Relationship of Age and Hormonal Status to Cell Kinetics and Morphology of the Fibroadenoma

By

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ACKNOWLEDGEMENTS

I would like to acknowledge and thank Professor A.G. Rose, Head of the Department of Anatomical Pathology of the University of Cape Town, for making facilities available for this study.

I would like to express my appreciation and gratitude to Professor A.J. Tiltman, of the Department of Anatomical Pathology of the University of Cape Town, for his encouragement, advice and direction throughout all stages of this work. I would also like to thank him for making available the immunocytochemical antibodies used in the study.

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ABBREVIATIONS

The following abbreviations are used in this dissertation:

PCNA	Proliferating cell nuclear antigen
BrdU	5-Bromodeoxyuridine
OC	Oral contraceptive
TLI	Thymidine labelling index

TABLE OF CONTENTS

Page

INTRODUCTION

The fibroadenoma is the most common benign tumour of the female breast and can occur in all age groups with a peak in the third decade. The pathological features of fibroadenomas are well known and have recently been reviewed by Fechner (1987). The typical fibroadenoma is characteristically well circumscribed, firm and spherical. Cut surface is white, glistening and sometimes slightly myxoid. Clefts are often discernible and light brown areas indicate the epithelial component.

As the name implies, the fibroadenoma is composed of fibrous and epithelial elements. Microscopically, stromal fibrous tissue comprises the major component. The quality of the stroma can be variable, either in the same lesion, or from lesion to lesion. It is sometimes loose and myxoid with stellate cells, or it may be quite cellular with frequent mitoses and a degree of cellular pleomorphism. The stroma can also be sparsely cellular with areas of hyalinised collagen. The stroma can compress ducts to slit-like spaces (the so-called intracanalicular pattern) or the ducts may retain their patency with circumferential stroma (the pericanalicular pattern). Rarely, the stroma may contain other elements such as mature adipose tