 o'clock	40	10.4 ± 4.5	15	0	−2.8 ± 6.0	12	−12
– 9 o'clock	18	11.0 ± 4.4	18	3	−0.9 ± 10.1	15	−21
– 12 o'clock	40	11.6 ± 6.2	24	0	−1.3 ± 7.7	12	−15
large cone probe (25 × 17)							
– 3 o'clock	10	13.2 ± 4.0	18	3	0 ± 8.4	9	−12
– 6 o'clock	24	14.3 ± 3.7	21	9	−1.5 ± 8.1	15	−18
– 9 o'clock	12	12.8 ± 8.6	27	0	0.8 ± 9.6	15	−12
– 12 o'clock	24	14.8 ± 5.8	24	0	−2.3 ± 8.3	12	−15
large cervix probe (25.4 × 7.87)							
– 3 o'clock	8	8.3 ± 7.8	15	−9	−0.8 ± 8.7	12	− 9
– 6 o'clock	16	11.4 ± 4.9	21	0	−3.8 ± 5.6	9	− 9
– 9 o'clock	7	8.6 ± 7.2	21	0	−2.6 ± 8.2	12	− 9
– 12 o'clock	16	13.3 ± 4.2	18	3	−4.7 ± 9.2	12	−18

It appeared that, apart from a significant difference of this distance in cryolesions of nulliparous and parous women at the 12 o'clock position, it did not vary with age, period of life and parity. Even between slit shaped and round external os no significant difference could be found in the mean distance from the distal border of the cryolesion to the reference point R (for documentation see appendix table 17 and 18).

The mean distance from the maximum extension of the cryolesion to the reference point varied from 8.3 to 14.8 mm ranging from 30 mm proximally to 9 mm distally from the reference point. This distance tends to increase with the use of cone probes (table VI.8). This tendency could also be observed in the graphs of the cryolesions of the different probes and at different clock positions (figure VI.5). However, only at the 6 o'clock position in the large cone group this increase in distance was significantly different ($p < 0.05$).

In general it seems that at the 3 and 9 o'clock the maximum extension of the cryolesions is located slightly nearer to the reference point than at the 6 and 12 o'clock positions. However these differences are not significant. The distance from the maximum extension of the cryolesion to the reference point does not vary with age,

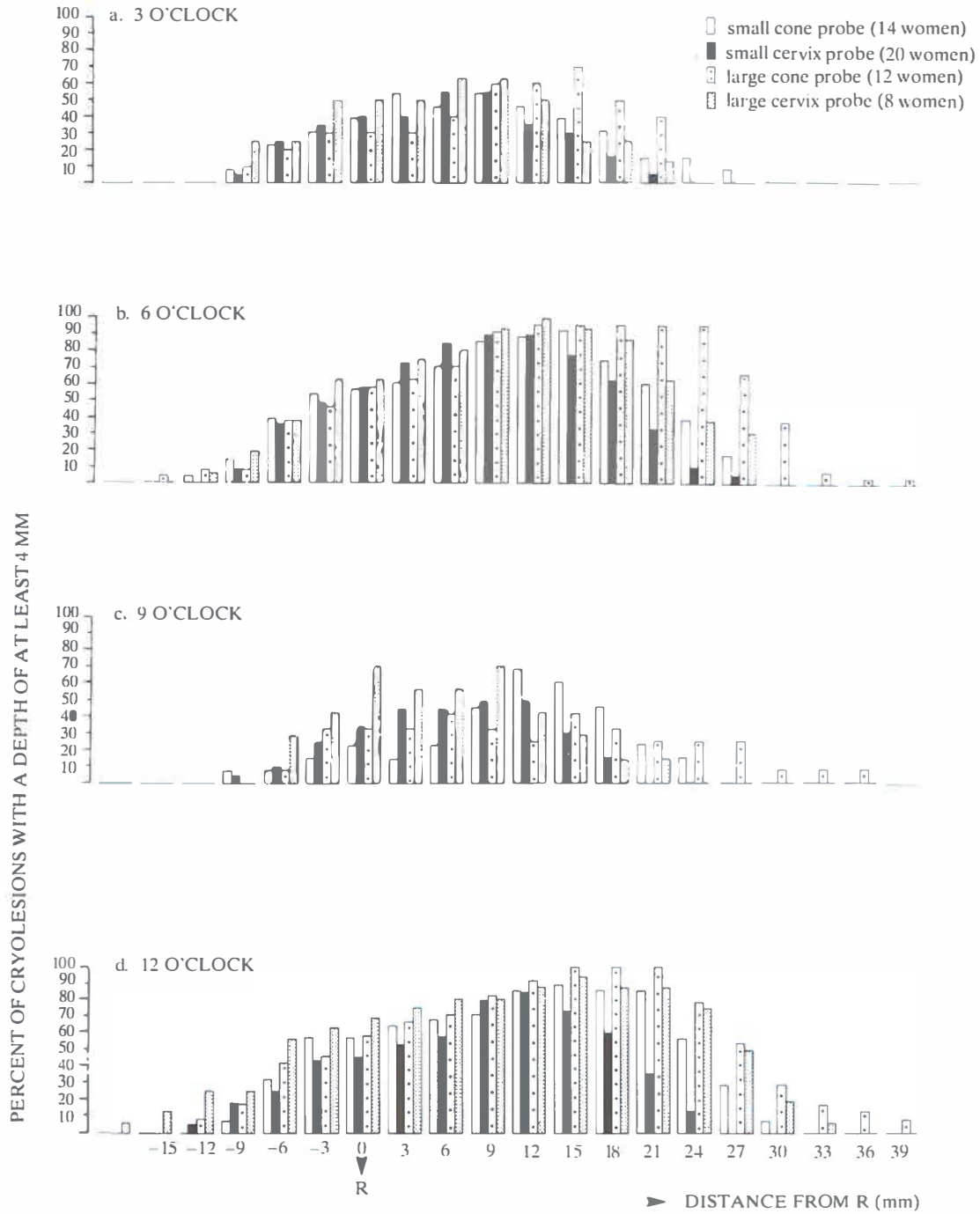


Figure VI.7: Frequency (%) of cryolesions with a depth of at least 4 mm at the various distances from the reference point at the 3, 6, 9 and 12 o'clock positions.

period of life, parity and shape of external os (for documentation see appendix table 17 and 18).

VI.3.4.2. Localization of the sufficient part of the cryolesion

For clinical purposes, it is interesting to evaluate with which frequency a sufficient lesion is found at the various distances from R. Therefore at each 3 mm distance the number of cryolesions, having a depth of 4 mm or more, was counted. This has been performed for all types of probes at different clock positions (figure VI.7a,b,c,d). For example, considering the 12 o'clock position (figure VI.7d), it can be seen that between 12 and 21 mm proximal from the reference point R only the cryolesions obtained with the large cone, large cervix and small cone probe have in more than 80% of the cases a depth of at least 4 mm. The small flat cervix probe produces only at a distance of 12 mm proximal from R in more than 80% of the cases cryolesions deeper than 4 mm. At the other distances from R this is the case in 80% or less and at more than 18 mm proximal from R this percentage even drops to 35% and less. It is clear that the other probes produce more effective cryolesions at this localization. Although to a lesser extent the same trend can be observed in distal direction. It is also evident that the large cone probe is most effective from 12 mm up to and including 21 mm proximal from the reference point (even 92-100% of the cryolesions are deeper than 4 mm). Comparing these results of the 12 o'clock positions with the 3 and 9 o'clock positions (figure VI.7a and c) it is conspicuous that at almost all distances from R at 3 and 9 o'clock the percentage of the cryolesions deeper than 4 mm is low. This phenomenon was observed for all types of probes, but most noticeable when the small flat cervix probe was used.

In conclusion, over a distance of 12-21 mm proximal from the reference point R only the large cone and cervix probe and the small cone probe, produce in 80-100% of the cases sufficient cryolesions at the 12 o'clock position, while at the 6 o'clock position the same is achieved over a distance of 9-15 mm from R (see figure VI.7b).

VI.4. Discussion

In accordance with the literature (Ostergard 1980, Monaghan and Townsend 1982, Disaia and Creasman 1984, Bryson et al. 1985), it was evident from the present study that insufficient freezing can arise from inadequate technique and equipment. A too low nitrous oxide tank pressure or diminished nitrous oxide flow by pollution of tubes resulting in an insufficiently low probe temperature, were very important causes of failures. In case the temperature did not drop to −70--80°C a prolonged freezing was necessary. Therefore probe-tip temperature monitoring was essential for a good application technique. The prolonged freeze time in younger women may be due to a more intensive circulation in the cervix at that age. In areas with deep cervical tears the cryolesion failed to develop properly.

Besides these obvious causes of failures, the results of this study revealed that type of probe and anatomical clock position were two independent factors influencing the size of the cryolesion.

By comparing the Mean Maximum Extension (MME) of the cryolesions performed with the four different probes, separately at each clock position, and by analysis of the failures it was evident that the small flat cervix probe produced lesions which were too small. The largest lesions as to depth and linear extension, were obtained with the large cone probe. The dimensions of the lesions produced with the small cone and large flat cervix probes were in between these two extremes.

The lesions at the 3 and 9 o'clock positions were much less extensive than at the 6 and 12 o'clock positions and often insufficient. This was not explained by the shape of the external os. Therefore, at these positions there must be another factor which limits the development of the cryolesion.

Conceivably differences in vascular supply between the lateral and the antero-

posterior aspects play a major role. The blood supply to the cervix is from three main sources: directly from the uterine artery, its cervicovaginal branch and from the vaginal artery. Some important features characterize the anatomical arrangement of the vascular supply (Gustafson 1976):

- First, the main stream of flow is along the lateral margins of the cervix.
- Second, there is a profuse anastomotic potential, maintaining a large blood flow at these specific sites (uterine and vaginal arteries).
- Third, the venous drainage flows laterally into a rich plexus on either side of the cervix.

This anatomical arrangement of the blood supply makes it very likely that the profuse vascular supply is the main cause of unsuccessful cryosurgery at the 3 and 9 o'clock position.

Bearing in mind that 21.6% of all the cryolesions are insufficient, and 67.4% sufficient, it must be concluded that the overall results are inacceptable. Even the probes with the best results, the cone shaped ones (small and large) and the large flat cervix probe, still produced insufficient cryolesions in about 17-21% (table VI.5). It is disappointing that more than 50% of the cryolesions at 3 and 9 o'clock were insufficient. Even when the probes with the best results were considered, the failure-rate at the 3 and 9 o'clock positions still varied between 38.5 and 54.5%. For the most ideal combinations (the small cone and large cone and cervix probes at 6 and 12 o'clock) the success rate varies between about 80-100%.

In general, it can be concluded that the way in which cryosurgery has been applied in this study is inadequate. In all probability, the duration of freezing, in this study determined by the 5 mm iceball around the probe, is too short to fully utilize all of the freezing potential. Further investigation will be necessary to establish an optimal freeze time.

From the graphs in figure VI.5 and 6 and the results on the mean distance of the maximum extension to the reference point R, it can be concluded that the most deepest part of the cryolesions, for all probe types, is located largely between the reference point and 24 mm proximally from it. In other words, topographically the sufficient part of the cryolesion extends in the endocervix over a distance of about 24 mm from the most caudal part of the ectocervix. This area includes the transformation zone in which the dysplastic changes mostly occur (Chapter II.2.3.2 and V). In Chapter V it was found that the CIN-III lesions are located at a mean distance of 8.2-13.3 mm proximal from the reference point R and that the crypt with the deepest involvement is found at a mean distance of 10.2 mm proximal from R.

In conclusion: The localization of the maximum extension of the cryolesions is suitable for the treatment of CIN, but with the current technique in many cases the cryolesions are not deep enough, especially not at the 3 and 9 o'clock positions and when the small flat cervix probe has been used.

VI.5. Summary and conclusions

The aim of this study was to measure the linear extension and depth of standard cryolesions and to evaluate whether the lesions were extensive enough for the treatment of CIN-III.

With four types of probes, a small and large cone probe, and a small and large flat cervix probe, a standardized cryolesion was applied in 54 women the day before they underwent a hysterectomy for a benign disease. The freeze-thaw-freeze technique was used. Freezing was sustained until a 5 mm ice-zone around the probe was achieved. After extirpation of the uterus the extension and localization of the cryolesion was morphometrically examined in histological slides using a computerized graphic tablet.

The required freeze time and the achieved extension of the cryolesion were studied in relation to the type of probe used, age, period of life (pre- and postmenopausal

state), and parity of the women. In addition it was investigated whether the extension of the cryolesion varied at the different anatomical clock positions (3, 6, 9 and 12 o'clock) and whether it was influenced by the shape of the external os.

A sufficiently high nitrous oxide tank pressure to guarantee an adequate low probe tip temperature was the first requirement for a good freeze procedure. This requirement being fulfilled we found the following:

1. The overall failure rate of 21.6% proved that determination of the freeze time by the size of the frosted area was inadequate. The resulting freeze time was too short to fully utilize the freezing potential. However, the too short freeze time allowed identification of factors causing insufficient cryolesions.
2. In younger patients a significantly longer freeze time was necessary to obtain a 5 mm ice-zone around the probe than in older patients. This was true for both small and large probes.
3. Cryolesions at the 3 and 9 o'clock positions were less extensive than at the 6 and 12 o'clock positions. More than 50% of the cryolesions at the 3 and 9 o'clock positions were insufficient as compared to 7.4% at the 6 and 12 o'clock positions. The failures at 3 and 9 o'clock were due to intensive circulation at those positions, and hardly due to a slit shaped external os.
4. The largest lesions, both as to depth and linear extension were obtained with the large cone probe. The small cone and large flat cervix probe produced smaller comparable extension of the cryolesion, with the exception that the lesions obtained with the large cervix probe penetrated deeper. The small flat cervix probe produced the smallest lesions.
5. The deepest part of the cryolesion is located in between the most caudal point of the ectocervix the reference point R and 24 mm proximally from it. This area includes the transformation zone in which the CIN-III is generally located and is therefore ideal for the treatment of CIN-III. However, the cryolesions as applied here, even when it was done with the cone probes (small and large) or with the large flat cervix probe, were only deep enough in too limited areas.
6. Therefore this study was carried on, applying cryosurgery with longer freeze time, in order to develop a better freeze technique.

CHAPTER VII

The effect of long freezing on the extension of the cryolesion

VII.1. Introduction

In a previous study (Chapter VI), the extension of a cryolesion achieved with a standardized freezing technique was evaluated. Freezing was sustained until a 5 mm wide frosted zone around the probe was achieved. The results showed that the overall failure rate was 21.6% and that 51.5% of the cryolesions at the 3 and 9 o'clock positions was insufficient.

Conceivably prolonged freezing produces a more extensive cryolesion thereby reducing the failure rate. To prove this assumption cryosurgery has been applied with long freeze time, in a small group of women.

VII.2. Material and methods

The study was carried out in 10 women, whose age, parity and period of life are summarized in table VII.1.

Table VII.1: Distribution of age, parity and period of life in 10 women with long freeze time cryosurgery.

	age		parity		period of life	
number of women	30-39	40-49	0	≥ 1	pre-menopause	post-menopause
10	4	6	3	7	10	0

All women were premenopausal and their age ranged between 30 and 49 years. Seven women were parous and three nulliparous.

With informed consent a cryolesion was applied on the uterine cervix the day before they had a hysterectomy. The freezing was carried out with a cryosurgical system with nitrous oxide as a refrigerant. Only the small cone probe (19 × 15.5 mm) was used. The probe was equipped with a tip temperature indicator and produced a temperature between –70 and –80°C. The system was also provided with a gas purifier to avoid inefficient and unreliable freezing, due to clogging of the tubes by pollution. The nitrous oxide tank pressure was maintained above 40 kg/cm^2. Before freezing a thin layer of water-soluble lubricant was applied to the probe, resulting in a better contact between probe and ectocervical surface.

In the previous experiments freezing was stopped at the moment that the frosted zone around the probe reached a width of 5 mm. The temperature in the transition zone from frozen to unfrozen tissue is 0°C. As the lethal tissue temperature is –20°C (see Chapter II.3.1) it is evident that this temperature was not achieved at the border of the ultimate cryolesion. It appeared that the cryolesions obtained in this manner, were insufficient (Chapter VI). To ensure that tissue necrosis will occur at 5 mm from the edge of the probe, an attempt was made to reach a temperature of –20°C at this location.

To control the temperature and the concomitant extension of the cryolesion, a thermocouple needle was inserted 5 mm from the edge of the probe in the ectocervical

surface. The intention was to continue freezing till the lethal temperature of –20°C was registered. The freeze-thaw-freeze technique was used and after the first and second freezing period the antero-posterior and transverse diameter of the frozen area on the portio was measured with a pair of compasses. The thaw cycle lasted 5 minutes.

After extirpation of the uterus the day after cryosurgery, the cervix was amputated and fixed in 8% formalin for 24 hours. From the fixed cervices tissue blocks were cut at the 3, 6, 9 and 12 o'clock positions. After processing, using standard methods, from each block single sections of 5 μm were cut in sagittal direction, perpendicularly to the mucosal surface, resulting into 40 slides. In the mounted, hematoxylin stained slides the easily observable border between frozen and unfrozen tissue was marked. All histological preparations were projected through a photographic magnifier onto white paper and eightfold magnifications were made. Then a drawing was made of the contour of the cervix and the border of the frozen area. From these drawings the measurements as described in Chapter VI.2 were obtained (figure VI.4).

VII.3. Results

VII.3.1. Freezing procedures

The first and second freeze time, with the acquired antero-posterior diameter of visibly frozen area on the ectocervix and the temperature at 5 mm from the edge of the probe are presented in table VII.2.

Initially in six women (I-VI in the table) an attempt was made to reach a temperature of –20°C. This was successful in four women after the first freezing period and in three after the second freezing period. Concerning the first freezing period it can be observed that the freeze time was about 5 minutes in three of the four women. In one freezing procedure 8 minutes were necessary, and in two others the temperature only dropped to 0°C and +2°C in spite of a freeze time of more than 10 and 6½ minutes respectively (woman II and IV).

On the basis of this experience in the last four women (VII-X) a first freeze time of about 5 minutes was used, independent of the resulting temperature at 5 mm from the edge of the probe. In those cases the temperature at that point ranged between –6°C and –11°C. Except in the last woman, the same was done with the second freeze time, resulting in temperatures at 5 mm from the probe ranging between –7°C to –20°C.

The mean antero-posterior diameter of the frozen area on the ectocervix was the same after the first and second freezing period, 34.3 ± 2.8 mm and 34.1 ± 2.9 mm respectively. The same applies to the mean transverse diameter, which was about 3 mm less than the antero-posterior diameter (Table VII.2).

VII.3.2. Extension of the cryolesion

The maximum extension of the cryolesion at the different clock positions ranged from 7.2 to 14.8 mm.

The mean maximum extension (MME) of the cryolesions are listed in table VII.2. The MME at the 3 and 9 o'clock positions (10.0 ± 2.1 mm and 10.6 ± 2.1 respectively) did not differ significantly from the 6 and 12 o'clock positions (10.8 ± 1.7 mm and 11.3 ± 1.1 mm respectively).
A diagram (Figure VII.1) shows the MME of the cryolesions at various distances from the reference point R at different clock positions. Neither at these various distances from R a statistical difference could be demonstrated between the cryolesions at the different clock positions.

VII.3.3. Analysis of failures

As in the previous study (Chapter VI.3.3) the cryolesion was defined as sufficient if

Table VII.2: Maximum Extension of the cryolesion, diameter of frozen area on the ectocervix and temperature at 5 mm from the edge of the probe after the first (T_1) and second (T_2) freezing period, and the required first (t_1) and second (t_2) freeze time (for each case and mean values). Small cone probe 19 × 15.5 mm.

	first freezing period				second freezing period				maximum extension of cryolesion			
woman	t_1	diameter of frozen area on ectocervix (mm) trans-verse	antero-poster.	T_1	t_2	diameter of frozen area on ectocervix (mm) trans-verse	antero-poster.	T_2	clock position 3	6	9	12
I	325	32	37	−20°C	189	31	32	−20°C	11.3	11.4	14.8	11.6
II	620	31	34	0°C	468	29	33	0°C	8.1	10.9	12.0	11.9
III	275	30	33	−20°C	222	30	35	−20°C	7.2	11.1	8.0	10.2
IV	403	33	34	+ 2°C	349	33	36	−10°C	11.7	8.7	10.4	11.0
V	479	35	41	−20°C	415	32	39	−12°C	8.1	11.9	8.7	10.3
VI	300	29	31	−20°C	180	28	29	−20°C	9.7	10.5	11.1	13.8
VII	302	30	33	−10°C	300	32	35	− 7°C	13.2	8.9	11.1	11.4
VIII	309	30	34	− 6°C	302	31	35	−15°C	11.1	13.6	9.3	11.3
IX	300	32	34	−11°C	300	33	36	−20°C	12.0	12.6	12.1	10.4
X	330	31	32	− 9°C	210	29	31	− 9°C	8.0	8.4	8.3	10.6
mean ± SD	364.3 ± 108.5	31.3 ± 1.8	34.3 ± 2.8		293.5 ± 96.8	30.8 ± 1.8	34.1 ± 2.9		10.0 ± 2.1	10.8 ± 1.7	10.6 ± 2.1	11.3 ± 1.1

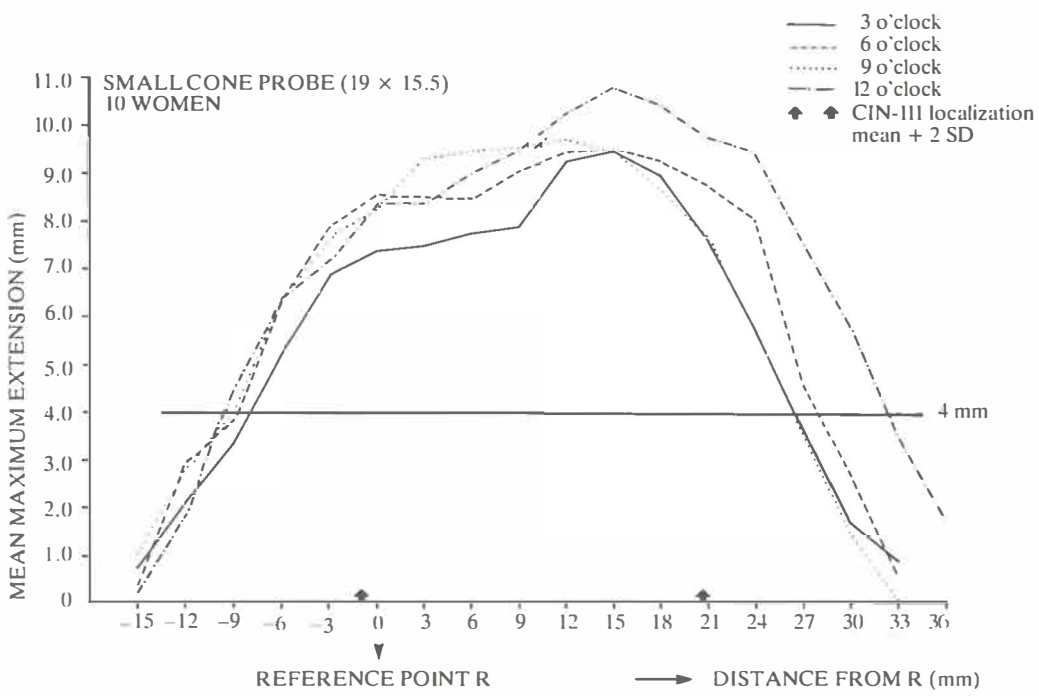


Figure VII.1: Mean Maximum Extension (MME) of the cryolesions after long freezing at 3, 6, 9 and 12 o'clock positions at various distances from the reference point R (for documentation: see appendix table 19).

over a distance of 15 mm or more the MME was at least 4 mm. It appeared that all cryolesions met this criterium as shown in table VII.3.

The minimum distance with a MME of at least 4 mm appeared only in one slide to be 15 mm. In two slides it was 18 mm and in a fourth one it was 27 mm. In the rest, 90% of the slides, a MME of the cryolesion of at least 4 mm was found over a distance of more than 30 mm, measured along the ecto- and endocervical surface.

On the bases of the afore-mentioned criteria, no insufficient lesions were found.

Table VII.3: Distribution of slides according to distance over which the MME of the cryolesion is at least 4 mm.

distance over which the MME of the cryolesion is at least 4 mm	number of slides	relative frequency %	cumulative frequency %
15 mm	1	2.5	2.5
18 mm	2	5.0	7.5
21 mm	–	–	7.5
24 mm	–	–	7.5
27 mm	1	2.5	10.0
30 mm	6	15.0	25.0
33 mm	6	15.0	40.0
36 mm	5	12.5	52.5
39 mm	6	15.0	67.5
42 mm	5	12.5	80.0
45 mm	4	10.0	90.0
48 mm	4	10.0	100.0
	40	100.0	

Table VII.4: Mean, Maximum, and Minimum distance of the maximum extension and the distal border of the cryolesions to the reference point at different clock positions (small cone probe 19 × 15.5; N = number of slides).

clock position	N	**distance from maximum extension of the cryolesion to the reference point R (mm)** mean ± SD	max.	min.	**distance from distal border of the cryolesion to the reference point R (mm)** mean ± SD	max.	min.
3 o'clock	10	9.9 ± 5.8	18	3	−5.7 ± 8.5	12	−15
6 o'clock	10	13.2 ± 9.4	27	0	−8.1 ± 6.2	6	−15
9 o'clock	10	10.2 ± 7.0	21	0	−8.7 ± 5.4	3	−15
12 o'clock	10	16.5 ± 6.2	24	3	−7.8 ± 6.4	6	−15

VII.3.4. Topography of the cryolesion

In table VII.4 the mean distance of the distal border of the cryolesion to the reference point is summarized for the different clock positions. As can be seen, the distance ranges from 5.7 ± 8.5 mm to 8.7 ± 5.4 mm distal from the reference point (negative value). The differences between the four clock positions are not statistically significant. The cryolesions in which the distal border was most removed from the reference point R, started at the distal side 15 mm from R and at the proximal side 12 mm from R.

The mean distances of the MME of the cryolesion to the reference point were also not significantly different for the four clock positions and ranged between 9.9 ± 5.8 mm and 16.5 ± 6.2 mm. It was of interest that at 6 and 12 o'clock the MME of the cryolesions was most removed from the reference point (13.2 ± 9.4 mm and 16.5 ± 6.2 mm respectively; Table VII.4).

The most distally located MME of the cryolesion was found at the reference point R and the most proximal one at a distance of 27 mm from R.

From the analysis of failures (see Chapter VII.3.3) it followed that 90% of the cryolesions over a distance of 30 mm had a depth of at least 4 mm. It is of interest to know at what distance from R this occurs, in other words. what is the topographic position of this sufficient part of the cryolesions. Therefore the different clock positions were examined to see how many of the cryolesions (%) had a depth of 4 mm or more at different distances from the reference point.

In a histogram in fig. VII.2a,b,c,d the results are summarized. It appears that from all the cryolesions at the 6, 9 and 12 o'clock positions, 90-100% have a MME of 4 mm or more, at least from the reference point as far as 21 mm proximal from the reference point. At the 3 o'clock position for some reason it is less and it is confined between 9 mm and 21 mm proximal from the reference point R.

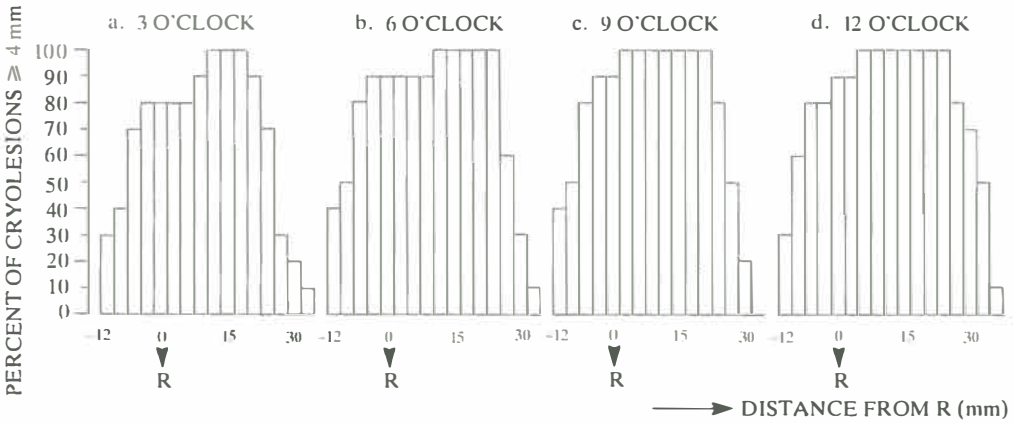


Figure VII.2a,b,c,d: Frequency of cryolesions with a Mean Maximum Extension (MME) of at least 4 mm at various distances from the reference point R (small cone probe; N = 10 women).

VII.4. Discussion

In a previous experiment (Chapter VI) the freeze time was determined by the extension of the visibly frozen area on the ectocervix. Using a small cone probe, freezing was stopped when the antero-posterior diameter of the ice-ball was 29 mm. The required mean first and second freeze times were 2 and 1.5 min, respectively. In this present study the mean first and second freeze time was 6 and 5 minutes, respectively (see Table VII.2). In spite of this much longer freeze time the mean increase of the antero-posterior diameter of the frozen area on the ectocervix was only 5 mm (from 29 to 34 mm).

It is noteworthy that after a certain period of freezing the frozen area on the ectocervix stabilizes, in other words after a certain freeze time a maximum diameter has been reached which hardly increases in spite of longer freezing. At that moment there is an equilibrium between the supplied cold and the heat transfer and the cryolesion has reached its minimum temperature and maximum extension. As was demonstrated the results of the last four freezing procedures (women VII-X in table VII.2) were similar compared with the first ones. Therefore, it can be concluded that after 5 minutes repetitive freezing this maximum surely has been reached. This is in

keeping with Gill et al. (1970) and Fraser (1975) who have shown that the development of the cryolesion has an initial rapid growth phase and a later slow phase.

It is imaginable that the insufficient drop in temperature 5 mm from the edge of the probe in a few cases arises because the thermocouple needle is inserted near a large vessel with a large amount of heat transfer. This idea was supported by the fact that in some cases, when the thermocouple, during the same freezing procedure, was inserted at another point, 5 mm from the edge of the probe, a lower temperature was found.

The difference in vascular supply between the 3 and 9 o'clock positions on the one side and at 6 and 12 o'clock positions at the other side, is still reflected in the difference between the transverse and antero-posterior diameter of the frozen area on the portio. However, this was not reflected in the MME of the cryolesion and the percentage of failures at the different clock positions. Apparently its influence was balanced by the long freeze time which resulted in a maximum extension of the cryolesion in all directions. This can also be concluded from the fact that all of the freezing curves, shown in figure VII.1, have a very similar form. However, it is evident that the cryolesions obtained with long freeze time are more extensive than those obtained with the short freeze time (Chapter VI.3.2; Figure VI.5).

In figure VII.3 it is shown how at all clock positions the extension of the cryolesions changes under influence of long freezing. The broken lines represent the extension of the cryolesion after short freezing. It is evident that after short freezing the extension at the 3 and 9 o'clock positions is less as compared to the 6 and 12 o'clock positions. After long freezing all lesions (black lines) extend to the same depth. The extension increases especially at the 3 and 9 o'clock positions to such a degree that an optimal cryolesion is achieved.

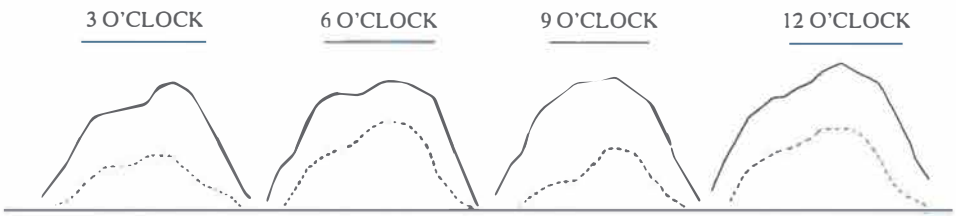


Figure VII.3: Comparison between the extension of the cryolesions after short (------) and long (——) freezing with the same probe (small cone probe) at 3, 6, 9 and 12 o'clock positions.

The long freeze time did not essentially change the topographic position of the maximum extension of the cryolesion, relative to the reference point, as can be seen in the diagram of these cryolesions (Chapter VI.3.4, Table VI.8, Table VII.4 and Figure VII.3). With both freezing procedures, the maximum extension, the top of the diagram lies between 9 and 18 mm from the reference point R. This is not true for the linear extension of the cryolesion. The cryolesions produced with the long freezing procedure begin more distally than those with the short freezing method and the proximal border is higher in the endocervical canal (Table VII.4, Table VI.8 and Figure VII.3).

This can also be observed by comparing the histograms in Figure VI.7d and Figure VII.2d. For example, 12 mm distal from the reference point, already 30% of the cryolesions achieved with long freezing have a depth of more than 4 mm against 0% in the lesions produced with short freezing, using the same small cone probe. At a distance of 9 mm distal from the reference point these percentages are 60% and 7%, respectively. The same applies to the proximal border of the cryolesions. At a distance

of 30 mm proximal from R still 70% of the cryolesions produced with long freezing proved to have a depth of at least 4 mm, against 7% in the other cryolesions and at a distance of 33 mm, these percentages are 50% and 0%. This means that by using a long freeze time the cryolesion also extends more in distal and proximal direction. In other words, the linear extension is also increased.

VII.5. Summary and conclusions

- A long freeze time increased both the linear extension and the depth of the cryolesions at all clock positions.
- Especially at the 3 and 9 o'clock positions a considerable increase in depth was achieved, to the effect that the extension of the cryolesion was similar at all clock positions.
- The MME of the cryolesions at the 3, 6, 9 and 12 o'clock positions were 10.0 ± 2.1 mm; 10.8 ± 1.7 mm; 10.6 ± 2.1 mm and 11.3 ± 1.1 mm, respectively.
- With the long freeze time the percentage of sufficient cryolesions (a MME of at least 4 mm over a distance of 15 mm) was 100% at all clock positions.
- In 90% of all the cryolesions the MME was 4 mm or more over a linear distance of 30 mm.
- The localization of the maximum extension of the cryolesions relative to the reference point was the same for both types of lesions (short and long). This maximum extension was located 9 to 18 mm proximally from the reference point R.
- Approximately 90-100% of the cryolesions at the 6, 9 and 12 o'clock positions had a MME of 4 mm or more over a distance of at least 21 mm proximal from the reference point. As to the 3 o'clock position this is in 80-100% of the cases.
- Long freezing produces a cryolesion approaching a maximal extension which is sufficient to cover the area at risk of the cervix in virtually all cases.

CHAPTER VIII

General discussion and conclusions

VIII.1. Aim of the study

This study was carried out to evaluate the reliability of cryosurgery in the treatment of cervical intraepithelial neoplasia (CIN). It is of importance to know how to select patients suitable for this method of treatment and how to apply it. A cryolesion is considered effective when it is more extensive than the extent of the CIN. Therefore, to define an adequate cryolesion it is necessary to know the maximum possible CIN extent in the cervical epithelium, both the linear extent and the extension in the cervical crypts.

Starting from the idea that the maximum depth of CIN crypt involvement is limited by the depth of the cervical crypts, first the depth and topography of the crypts in healthy cervices were ascertained. Next the depth of CIN-III crypt involvement, the linear extent of the CIN-III and the topography of both were measured in cone specimens. Also the ratio between the deepest CIN-III crypt involvement and the deepest crypt was determined. The results of these studies showed the extension of CIN-III and as a consequence the required extension of the cryolesion could be defined. From this, criteria for a sufficient and an insufficient cryolesion were set.

On the basis of these criteria, the extension of the cryolesion in the uterine cervix on the 3, 6, 9 and 12 o'clock positions, obtained with different types of probes were evaluated.

VIII.2. Results

Depth of crypts

The overall Mean Maximum Depth (MMD) of the crypts, that is the mean of the deepest crypt in each slide, was 3.8 mm with a maximum of 12.4 mm. The crypts at the 3, 6, 9 and 12 o'clock positions had the same depth. Therefore the 12 o'clock position was considered representative for the entire population. Slides at 12 o'clock were used for analysis of the distribution of the deepest crypts proximally and distally from the most caudal point of the ectocervix (reference point R). To this end the MMD of crypts was determined at 3 mm intervals from reference point R. The MMD of the crypts at various distances from R, varied from 2.0 ± 0.8 mm to 2.7 ± 1.5 mm. In general it increased in proximal direction. With the idea that it would be possible to select a group of women with shallow crypts, being more suitable for cryosurgery, the depth of the crypts was related to age, period of life, parity and portio diameter.

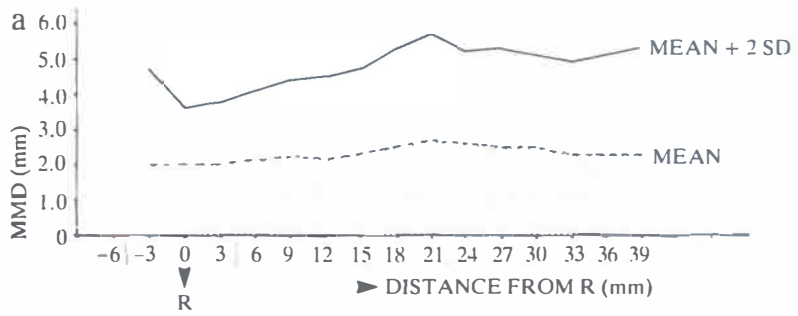
The depth of crypts varied with age, such that the MMD is the least in women older than 60 years. Independent of age the MMD is smaller in nulliparous than in parous women. The variations of the depth of crypts with period of life and diameter of the portio were related to age and parity.

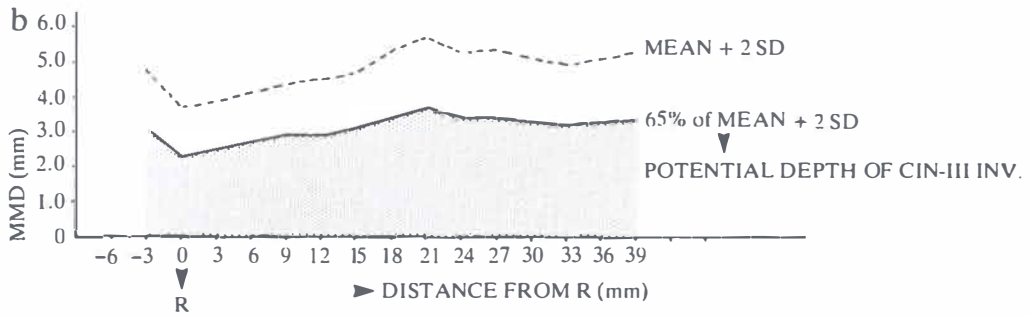
However, in the distal part of the cervix, the area at risk, the difference in crypt depth is small and therefore age and parity are neglectable in selecting patients.

Linear extent and depth of CIN-III lesions

As previously mentioned, the most caudal point of the ectocervix was used as reference point R. It appeared that in about 95% of the patients (mean ± 2 SD) the CIN-III is confined to the area from 0.6 mm distally to 20.7 mm proximally of the reference point (Chapter V.3.3.1). In this area (at the 12 o'clock position) the Mean Maximum Depth (MMD) of the crypts did not exceed 2.7 ± 1.5 mm (Chapter IV.3.2, appendix Table 1). That implies, that in at least 95% of the women (mean + 2 SD) in that area the deepest crypts is less than 5.7 mm. In figure VIII.1a this mean and mean + 2 SD

value for the entire cervix is shown. It was found that on average CIN-III involvement occupies 65% of the depth of the deepest crypts. Calculated from the depth of healthy crypts one would expect that CIN-III does not exceed 3.7 mm (being 65% from 5.7 mm). The 65% level from the mean + 2 SD for the entire cervix is shown in figure VIII.1b. Actual measurement of the depth of CIN-III were in complete agreement with this theoretical figure.





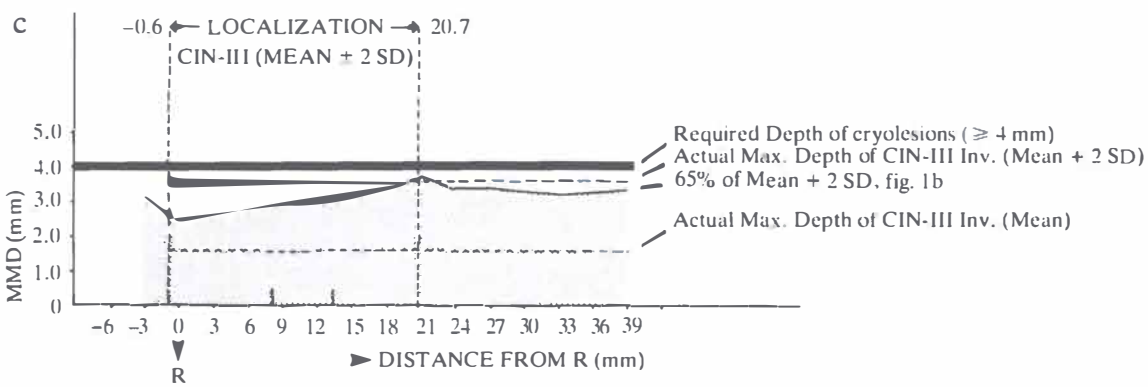


Figure VIII.1a,b,c: Diagram of depth of cervical crypts and CIN-III involvement with required depth of cryolesion.

The mean maximum depth of CIN-III crypt involvement was 1.6 ± 1.0 mm, and the mean + 2 SD (about 95% of the patients) value 3.6 mm (Chapter V.3.1). In a diagram in figure VIII.1c besides the 65% value of the mean + 2 SD of the MMD of the crypts, also the mean + 2 SD level of the mean maximum depth of crypt involvement, is shown. The localization of the CIN-III (mean ± 2 SD) is indicated by arrows.

On the basis of these results it can be concluded that in almost all patients (> 95%) the depth of crypt involvement does not exceed the 4 mm level.

Depth of Nabothian cysts:

The overall MMD of the Nabothian cysts, that is the mean of the deepest Nabothian cyst in each slide appeared to be 4.0 ± 2.0 mm with a maximum of 11.2 mm (Chapter IV.3.8). This is hardly deeper than the overall MMD of the deepest crypts, but deeper than the MMD of the crypts at various distances from the reference point R. In accordance with literature (Anderson and Hartley 1980) this study revealed that Nabothian cysts can be involved with CIN-III. Using cryosurgery in CIN it must be kept in mind that grossly visible cysts can penetrate deeper than 4 mm into the cervical stroma.

Definition of a sufficient extension of the cryolesion

On the basis of the CIN-III crypt involvement the extension of a cryolesion should also have a depth of at least 4 mm. Moreover, regarding the fact that in about 95% of the patients (mean + 2 SD) the linear extent of the CIN-III does not exceed 14.8 mm (Chapter V.3.2), the cryolesion should have a depth of at least 4 mm over a distance of 15 mm.

For that reason a sufficient cryolesion is defined as a cryolesion with a depth of ≥ 4 mm over a distance of 15 mm or more in the area at risk.

The application technique of a cryolesion

In this study cryosurgery was applied with essentially two different freeze procedures. In the first experiment freezing was continued till a 5 mm frozen area around the probe was visible, resulting in a freeze-thaw-freeze cycle of about 2 min - 5 min - 1½ min (short freezing).

In a second experiment a cryolesion was applied with a freeze-thaw-freezecycle of about 5 min - 5 min - 5 min (long freezing). In all freeze procedures a water-soluble lubricant was applied to the probes.

Factors influencing the extension of the cryolesion:

Clock position

Out of the cryolesions applied with the short freeze time 21.6% were insufficient. It appeared that in particular the percentage of insufficient lesions at the 3 and 9 o'clock positions was very high (51.5%), compared to the 6 and 12 o'clock positions (7.4%). Initially it was thought that the slit shaped external os might be the cause of the high failure rate at the 3 and 9 o'clock positions. However, in cervices with slit shaped external os the percentage of insufficient cryolesions was 53.4% at the 3 and 9 o'clock positions, as compared to 46.7% in cervices with a round external os (Table VI.6).

The difference is small and comparing these percentages with the relatively low percentage failures at the 6 and 12 o'clock positions it must be concluded that there is another factor causing such an insufficient extension of the cryolesions at the 3 and 9 o'clock positions.

The most likely explanation is that the arterial and venous vascular supply of the cervix is localized at 3 and 9 o'clock. In these areas the large supply of heat by the circulation interferes with the extension of the cryolesion.

Type of probe

The type of probe also appeared to influence the extension of the cryolesions. The large cone probe gave the best results and the small flat cervix probe the worst. In a

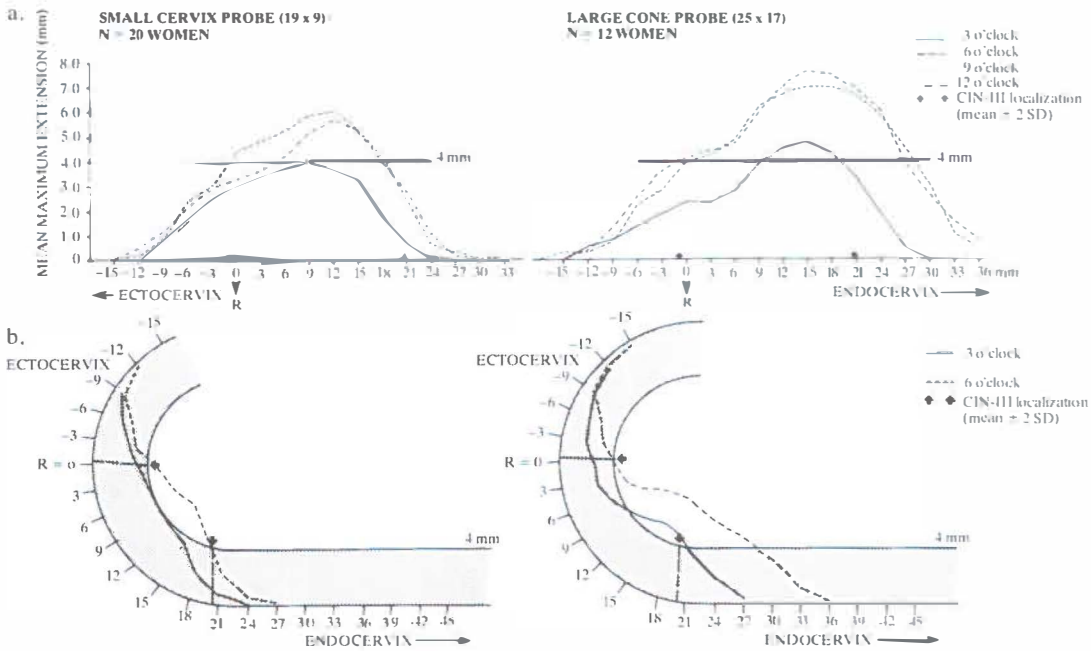


Figure VIII.2a,b: Extension of the cryolesion applied with small cervix probe and large cone probe in a diagram for the 3, 6, 9 and 12 o'clock positions (VIII,2a), and in a drawing of a sagittal section of the cervix with cryolesions at 3 and 6 o'clock positions (VIII.2b).

diagram (figure VIII.2a) this is shown for the different clock positions. The 4 mm depth level is indicated with a thick line and the localization of CIN-III with arrows. Both the insufficient extension of the cryolesion at 3 and 9 o'clock positions and the inferior freezing with the small flat cervix probe are evident. A drawing representing a sagittal section of the cervix (Figure VIII.2b) with the 4 mm depth level shaded, demonstrates what this difference in probe type in reality implies. For the sake of clearness it is shown only for the 3 and 6 o'clock positions, but at the 9 and 12 o'clock positions the results are similar. The localization of CIN-III (Mean ± 2 SD) is indicated by arrows. It is evident that only cryolesions performed with the large cone probe are sufficient at 6 (and 12) o'clock.

Freeze time

Using the long freeze time the cryolesions both at the 6 and 12 o'clock positions as well as those at the 3 and 9 o'clock positions were sufficient. In a diagram (Figure VIII,3a) and in a drawing of a sagittal section of the cervix (Figure VIII.3b) this is shown for the small cone probe at different clock positions and compared with short freezing with the same probe. From the diagram and drawing, in which also the 4 mm level is shaded and the localization of CIN-III indicated with arrows, it is evident that after long freezing the extension of the cryolesions is sufficient and almost similar at all clock positions. With the long freezing a nearly maximum extension of the cryolesions is obtained.

From the diagrams it can be seen that the distance of the maximum extension of the cryolesions to the reference point, achieved with short freezing and with long freezing (Figure VIII.3a) is more or less the same (varying between 9-15 mm proximally from the reference point). The sufficient part of all cryolesions achieved with long freezing is on average localized from 9 mm distally as far as 27 mm proximally

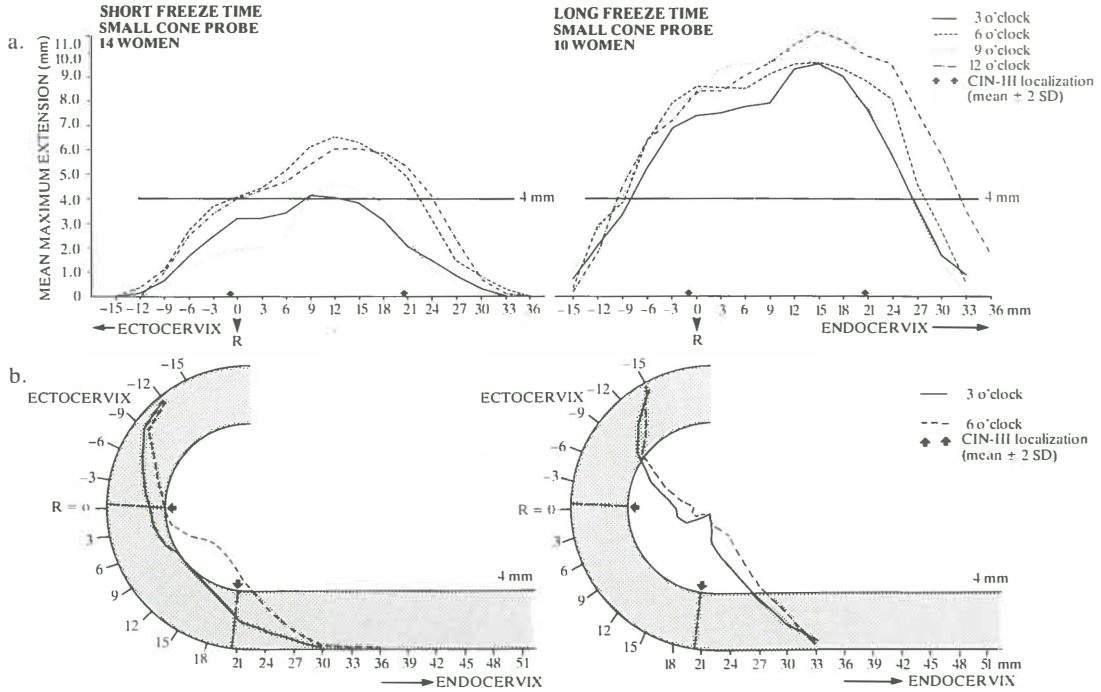


Figure VIII.3a,b: Extension of the cryolesions after short and long freeze time in a diagram for the 3, 6, 9 and 12 o'clock positions (VIII.3a) and in a drawing of a sagittal section of the cervix with cryolesions at the 3 and 6 o'clock positions (VIII.3b).

from the reference point. This area includes largely the area in which CIN-III of the cervix occurs (from 0.6 mm distally as far as 20.7 mm proximally, in the figures indicated by arrows). The sufficient parts of the cryolesions achieved with the short freezing are all located proximally from the reference point, even when the large probes were used. This implies that when CIN-III is located distally on the ectocervix long freezing is required.

VIII.3 Conclusions

Cryosurgery as local destructive treatment of CIN can only be used when the well-known criteria, which are described in Chapter II have been met. The results of this study show that the linear extension and the depth of the cryolesion depend on the freeze time and the type of probe used and vary at the different anatomical clock positions. Notably at the 3 and 9 o'clock positions, the cryolesion developes poorly. Therefore CIN-III lesions at those positions are at risk and require a long freezing procedure. A 5 min freeze – 5 min thaw – 5 min freeze proved to be sufficient. If preferable at the 6 and 12 o'clock positions, provided that a selected probe is used, a shorter freezing procedure (3 min freeze – 5 min thaw – 3 min freeze) seems justified.

However, extensive ectocervical lesions, even if located at the 6 and 12 o'clock positions, are also at risk. The best result will be achieved when the CIN-lesion is completely covered by the probe. Therefore, in large lesions large probes should be used. When a CIN-III lesion extends beyond the probe the long freezing procedure is obligatory. If the CIN-III extends more than 5 mm beyond the probe, cryosurgery is not the treatment of choice.

Selection of the size of the probe also depends on the diameter of the portio. After freezing the diameter of the visibly frozen area is about 10-15 mm more than the

diameter of the probe. Therefore large probes (diameter 25 mm) cannot be used in cervices with a portio diameter smaller than about 35 mm.

In patients with grossly visible Nabothian cysts long freezing is recommended.

Summarizing it can be stated that using a long freezing procedure of 5 min freeze – 5 min thaw – 5 min freeze leads to a close to maximal extension of the cryolesions. This is the safest application technique which is obligatory to use in CIN-III lesions at risk. When a conservative approach is preferred, in certain selected cases and with selected probes cryosurgery can be applied with a 3 mm freeze – 5 min thaw – 3 min freeze cycle.

Samenvatting

Hoofdstuk I

INLEIDING EN DOELSTELLING VAN HET ONDERZOEK

In de inleiding wordt ingegaan op het feit dat het merendeel van de plaveiselcel-carcinomen van de cervix uteri ontstaan uit de voorstadia dysplasie en carcinoma in situ.

Uit de literatuur is bekend dat niet alle dysplastische epitheelafwijkingen overgaan in ernstiger aandoeningen. Een gedeelte gaat in regressie en een ander deel progredieert tot carcinoma in situ of tot invasief carcinoom. In de loop van 5-10 jaar wordt een groot gedeelte (54-71%) van de in situ carcinomen invasief.

De traditionele therapie, exconisatie en eventueel hysterectomie, is in veel gevallen overbehandeling gebleken en heeft met name voor de patiënt met kinderwens vaak ingrijpende consequenties. Vooral in de jonge leeftijdsgroepen neemt het aantal patiënten toe bij wie deze premaligne voorstadia van cervixcarcinomen worden waargenomen. Om deze redenen is er naarstig gezocht naar minder ingrijpende behandelingsmethoden van deze voorstadia. Dit heeft geleid tot de zogenaamde conserverende behandelingsmethoden, zoals laser verdamping, cryocoagulatie, electrocoagulatie, infraroodcoagulatie en andere.

De vraag hierbij is of de weefseldestructie voldoende uitgebreid is om al het afwijkende epitheel aan het oppervlak en in de crypten van de cervix te vernietigen. Het doel van deze studie is om dit na te gaan in geval cryocoagulatie wordt gebruikt als therapie bij de cervicale intra-epitheliale neoplasie graad III (CIN-III).

De vereiste uitbreiding van het cryoletsel wordt bepaald door de uitbreiding van de CIN-III aan het oppervlak, de zogenaamde lineaire extensie, en de mate van uitbreiding in de crypten.

Omdat de diepte van de crypten een maat is voor de maximale uitbreidings-mogelijkheid van het CIN in de crypten, is in deze studie in de eerste plaats de diepte van de crypten gemeten en nagegaan welke factoren hierop van invloed zijn. Dit laatste om een groep patiënten te selecteren met ondiepere crypten, die bij uitstek geschikt zou zijn om met cryocoagulatie te behandelen.

Vervolgens is onderzocht tot welke diepte het CIN-III zich in de crypten uitbreidt en waar en over welke afstand het afwijkende epitheel aan het oppervlak van de cervix voorkomt. Tenslotte is een morfometrisch onderzoek verricht naar de lokalisatie en uitbreiding van het vriesletsel.

Hoofdstuk II

LITERATUUROVERZICHT

In dit hoofdstuk is in de eerste plaats de voor dit onderzoek relevante macro- en microscopische anatomie van de cervix beschreven. Vervolgens is een literatuur-overzicht gegeven over cervicale intra-epitheliale neoplasie (CIN). Hierbij is aandacht geschonken aan de definitie, de ontstaanswijze, de uitbreiding zowel aan het oppervlak (lineaire extensie) als in de crypten ('crypt involvement') en aan de topografie van de epitheelafwijkingen.

Vervolgens is ingegaan op de voor deze studie relevante gegevens betreffende epidemiologie, diagnostiek en behandeling.

In het laatste gedeelte van dit hoofdstuk is een literatuuroverzicht gegeven over de cryocoagulatie, waarin de geschiedenis en de ontwikkeling van de cryocoagulatie worden behandeld. Dit gedeelte is afgerond met een samenvatting over cryobiologie, histologische veranderingen na bevriezing en uitbreiding van het vriesletsel.

Hoofdstuk III

SCHROMPELING VAN HET CERVICALE WEEFSEL ONDER INVLOED VAN HISTOLOGISCHE BEWERKING

Het is bekend dat weefsel dat bewerkt wordt voor histologisch onderzoek, schrompelt. Daar een morfometrisch onderzoek verricht moest worden, was het nodig om de mate van schrompeling van het cervicale weefsel te onderzoeken. Door het bepalen van de afmetingen van een geamputeerde cervix voor en na fixatie, was de schrompelingsfactor door formaline fixatie te bepalen. Deze bleek 2,7% te zijn. De schrompeling door paraffine inbedding bedroeg 12,6%, terwijl de schrompeling door snijden en monteren te verwaarlozen was. Dit leverde een totale schrompeling van 2,7 + 12,6 = 15,3% op.

Hoofdstuk IV

DIEPTE EN TOPOGRAFIE VAN DE CRYPTEN VAN DE CERVIX UTERI

Met gebruikmaking van een grafisch tablet werd in 172 hysterectomie-preparaten de diepte van de cervicale crypten gemeten. De metingen werden verricht op intervallen van 3 mm distaal- en proximaalwaarts van het meest caudale punt van de ectocervix (referentiepunt R) in coupes op 3, 6, 9 en 12 uur. De verkregen gegevens van 635 coupes werden per computer verwerkt.

Het bleek dat het meest frequent een maximale cryptediepte tussen de 3 en 4 mm werd gevonden. In 38,3% van de coupes bleek de maximale cryptediepte groter dan 4 mm te zijn en in 16,9% groter of gelijk aan 5 mm.

De gemiddelde maximale diepte van de crypten bleek 3,8 ± 1,5 mm te zijn, met een maximale waarde van 12,4 mm. De gemiddelde maximale diepte van de crypten varieerde op de verschillende anatomische posities tussen 3,6 ± 1,4 mm en 4,0 ± 1,6 mm. Dit bleken geen significante verschillen te zijn. Ook op de verschillende intervallen van het referentiepunt bleek de gemiddelde maximale diepte van de crypten niet te verschillen op de vier uurposities. Daarom werden de waarden op de 12 uurpositie als representatief beschouwd voor de hele populatie. Op deze positie bleek op elke 3 mm afstand van R de gemiddelde maximale diepte niet meer dan 2,7 ± 1,5 mm te bedragen.

De gemiddeld maximale cryptediepte was significant geringer bij vrouwen boven de 60 jaar, bij postmenopauzale vrouwen en in portio's met een diameter kleiner dan 25 mm. Tussen leeftijd, menopauze en portiodiameter bleek een onderlinge relatie te bestaan. Door uitsluiting van de leeftijdsfactor bleek pariteit een tweede onafhankelijke variabele te zijn, die van invloed was op de cryptediepte. De gemiddelde maximale diepte van de crypten was significant groter bij vrouwen die één of meer keren waren bevallen. De vorm van het ostium externum bleek niet van invloed te zijn op de diepte van de crypten.

De gemiddelde diepte van de meest distale crypte, de zogenaamde 'last gland' was 1,6 ± 1,0 mm (12 uurpositie).

De ovula Nabothi bleken gemiddeld maar weinig dieper te liggen dan de crypten.

Over 't algemeen bleek dat de diepste crypten meer in endocervicale richting gelegen waren. De afstand van de diepste crypten tot het meest distale punt van de ectocervix varieerde van 16,8-19,8 mm op de verschillende uren.

In de postmenopauzale vrouw met een kleine portio was deze afstand significant kleiner dan in de premenopauzale vrouw met een meer plompe portio.

Hoofdstuk V

CERVICALE INTRAEPITHELIALE NEOPLASIE GRAAD III, MATE VAN UITBREIDING IN DE CRYPTEN, LINEAIRE UITBREIDING EN TOPOGRAFIE

In dit hoofdstuk wordt het onderzoek beschreven naar de uitbreiding en de topografie van CIN-III in het oppervlakteepitheel van de cervix en in het epitheel van de cervicale crypten.

De uitbreiding van het CIN-III in de crypten werd onderzocht in 57 exconisatiepreparaten. De diepte van de CIN-III werd gemeten vanaf het cervicale oppervlak. In 48 exconisatiepreparaten werd de lineaire uitbreiding van het CIN-III gemeten en de lokalisatie bepaald. Tenslotte kon in 37 exconisatiepreparaten de topografie van de diepste CIN-III uitbreiding in de crypte vastgelegd worden.

De topografie werd bepaald door de afstand van de CIN-III tot het meest caudale punt van de ectocervix (het referentiepunt R) te meten. Alle metingen werden verricht met behulp van een grafisch tablet en verwerkt met een computer.

De gemiddelde maximale diepte van de CIN-III uitbreiding in de crypten bleek 1,6 ± 1,0 mm te zijn. De diepste uitbreiding was 4,5 mm. De diepte van de diepste CIN-III uitbreiding in de crypten bedroeg gemiddeld ongeveer 65% van de diepte van de diepste crypte. Deze verhouding en de diepte van de CIN-III was het grootst bij patiënten ouder dan 50 jaar.

De gemiddelde lineaire uitbreiding van de CIN-III-aandoening bleek 7,4 ± 3,7 mm te zijn. De kleinste uitbreiding was 1,4 mm en de grootste 17,6 mm.

Gemiddeld strekten de door CIN-III aangedane epitheelgebieden zich endocervicaalwaarts uit van 8,2-13,3 mm van het meest caudale punt van de ectocervix (R). Bij ruim 95% van de patiënten (gemiddelde ± 2 SD) was dit het geval tussen 0,6 mm distaal van het referentiepunt R tot 20,7 mm proximaal hiervan. Slechts 4,2% van de CIN-III aandoeningen breidde zich distaal van het referentiepunt R uit.

Bij jonge patiënten was de CIN-III meer ectocervicaal gelegen en waren er statistisch niet te bevestigen aanwijzingen, dat de lineaire uitbreiding ervan geringer was. Het bleek dat ovula Nabothi ook door CIN-III aangedaan kunnen worden.

Hoofdstuk VI

EEN GESTANDAARDISEERD VRIESLETSEL VAN DE CERVIX UTERI; METHODE VAN TOEPASSING, DE UITBREIDING EN OORZAAK VAN TEKORT SCHIETEN

Het doel van dit onderdeel van de studie was om na te gaan of de lineaire uitbreiding en de uitbreiding in de diepte van een gestandaardiseerd cryoletsel voldoende was voor de behandeling van de CIN-III.

Met een kleine en grote conusvormige probe en een kleine en grote platte cervix probe werd, na informed consent, volgens een vast omlijnd protocol een vriesletsel aangebracht op de portio van 54 vrouwen, de dag voordat zij een hysterectomie ondergingen voor een goedaardige aandoening. De vries-dooi-vries techniek werd gebruikt en er werd gevroren totdat een 5 mm brede berijpte zone rondom de probe was ontstaan. Na verwijdering van de uterus werd de uitbreiding en lokalisatie van het vriesletsel gemeten met behulp van het grafisch tablet. De resultaten werden met een computer verwerkt.

De resultaten werden geanalyseerd in relatie tot het gebruikte probetype, de leeftijd, de levensfase en de pariteit van de vrouw. Tevens werd nagegaan of de uitbreiding van het vriesletsel varieerde op de verschillende anatomische posities (3, 6, 9 en 12 uur) en of deze beïnvloed werd door de vorm van het ostium externum. De volgende resultaten werden verkregen:

Vriestijd: Om een optimale daling van de probetip-temperatuur te bereiken, was een voldoende hoge druk ($\geq$ 40 kg/cm^2) van de stikstofdioxide in de cylinder nodig. Bij jongere vrouwen moest langer gevroren worden om tot de vereiste afmeting van het uitwendige, zichtbare vriesletsel te komen dan bij oudere vrouwen. De grote probes vereisten een langere vriesduur dan de kleine probes.

Uitbreiding van het vriesletsel: In hoofdstuk V werd gevonden dat de CIN-III bij ruim 95% van de patiënten gelokaliseerd was tussen 0,6 mm distaal van het referentiepunt en 20,7 mm proximaal daarvan. In dat gebied werd een gemiddelde maximale cryptediepte van 2,7 ± 1,5 mm niet overschreden (hoofdstuk IV). De gemiddelde waarde + 2 SD is 5,7 mm, dat betekent dat in meer dan 95% van de patiënten de

cryptediepte van 5,7 mm niet overschreden wordt. Tevens werd gevonden dat de crypten gemiddeld voor maximaal 65% aangedaan worden door CIN-III, dat wil zeggen dat de CIN-III uitbreiding in ruim 95% van de patiënten niet dieper reikt dan 3,7 mm. Dit komt overeen met de werkelijk gemeten diepte van 3,6 mm (gemiddelde diepte CIN-III: 1,6 ± 1,0 mm; gemiddelde + 2 SD: 3,6 mm, hoofdstuk V). Dit betekent dat een voldoende uitgebreid vriesletsel tenminste 4 mm diep moet zijn.

De gemiddelde lineaire uitbreiding van het CIN-III is 7,4 ± 3,7 mm, dus bij ruim 95% van de patiënten is de lineaire uitbreiding niet groter dan 14,8 mm (= gemiddelde + 2 SD). Hieruit volgt de tweede voorwaarde voor een voldoende uitgebreid vriesletsel, het moet namelijk een lineaire uitbreiding hebben van tenminste 15 mm, in het relevante gebied van de cervix.

Samenvattend is een voldoende vriesletsel als volgt gedefinieerd: „Een voldoende uitgebreid vriesletsel moet tenminste een diepte van 4 mm hebben over een afstand van 15 mm of meer in het bedreigde gebied van de cervix."

Op grond van dit criterium bleek 21,6% van de vriesletsels onvoldoende te zijn. Van de vriesletsels op 3 en 9 uur bleek zelfs meer dan 50% onvoldoende. Het aantal onvoldoende vriesletsels bleek het kleinst te zijn met de grote conusvormige probe (17,1%) en het grootst met de kleine platte cervixprobe (25,8%). Het gunstige effect van de grote conusvormige probe komt het sterkst tot uiting op 6 en 12 uur, daar bleken alle vriesletsels voldoende te zijn.

Topografie van het vriesletsel: Gemiddeld strekte het voldoende deel van het vriesletsel zich uit van het meest caudale punt van de ectocervix (referentiepunt R) tot 24 mm proximaal (endocervicaal) hiervan. Deze lokalisatie omsluit de transformatiezone, waarbinnen de CIN-III meestal voorkomt. Dit houdt in dat het cryoletsel optimaal gelegen is voor de behandeling van het CIN-III. Echter hierbij dient opgemerkt te worden dat met de boven beschreven vriesmethode de uitbreiding van het vriesletsel slechts op 6 en 12 uur voldoende diep was (≥ 4 mm) over een afstand van respectievelijk 9-15 mm en 12-21 mm van het referentiepunt. Bovendien was dit alleen het geval bij 80-100% van de vriesletsels, aangebracht met de grote en kleine conusvormige probe en de grote platte cervix probe. Indien de kleine platte cervix probe werd gebruikt, daalde dit percentage. Terwijl het percentage voldoende diepe vriesletsels (≥ 4 mm) op de verschillende afstanden van R op de 3 en 9 uurposities opvallend veel lager was bij gebruik van alle vier soorten probes.

Conclusie: Zowel het aantal vriesletsels als de afstand waarover de vriesletsels een diepte hebben van tenminste 4 mm is in vele gevallen onvoldoende. Op grond hiervan is verder onderzoek verricht naar de uitbreiding van de vriesletsels welke verkregen zijn door langduriger vriezen.

Hoofdstuk VII

HET EFFECT VAN LANGER VRIEZEN OP DE UITBREIDING VAN HET VRIESLETSEL

In dit hoofdstuk worden de resultaten weergegeven van het onderzoek naar de invloed van langere vriesduur op de uitbreiding van het vriesletsel. Ook nu werd met informed consent van de patiënt, met de kleine conusvormige probe een vriesletsel aangebracht op de portio van patiënten die wegens een goedaardige aandoening een uterus-extirpatie ondergingen. De cryocoagulatie werd de avond vóór de operatie aangebracht door middel van de zogenaamde 'vries-dooi-vries'-techniek. In eerste instantie werd bij de bevriezingen gepoogd een temperatuur van –20°C op 5 mm afstand van de probe te krijgen. De hiervoor benodigde vriesduur was zeer wisselend, variërend van ongeveer 4,5-8 minuten (eerste keer vriezen) en 3-4 minuten (tweede keer vriezen). Echter in enkele gevallen lukte het in het geheel niet deze temperatuur van –20°C te bereiken, zelfs niet na 6,5-10 minuten vriezen. Om deze reden werd in de proefopstelling bij de volgende vier vrouwen een eerste en tweede vriesduur van ± 5 minuten toegepast.

Bij het toepassen van langere vriesduur was er een toename van zowel de lineaire uitbreiding als van de uitbreiding in de diepte van het cryoletsel. Met name op 3 en 9 uur was er een opvallende toename van de uitbreiding in de diepte, zodanig dat vergeleken met 6 en 12 uur geen verschil meer aanwezig was. Dit houdt in dat bij langdurig vriezen (eerste keer gemiddeld 6 min en tweede keer gemiddeld 5 min), de vriestijd lang genoeg geweest is om in alle richtingen een maximale uitbreiding van het letsel te bereiken. De vriestijd was lang genoeg om de door de intensivere bloedcirculatie extra toegevoerde warmte op 3 en 9 uur te compenseren. Met deze langere vriesduur bleek het percentage voldoende vriesletsels 100% te zijn en 90% van alle vriesletsels hadden zelfs over een afstand van 30 mm een diepte-uitbreiding van 4 mm of meer. De lokalisatie van het vriesletsel bleek dezelfde te zijn als die verkregen met korter vriezen. Van de vriesletsels op 6, 9 en 12 uur hadden 90-100% een diepte-uitbreiding van tenminste 4 mm vanaf het referentiepunt R tot 21 mm proximaal daarvan, terwijl dit op 3 uur in 80-100% van de bevriezingen het geval was.

Hoofdstuk VIII

ALGEMENE DISCUSSIE EN CONCLUSIES

In dit laatste hoofdstuk worden de resultaten van de verschillende onderzoekingen kort samengevat en met elkaar in verband gebracht. Naar aanleiding hiervan worden enkele algemene conclusies getrokken betreffende het toepassen van cryocoagulatie als therapie bij de CIN-III. Cryocoagulatie als sparende behandelingsmethode bij CIN kan alleen toegepast worden als aan de algemeen bekende voorwaarden is voldaan. De resultaten van deze studie tonen aan dat de lineaire uitbreiding en de diepte van het vriesletsel afhankelijk is van de vriestijd en de gebruikte probe en varieert op de verschillende anatomische posities (3, 6, 9 en 12 uur). Opvallend is dat het vriesletsel zich op de 3 en 9 uurposities matig ontwikkelt. De behandeling van CIN-III op deze posities met cryocoagulatie brengt dan ook een extra risico met zich mee. Een lange vriesprocedure van 5 min vriezen – 5 min dooien – 5 min vriezen bleek voldoende te zijn. Indien wenselijk lijkt het gerechtvaardigd om op 6 en 12 uur, met een bepaalde probe, cryocoagulatie toe te passen met een kortere vriesduur (3 min vriezen – 5 min dooien – 3 min vriezen).

Echter, patiënten met uitgebreide ectocervicale afwijkingen, zelfs als deze op 6 en 12 uur gelokaliseerd zijn, behoren tot de risicogroep. De beste resultaten worden verkregen als de CIN-afwijking volledig bedekt is met de probe. Vandaar dat bij uitgebreide CIN een grote probe gebruikt moet worden. Als de CIN-III afwijking niet geheel door de probe bedekt wordt, dan dient de vriesprocedure met de lange vriestijd toegepast te worden. Echter hierbij moet opgemerkt worden dat indien de CIN-III meer dan 5 mm buiten de probe uitsteekt, geen cryocoagulatie toegepast moet worden.

De keuze van de grootte van de probe hangt ook af van de diameter van de portio. Doordat na vriezen de diameter van het berijpte gebied op de portio ongeveer 10-15 mm groter is dan de diameter van de portio, kunnen de grote probes (diameter 25 mm) niet gebruikt worden bij portio's waarvan de diameter kleiner dan ongeveer 35 mm is. Bij patiënten met duidelijk zichtbare ovula Nabothi wordt de vriesprocedure met lange vriestijd aanbevolen.

Samenvatting: Het toepassen van cryocoagulatie met lange vriesduur (5 min vriezen – 5 min dooien – 5 min vriezen) heeft een nagenoeg maximale uitbreiding van het vriesletsel tot gevolg. Deze toepassingsmethode is de meest veilige en moet zeker toegepast worden bij CIN-III gebieden welke door ligging en uitbreiding extra risico meebrengen. Indien de voorkeur aan een meer behoudende benadering gegeven wordt, kan in bepaalde geselecteerde gevallen en met geselecteerde probes cryocoagulatie toegepast worden met een vriesprocedure van 3 min vriezen – 5 min dooien – 3 min vriezen.

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Appendix

Table 1:
Mean Maximum Depth (MMD) and maximum depth (mm) of cervical crypts at various distances from the reference point R at the 3, 6, 9 and 12 o'clock positions (n = number of measurements)

clock position	no. of slides	distance from R —• -3 mm	R 0	3 mm	6 mm	9 mm	12 mm	15 mm	18 mm	21 mm	24 mm	27 mm	30 mm	33 mm	36 mm	39 mm	42 mm	45 mm
3 o'clock	153	n	16	30	49	78	108	127	132	127	128	120	90	64	42	13	3	3
		MMD ± SD	2.5±1.0	2.3±1.3	2.2±1.1	2.2±1.0	2.3±1.2	2.4±1.4	2.5±1.5	2.4±1.3	2.3±1.2	2.3±1.3	2.3±1.4	2.4±1.4	2.1±1.1	2.0±1.1	2.9±0.5	1.1±0.4
		Maximum	3.9	7.2	5.3	5.5	6.4	6.6	8.2	7.4	6.1	5.7	6.5	6.6	5.2	4.6	3.3	1.5
6 o'clock	171	n	15	26	56	78	115	135	147	153	157	143	129	92	67	36	11	5
		MMD ± SD	2.3±1.1	1.9±0.9	2.2±1.0	2.1±1.0	2.1±1.2	2.3±1.3	2.5±1.7	2.8±1.6	2.9±1.5	2.7±1.5	2.7±1.5	2.8±1.2	2.5±1.5	2.3±1.3	2.9±1.6	2.2±1.3
		Maximum	4.8	4.4	4.8	5.9	7.8	7.3	10.1	12.4	9.4	8.4	8.5	7.1	7.3	4.7	5.1	4.2
9 o'clock	137	n	9	15	42	69	98	113	116	124	118	100	80	59	37	13	8	3	1
		MMD ± SD	2.6±1.0	2.1±0.7	2.5±1.1	2.2±1.1	2.2±1.0	2.4±1.1	2.6±1.4	2.5±1.3	2.5±1.3	2.8±1.6	2.4±1.5	2.2±1.2	2.2±1.0	2.1±0.9	2.1±1.0	2.4±0.7	1.2±0
		Maximum	4.3	3.7	6.2	5.8	5.3	7.9	8.2	8.9	7.1	10.3	10.8	4.8	4.8	3.8	4.8	2.9	1.2
12 o'clock	171	n	25	31	68	74	112	137	142	141	144	133	125	117	80	54	21	3	1
		MMD ± SD	2.0±1.4	2.0±0.8	2.0±0.9	2.1±1.0	2.2±1.1	2.1±1.2	2.3±1.2	2.5±1.4	2.7±1.5	2.6±1.3	2.5±1.4	2.5±1.3	2.3±1.3	2.3±1.4	2.3±1.5	0.9±0.2	1.5±0
		Maximum	7.1	3.9	4.3	4.8	6.7	6.9	6.6	8.3	7.8	8.3	8.5	6.7	6.1	6.0	6.2	1.1	1.5
total	632																		

Table 2:
Mean Maximum Depth (MMD) and maximum depth of cervical crypts and mean depth and maximum depth of 'last gland' in relation to age (at 12 o'clock)

age	no. of slides	%	depth of crypts (mm) MMD±SD	max.	depth of 'last gland' (mm) mean depth±SD	max.
20 - 29 years	10	5.8	3.9 ± 1.9	7.5	1.3 ± 0.5	1.9
30 - 39 years	47	27.5	3.8 ± 1.4	7.1	1.9 ± 1.2	7.1
40 - 49 years	66	38.6	3.8 ± 1.5	8.3	1.6 ± 0.9	4.2
50 - 59 years	26	15.2	4.6 ± 1.3	7.4	1.5 ± 1.0	4.1
60 - 69 years	13	7.6	2.8 ± 1.8	6.4	1.3 ± 0.8	3.0
≥ 70 years	9	5.3	2.5 ± 1.2	4.8	1.4 ± 1.2	3.9
total	171	100				
< 60 years	149	87.1	4.0 ± 1.5	8.3	1.7 ± 1.0	7.1
≥ 60 years	22	12.9	2.7 ± 1.3	6.8	1.4 ± 0.9	3.9
	171	100				

Table 3:
Mean Maximum Depth (MMD) of cervical crypts (mm) at various distances from the reference point R in relation to age (12 o'clock position; n = number of measurements)

age	no. of slides	%	distance from R —•	-3 mm	R 0	3 mm	6 mm	9 mm	12 mm	15 mm	18 mm	21 mm	24 mm	27 mm	30 mm	33 mm	36 mm	39 mm
< 60 years	149	86.6	n	19	25	43	64	100	121	126	134	128	118	113	107	72	48	19
			MMD ± SD	2.0±1.5	2.1±0.8	2.1±0.8	2.1±1.0	2.2±1.2	2.2±1.2	2.4±1.2	2.6±1.4	2.8±1.5	2.7±1.3	2.6±1.4	2.5±1.4	2.4±1.3	2.3±1.4	2.4±1.5
≥ 60 years	22	13.4	n	6	6	5	10	12	16	16	17	16	15	12	10	8	6	2
			MMD ± SD	2.0±1.0	1.7±0.9	1.4±0.9	2.0±1.0	2.2±1.0	1.6±0.6	1.7±1.1	1.8±1.1	2.0±1.1	1.7±0.6	1.9±1.2	2.4±1.3	2.0±1.3	1.7±0.9	1.8±1.3
total	171	100																

Table 4:
Mean Maximum Depth (MMD) and maximum depth of cervical crypts and mean depth and maximum depth of 'last gland' in relation to period of life (at 12 o'clock position)

period of life	no. of slides	%	depth of crypts (mm)		depth of 'last gland' (mm)	
			MMD ± SD	max.	mean depth ± SD	max.
premenopause	135	78.9	4.0 ± 1.5	8.3	1.7 ± 1.1	7.1
postmenopause	36	21.1	3.0 ± 1.1	6.2	1.4 ± 0.9	3.9
total	171	100				

Table 5:
Mean Maximum Depth (MMD) of cervical crypts (mm) at various distances from the reference point R in relation to period of life (12 o'clock position; n = number of measurements)

period of life	no. of slides	%	distance from R	⟵ −3 mm	R 0	3 mm	6 mm	9 mm	12 mm	15 mm	18 mm	21 mm	24 mm	27 mm	30 mm	33 mm	36 mm	39 mm	42 mm
pre-menopause	135	78.9	n	19	24	41	59	85	109	114	112	115	109	106	100	70	45	16	3
			MMD ± SD	2.0±1.5	2.0±0.8	2.1±0.8	2.1±1.0	2.2±1.2	2.2±1.2	2.4±1.3	2.7±1.4	2.9±1.5	2.7±1.3	2.6±1.4	2.5±1.4	2.3±1.3	2.4±1.5	2.3±1.3	0.9±0.3
post-menopause	36	21.1	n	6	7	7	15	22	28	28	29	29	24	18	17	10	9	5	1
			MMD ± SD	2.0±1.0	1.9±0.9	1.5±0.9	1.9±1.0	2.0±1.0	1.8±0.8	2.0±1.0	1.9±1.2	2.2±1.1	1.9±0.8	2.2±1.1	2.3±1.2	2.3±1.4	1.7±0.9	2.4±2.3	1.0±0
total	171	100																	

Table 6:
Mean Maximum Depth (MMD) and maximum depth of cervical crypts and mean depth and maximum depth of 'last gland' in relation to parity (at 12 o'clock position)

parity	no. of slides	%	depth of crypts (mm)		depth of 'last gland' (mm)	
			MMD±SD	max.	mean depth±SD	max.
0	14	8.2	3.0 ± 1.0	5.1	1.1 ± 0.6	2.1
1	11	6.4	3.6 ± 1.3	6.0	1.6 ± 0.9	3.3
2	85	49.7	3.8 ± 1.7	8.3	1.7 ± 1.1	7.1
3	39	22.8	4.0 ± 1.5	6.9	1.7 ± 1.0	4.2
≥ 4	22	12.9	3.9 ± 1.2	6.1	1.9 ± 1.0	3.9
total	171	100				
0	14	8.2	3.0 ± 1.0	5.1	1.1 ± 0.6	2.1
≥ 1	157	91.8	3.9 ± 1.5	8.3	1.7 ± 1.0	7.1
total	171	100				

Table 7:
Mean Maximum Depth (MMD) of cervical crypts (mm) at various distances from the reference point R in relation to parity (12 o'clock position; n = number of meaurements)

parity	no. of slides	%	distance from R	⟵ −3 mm	R 0	3 mm	6 mm	9 mm	12 mm	15 mm	18 mm	21 mm	24 mm	27 mm	30 mm	33 mm	36 mm	39 mm
0	14	8.2	n	2	4	5	5	9	12	10	11	9	10	10	8	7	3	3
			MMD ± SD	1.3±0.4	1.6±0.1	1.9±0.7	1.3±0.4	1.9±0.9	1.8±0.8	1.6±1.0	2.1±1.2	2.4±1.1	2.4±1.3	2.0±1.3	2.2±1.1	1.9±1.2	1.4±1.0	2.0±1.1
≥ 1	157	91.8	n	23	27	43	69	103	125	132	130	135	123	115	109	73	49	18
			MMD ± SD	2.1±1.4	2.1±0.9	2.0±0.9	2.2±1.0	2.2±1.2	2.1±1.2	2.4±1.2	2.6±1.4	2.8±1.5	2.6±1.3	2.6±1.4	2.5±1.4	2.4±1.4	2.3±1.4	2.4±1.6
total	171	100																

Table 8:
Mean Maximum Depth (MMD) of cervical crypts (mm) at various distances from the reference point R in relation to parity and age (12 o'clock position; n = number of measurements)

age and parity	no of slides	%	distance from R	−3 mm	0	3 mm	6 mm	9 mm	12 mm	15 mm	18 mm	21 mm	24 mm	27 mm	30 mm	33 mm	36 mm	39 mm
<60 years																		
parity: 0	12	7.0	n						11	8	9	8	8	8	7	6	4	2
			MMD ± SD						1.8±0.8	1.8±1.0	2.4±1.1	2.5±1.1	2.5±1.3	2.2±1.4	2.2±1.2	2.1±1.2	1.5±1.2	2.6±0.8
≥1	137	80.1	n	17	21	38	59	91	110	118	115	120	109	105	100	66	44	17
			MMD ± SD	2.1±1.5	2.2±0.9	2.1±0.9	2.2±1.0	2.2±1.2	2.2±1.2	2.5±1.2	2.6±1.4	2.9±1.5	2.7±1.3	2.6±1.4	2.5±1.4	2.4±1.4	2.4±1.4	2.3±1.6
≥60 years																		
parity: 0	2	1.2	n						1	2	2	1	1	2	1	1	1	1
			MMD ± SD						2.1	0.7±0.3	0.8±0.2	1.5±0	1.3±0	1.4±0.4	1.8±0	0.7±0	1.1±0	0.9±0
≥1	20	11.7	n	6	6	5	10	12	15	14	15	15	14	10	9	7	5	1
			MMD ± SD	2.0±1.0	1.7±0.9	1.4±0.9	2.0±1.0	2.2±1.0	1.6±0.7	1.8±1.1	1.9±1.1	2.1±1.1	1.7±0.6	2.0±1.3	2.5±1.3	2.2±1.2	1.8±0.9	2.7±0
total	171	100																

Table 9:
Mean Maximum Depth (MMD) of cervical crypts (mm) at various distances from the reference point R in relation to shape of external os and clock postion (n = number of measurements)

shape of external os and clock position	no of slides	%	distance from R	−3 mm	0	3 mm	6 mm	9 mm	12 mm	15 mm	18 mm	21 mm	24 mm	27 mm	30 mm	33 mm	36 mm	39 mm	42 mm
3 o'clock:																			
slit shaped	127	89.5	n	12		48	73	96	114	119	116	116	108	80	59	39	12	2	1
			MMD ± SD	2.1±0.8		2.2±1.1	2.1±1.0	2.2±1.2	2.4±1.4	2.5±1.4	2.4±1.3	2.2±1.8	2.3±1.3	2.4±1.4	2.4±1.4	2.1±1.2	1.9±1.1	2.9±0.6	1.2±0.5
round	14	9.2	n	3		1	5	10	11	11	9	10	10	8	5	3	1	1	1
			MMD ± SD	3.7±0.2		1.2±0	2.4±1.0	2.5±1.3	3.0±1.8	2.8±1.7	2.6±1.7	3.1±1.4	2.7±1.5	2.0±1.2	2.0±1.1	1.9±0.2	3.0±0	2.9±0	0.9±0
unknown	2	1.3																	
6 o'clock:																			
slit shaped	150	87.7	n	13	24	49	71	99	110	129	135	138	127	116	84	62	33	9	4
			MMD ± SD	2.2±1.0	2.0±0.9	2.2±1.0	2.2±1.0	2.1±1.2	2.4±1.3	2.6±1.7	2.8±1.7	2.8±1.7	2.7±1.6	2.8±1.5	2.5±1.3	2.5±1.5	2.2±1.2	2.5±1.3	1.8±0.8
round	19	11.1	n	2	2	6	6	14	13	16	16	17	14	13	8	4	3	2	1
			MMD ± SD	2.7±1.9	1.3±0.4	2.0±1.3	1.4±0.4	1.7±0.9	2.2±1.0	3.1±1.0	2.7±1.0	3.0±1.3	2.7±1.5	2.1±1.0	2.2±0.8	2.4±1.2	3.1±2.1	5.1±0.1	4.2±0
unknown	2	1.2																	
9 o'clock:																			
slit shaped	118	86.1	n		13	39	59	74	99	99	106	94	86	82	52	31			
			MMD ± SD		2.1±0.7	2.5±1.1	2.2±1.1	2.2±1.0	2.3±1.1	2.5±1.3	2.4±1.2	2.4±1.3	2.8±1.6	2.4±1.5	2.1±1.1	2.2±0.9			
round	17	12.4	n		2	3	10	12	12	15	16	14	12	5	5	4			
			MMD ± SD		2.1±1.1	2.4±0.9	2.4±1.4	1.8±0.8	2.5±0.9	2.7±1.2	2.9±1.4	2.6±1.2	2.6±1.5	1.7±0.9	2.4±1.4	2.8±1.8			
unknown	2	1.5																	
12 o'clock:																			
slit shaped	151	88.3	n	23	26	42	66	98	119	126	123	128	119	111	106	73	48	18	
			MMD ± SD	2.1±1.4	2.0±0.9	2.0±0.9	2.2±1.1	2.2±1.2	2.2±1.2	2.4±1.2	2.6±1.4	2.8±1.4	2.6±1.3	2.5±1.4	2.5±1.4	2.3±1.4	2.4±1.4	2.4±1.5	
round	18	10.5	n	2	5	6	7	12	16	14	16	14	13	12	9	6	6	3	
			MMD ± SD	1.3±0.4	1.8±0.6	1.8±0.8	1.5±0.6	1.8±0.9	1.8±0.8	1.8±0.8	2.1±1.2	2.3±1.2	2.3±1.3	2.3±1.2	2.1±1.1	1.9±1.2	1.0±0.4	1.6±1.3	
unknown	2	1.2																	
total	635	100																	

Table 10:
Mean Maximum Depth (MMD) and maximum depth of cervical crypts and mean depth and maximum depth of 'last gland' in relation to portio diameter (at 12 o'clock position)

portio diameter	no of slides	%	depth of crypts (mm) MMD ± SD	max.	depth of 'last gland' (mm) mean depth ± SD	max.
< 25 mm	30	17.5	2.8 ± 0.9	5.0	1.4 ± 0.8	3.3
25 – 35 mm	107	62.6	4.0 ± 1.6	8.3	1.7 ± 1.1	7.1
> 35 mm	33	19.3	4.1 ± 1.4	7.1	1.8 ± 1.0	4.1
unknown	1	0.6				
total	171	100				

Table 11:
Mean Maximum Depth (MMD) of cervical crypts (mm) at various distances from the reference point R in relation to portio diameter (12 o'clock position; n = number of measurements)

portio diameter	no of slides	%	distance from R	R	3 mm	6 mm	9 mm	12 mm	15 mm	18 mm	21 mm	24 mm	27 mm	30 mm	33 mm	36 mm	39 mm	42 mm
< 25 mm	30	17.5	n	3	4	6	11	20	25	26	26	22	20	14	12	9	6	3	1
			MMD ± SD	1.5±0.3	1.6±0.6	1.7±0.8	1.6±1.0	1.8±0.8	1.7±0.8	1.7±0.8	1.7±0.9	1.8±0.9	2.0±1.0	2.0±0.7	1.9±0.7	1.4±0.9	1.5±1.5	1.7±1.2	1.0±0
25-35 mm	107	62.6	n	20	18	32	46	66	84	87	88	92	86	85	80	51	35	15	1
			MMD ± SD	2.1±1.5	1.8±0.6	2.0±0.9	2.1±1.0	2.2±1.3	2.2±1.3	2.5±1.3	2.7±1.4	2.9±1.5	2.6±1.3	2.6±1.4	2.5±1.3	2.4±1.3	2.3±1.4	2.4±1.7	0.7±0
> 35 mm	33	19.3	n	2	8	10	16	25	27	28	28	29	27	26	25	20	13	3	1
			MMD ± SD	1.5±0	2.5±1.2	2.2±0.8	2.5±1.1	2.4±0.9	2.2±1.0	2.5±1.2	2.8±1.4	3.1±1.4	2.8±1.3	2.8±1.7	2.8±1.5	2.6±1.5	2.5±1.4	2.2±0.3	1.1±0
unknown	1	0.6	n																
total	171	100																	

Table 12:
Distance of deepest crypt and 'last gland' to reference point R in relation to age, period of life. parity, portio diameter at 12 o'clock position (mm)

groups	no of slides	distance deepest crypt to reference point R mean ± SD	maximum proximally	maximum distally	distance 'last gland' to reference point R mean ± SD	maximum proximally	maximum distally
age:							
20-29 years	10	19.8±10.5	39	3	3.6±6.9	12	-9
30-39 years	47	20.4± 9.6	39	-3	6.3±6.0	21	-6
40-49 years	66	20.4± 8.4	36	0	3.9±6.0	15	-9
50-59 years	26	21.3± 8.1	39	6	7.8±5.1	18	-3
60-69 years	13	17.1± 9.3	33	-3	5.1±7.5	12	-6
≥ 70 years	9	14.7± 6.6	27	6	3.9±9.6	18	-9
< 60 years	149	20.4± 8.7	39	-3			
≥ 60 years	22	16.2± 8.4	33	-3	5.4±6.0	21	-9
					4.5±8.1	18	-9
period of life:							
premenopause	135	21.0± 8.7	39	-3			
postmenopause	36	16.5± 8.7	39	-3	5.1±6.3	21	-9
					5.1±6.9	18	-9
parity:							
nulliparous	14	22.5±10.5	39	3			
parous	157	19.8± 8.7	39	-3	5.4±6.6	15	-6
					5.1±6.3	21	-9
portio diameter:							
< 25 mm	30	16.2±10.8	39	-3			
25-35 mm	107	21.3± 7.8	39	-3	6.0±5.4	12	-6
> 35 mm	33	19.5± 8.7	36	0	5.1±6.6	21	-9
unknown	1				4.8±6.0	15	-9

Table 13:
Distance of deepest crypt and 'last gland' to reference point R in relation to shape of external os at different clock positions (mm)

shape of external os and clock position	no of slides	distance deepest crypt to reference point R mean ± SD	maximum proximally	maximum distally	distance 'last gland' to reference point R mean ± SD	maximum proximally	maximum distally
3 o'clock:							
- slit shaped	137	19.8± 9.0	33	-3	5.1±5.4	21	-6
- round	14	14.7± 8.7	24	-6	5.2±6.8	21	-6
- unknown	2						
6 o'clock:							
- slit shaped	150	19.2± 8.7	36	-6	4.8±5.7	27	-9
- round	19	19.2±11.1	39	-9	5.1±7.5	15	-9
- unknown	2						
9 o'clock:							
- slit shaped	118	16.5± 7.8	36	-3	5.1±5.4	27	-9
- round	17	19.2± 7.8	33	6	5.4±3.9	12	0
- unknown	2						
12 o'clock:							
- slit shaped	151	19.8± 8.7	39	-3	5.1±6.3	21	-9
- round	18	19.8±10.2	39	0	5.4±6.0	12	-6
- unknown	2						

Table 14:
Absolute (number) and relative (%) frequency of topographical distribution of deepest crypts, the 'last glands' and the Nabothian cysts (12 o'clock position)

distance to R (mm)	deepest crypt frequency		last gland frequency		Nabothian cysts frequency	
	absolute	relative	absolute	relative	absolute	relative
-9			5	2.9%		
-6			7	4.1%		
-3	2	1.1%	18	10.5%	2	0.7%
0	3	1.8%	18	10.5%	1	0.4%
3	2	1.1%	24	14.1%	2	0.7%
6	3	1.8%	28	16.4%	3	1.1%
9	13	7.6%	34	19.9%	1	0.4%
12	18	10.5%	28	16.4%	12	4.4%
15	21	12.3%	4	2.3%	21	7.8%
18	20	11.7%	4	2.3%	31	11.5%
21	25	14.6%	1	0.6%	36	13.3%
24	16	9.4%			40	14.8%
27	18	10.5%			32	11.9%
30	13	7.6%			27	10.0%
33	7	4.1%			20	7.4%
36	7	4.1%			23	8.5%
39	3	1.8%			12	4.4%
42					6	2.2%
45					1	0.4%
48						
Total	171	100%	171	100%	270	100%

Table 15:
Maximum, Minimum, and Mean Maximum Extension (MME) of the cryolesion in relation to age, period of life and parity at 3, 6, 9 and 12 o'clock positions (mm) (small cervix probe 19 x 9; N = number of women/slides)

population			N		extension of cryolesion		
			women	slides	MME±SD	max.	min.
3 o'clock	age	30-39 years	6	6	5.7±2.4	9.1	3.1
		40-49 years	9	9	4.6±1.6	7.9	3.0
		50-59 years	5	5	3.8±2.2	5.6	0
	period of life	premenopause	15	15	5.2±1.9	9.1	3.0
		postmenopause	5	5	3.3±2.0	4.9	0
	parity	nulliparous	2	2	5.9±0.4	6.2	5.6
		parous	18	18	4.6±2.1	9.1	0
6 o'clock	age	30-39 years	6	12	6.8±1.1	8.9	5.4
		40-49 years	9	18	6.0±1.4	9.1	3.5
		50-59 years	5	10	7.0±1.1	8.3	5.8
	period of life	premenopause	13	30	6.3±1.3	9.1	3.5
		postmenopause	5	10	7.2±1.0	8.3	5.8
	parity	nulliparous	2	4	5.9±0.2	6.1	5.6
		parous	18	36	6.6±1.4	9.1	3.5
9 o'clock	age	30-39 years	6	6	4.4±2.5	6.9	0
		40-49 years	9	9	4.1±1.8	7.3	1.9
		50-59 years	5	5	4.0±2.6	6.5	0
	period of life	premenopause	13	15	4.2±2.1	7.3	0
		postmenopause	5	5	4.0±2.6	6.5	0
	parity	nulliparous	2	2	4.7±2.4	6.4	3.0
		parous	18	18	4.1±2.2	7.3	0
12 o'clock	age	30-39 years	6	12	6.0±2.2	9.6	2.6
		40-49 years	9	18	6.4±1.5	8.8	3.8
		50-59 years	5	10	6.2±1.3	8.4	4.2
	period of life	premenopause	15	30	6.1±1.8	9.6	2.6
		postmenopause	5	10	6.6±1.2	8.4	4.2
	parity	nulliparous	2	4	6.2±1.0	7.1	5.2
		parous	18	36	6.2±1.7	9.6	2.6

Table 16:
Mean Maximum Extension (MME) of the cryolesion (mm) at various distances from the reference point R, achieved with four types of probes at 3, 6, 9 and 12 o'clock positions (N = number of women/slides)

probe and clock position	N women	N slides	distance from R ⟶	-15	-12	-9	-6	-3	R 0	3	6	9	12	15	18	21	24	27	30	33	36
small cone																					
3 o'clock	13	13	MME±SD	0	0.2±0.7	0.7±1.6	1.7±2.3	2.5±2.7	3.2±2.8	3.2±2.8	3.4±2.7	4.1±2.7	4.0±2.7	3.8±2.7	3.1±2.8	2.0±2.6	1.4±2.4	0.8±1.8	0.3±0.7	0	0
6 o'clock	14	28	MME±SD	0	0.4±1.1	1.1±2.4	2.7±3.2	3.7±3.5	4.1±3.5	4.4±3.3	5.1±3.0	6.1±2.6	6.5±2.3	6.3±2.0	5.8±2.4	4.9±2.7	3.1±2.9	1.4±2.1	0.3±0.8	0.2±0.6	0.2±0.6
9 o'clock	13	13	MME±SD	0	0	0.4±1.3	1.2±2.0	1.7±2.5	1.9±2.8	2.0±2.7	2.5±2.4	4.1±2.1	4.6±2.2	4.4±2.3	3.9±2.0	2.3±2.0	1.1±1.8	0.6±1.1	0.1±0.5	0	0
12 o'clock	14	28	MME±SD	0	0.0±0.1	1.0±2.2	2.5±2.7	3.4±3.1	4.0±3.2	4.4±2.9	4.7±2.6	5.4±2.0	6.0±1.7	6.0±1.8	5.9±1.6	5.3±1.7	4.1±2.6	2.3±2.4	0.7±1.6	0.1±0.5	0
small cervix																					
3 o'clock	20	20	MME±SD	0	0.0±0.2	0.7±1.5	1.6±2.4	2.6±2.8	3.1±3.0	3.5±2.8	3.8±2.5	4.0±2.4	3.7±2.3	3.3±2.2	1.8±2.3	0.7±1.4	0.1±0.2	0	0	0	0
6 o'clock	20	40	MME±SD	0	0.2±0.7	0.9±1.8	2.4±2.7	3.0±3.2	4.4±2.7	4.8±2.5	5.3±2.1	5.9±1.7	6.0±1.4	5.3±1.9	4.2±2.4	2.4±2.4	0.7±1.7	0.3±1.1	0.1±0.4	0	0
9 o'clock	20	20	MME±SD	0	0.1±0.3	0.4±1.4	1.0±1.9	1.5±2.2	2.1±2.2	2.4±2.3	2.9±2.4	3.2±2.5	3.4±2.4	3.2±2.0	1.6±1.6	0.6±1.1	0	0	0	0	0
12 o'clock	20	40	MME±SD	0.1±0.4	0.5±1.3	1.2±2.4	2.2±2.9	2.9±3.2	3.3±3.3	3.6±3.2	4.2±2.9	5.2±2.3	5.7±1.7	5.3±1.8	4.0±2.8	2.8±2.4	1.1±1.8	0.2±0.7	0	0	0
large cone																					
3 o'clock	10	10	MME±SD	0	0.6±1.3	0.9±1.9	1.5±2.3	1.9±2.8	2.4±3.0	2.4±2.9	2.9±2.6	4.0±1.8	4.6±1.7	4.8±1.9	4.4±2.1	3.4±2.4	1.9±2.0	0.5±1.0	0	0	0
6 o'clock	12	24	MME±SD	0.3±0.9	0.4±1.4	0.8±1.8	2.3±2.8	2.9±3.3	4.0±3.3	4.3±3.2	5.0±2.8	6.3±2.4	6.9±2.1	7.1±1.7	7.1±1.4	6.7±1.4	5.9±1.6	4.6±2.1	3.1±2.4	1.1±2.0	0.4±1.5
9 o'clock	12	12	MME±SD	0	0.1±0.2	0.5±1.1	1.7±1.9	2.2±2.4	2.5±2.8	2.5±2.8	2.7±2.5	3.1±2.1	3.1±2.1	3.5±1.7	3.3±1.9	2.9±2.5	2.8±2.4	1.8±2.6	0.8±2.1	0.6±1.9	0.5±1.8
12 o'clock	12	24	MME±SD	0.1±0.5	0.5±1.3	1.4±2.5	2.6±3.2	3.7±3.5	4.2±3.7	4.5±3.7	4.9±3.6	6.2±2.9	7.3±2.1	7.8±1.5	7.6±1.5	7.0±1.7	6.0±2.1	3.9±3.2	2.6±3.1	1.6±2.9	0.9±2.1
large cervix																					
3 o'clock	8	8	MME±SD	0	0	1.7±2.9	2.2±2.6	2.7±3.0	3.1±3.0	3.2±2.9	3.4±2.9	3.9±2.5	4.3±1.9	3.7±1.5	2.9±1.8	1.9±1.8	0.4±1.2	0.3±0.8	0	0	0
6 o'clock	8	16	MME±SD	0	0.4±1.6	1.3±2.3	3.0±3.3	4.8±3.6	5.6±3.6	6.3±3.0	6.5±2.6	7.1±1.9	7.4±1.9	7.0±2.2	6.4±2.9	4.5±3.2	2.5±3.2	1.6±2.1	0.8±1.3	0	0
9 o'clock	7	7	MME±SD	0	0	0.5±1.1	2.5±2.0	3.3±2.4	3.7±2.6	3.7±2.7	3.7±2.4	3.9±1.9	3.9±1.5	3.2±1.5	2.2±1.7	1.5±1.9	0.9±1.2	0.5±1.3	0	0	0
12 o'clock	8	16	MME±SD	0.8±2.2	1.8±3.3	2.6±3.8	4.5±4.2	5.6±4.4	6.1±4.5	6.9±4.1	7.1±3.8	7.4±3.8	8.2±3.2	8.4±3.2	7.7±3.3	6.9±3.4	5.3±3.0	3.4±3.0	1.0±1.9	0.5±1.4	0.2±0.8

Table 17:
Mean, maximum and minimum distance (mm) of maximum extension and distal border of cryolesion to reference point R in relation to age, period of life and parity (small cervix probe 19 x 9; N = number of women/slides)

	groups		N		distance of maximum extension of the cryolesion to reference point R			distance of distal border of the cryolesion to reference point R		
			women	slides	mean ± SD	max.	min.	mean ± SD	max.	min.
3 o'clock	age	30-39 years	8	8	9.0±4.6	15.0	3.0	-1.5± 7.8	6.0	-12.0
		40-49 years	8	8	10.0±6.9	21.0	0	0± 9.1	15.0	-9.0
		50-59 years	6	6	9.8±4.5	15.0	6.0	-6.0± 9.7	6.0	-21.0
	period of life	premenopause	15	15	9.6±4.5	21.0	0	-0.8± 8.4	15.0	-12.0
		postmenopause	6	6	9.8±4.5	15.0	6.0	-5.4± 9.8	6.0	-21.0
	parity	nulliparous	2	2	7.5±2.1	9.0	6.0	-6.0± 0	-6.0	-6.0
		parous	17	17	9.9±5.8	21.0	0	-1.5± 9.2	15.0	-21.0
6 o'clock	age	30-39 years	6	12	9.2±3.9	15.0	0	-4.5± 5.5	3.0	-12.0
		40-49 years	9	18	9.7±5.1	15.0	0	-2.7± 6.9	12.0	-12.0
		50-59 years	5	10	12.9±3.2	15.0	6.0	-0.9± 4.7	6.0	-9.0
	period of life	premenopause	15	30	9.8±4.5	15.0	0	-2.5± 6.5	12.0	-12.0
		postmenopause	5	10	12.0±4.5	15.0	3.0	-3.6± 4.6	0	-9.0
	parity	nulliparous	2	4	10.5±1.7	12.0	9.0	-2.3± 2.9	6.0	0
		parous	18	36	10.3±4.7	15.0	0	-3.3± 6.0	12.0	-12.0
9 o'clock	age	30-39 years	5	9	10.8±3.4	15.0	6.0	-4.0±11.6	12.0	-21.0
		40-49 years	9	9	10.7±5.0	18.0	3.0	-1.0± 8.4	15.0	-9.0
		50-59 years	4	4	12.0±4.9	18.0	6.0	-0.5±12.3	9.0	-21.0
	period of life	premenopause	14	14	11.6±4.5	18.0	3.0	-0.4±10.1	15.0	-21.0
		postmenopause	4	4	9.0±3.5	12.0	6.0	-2.4±11.1	6.0	-21.0
	parity	nulliparous	2	2	13.5±6.4	18.0	9.0	-3.0± 8.5	9.0	-3.0
		parous	16	16	10.7±4.2	18.0	3.0	-1.3±10.4	15.0	-21.0
12 o'clock	age	30-39 years	6	12	11.5±7.0	24.0	0	-2.0± 8.3	12.0	-12.0
		40-49 years	9	18	10.3±6.2	18.0	0	-0.3± 7.8	9.0	-15.0
		50-59 years	5	10	14.1±4.7	21.0	9.0	-1.2± 7.6	9.0	-12.0
	period of life	premenopause	15	30	11.5±6.9	24.0	0	-0.7± 8.3	12.0	-15.0
		postmenopause	5	10	12.0±3.2	18.0	9.0	-3.0± 5.8	6.0	-12.0
	parity	nulliparous	2	4	18.8±2.9	21.0	15.0	9.0± 0	9.0	9.0
		parous	18	36	10.8±5.9	24.0	0	2.4± 7.3	12.0	-15.0

Table 18:
Mean, maximum and minimum distance (mm) of maximum extension and distal border of cryolesion to reference point R in relation to shape of external os at 3, 6, 9 and 12 o'clock positions (small cervix probe 19 x 9; N = number of women/slides)

clock position	shape of external os	N		distance of maximum extension of the cryolesion to reference point R			distance of distal border of the cryolesion to reference point R		
		women	slides	mean ± SD	max.	min.	mean ± SD	max.	min.
3 o'clock	slit shaped ostium	11	11	12.3±4.7	21.0	6.0	-1.0±10.3	15.0	-21.0
	round ostium	8	8	8.3±4.5	15.0	0	-6.4± 2.5	-3.0	-9.0
6 o'clock	slit shaped ostium	12	24	10.0±4.4	15.0	0	-1.6± 8.1	12.0	-12.0
	round ostium	8	16	10.9±4.9	15.0	0	-4.5± 5.7	6.0	-12.0
9 o'clock	slit shaped ostium	10	10	10.5±4.9	18.0	3.0	1.7±12.3	15.0	-21.0
	round ostium	8	8	11.6±3.7	18.0	6.0	0.4± 5.9	9.0	-6.0
12 o'clock	slit shaped ostium	12	24	11.4±6.5	24.0	0	0.5± 7.1	12.0	-12.0
	round ostium	8	16	12.0±5.8	21.0	0	-2.4± 8.4	9.0	-15.0

Table 19:
Mean Maximum Extension (MME) of the cryolesion (mm) after long freezing at 3, 6, 9 and 12 o'clock positions at various distances from the referenc point R (small cone probe 19 x 15.5)

clock position	no of slides	distance from R →	-15	-12	-9	-6	-3	R	3	6	9	12	15	18	21	24	27	30	33	36
3 o'clock	10	MME±SD	0.7±2.3	2.1±3.5	3.4±3.8	5.3±4.1	6.9±4.0	7.4±4.2	7.5±4.3	7.8±3.9	7.9±3.7	9.4±1.9	9.6±1.9	9.0±2.2	7.7±2.6	5.9±3.4	3.7±4.2	1.8±3.3	0.9±2.3	0
6 o'clock	10	MME±SD	0.3±0.9	2.9±3.4	3.9±3.9	6.4±3.8	7.9±3.5	8.6±4.1	8.5±4.1	8.5±3.4	9.1±2.5	9.5±1.9	9.6±1.8	9.3±1.7	8.8±1.7	8.1±2.1	4.6±4.6	2.7±3.7	0.5±1.7	0
9 o'clock	10	MME±SD	1.0±2.2	2.8±3.7	4.0±3.9	6.4±3.4	7.7±3.5	8.3±3.9	9.4±3.0	9.5±3.0	9.6±2.5	9.8±1.9	9.5±1.5	8.7±1.7	7.8±1.8	5.8±2.2	3.6±3.4	1.6±2.3	0.1±0.4	0
12 o'clock	10	MME±SD	0.2±0.6	2.0±3.0	4.6±3.6	6.4±3.7	7.3±4.2	8.4±3.6	8.4±3.5	9.1±1.8	9.5±1.3	10.3±1.0	10.9±1.0	10.6±1.4	9.9±2.2	9.5±2.3	7.7±3.8	5.9±4.0	3.6±3.6	1.8±2.9

Dankwoord

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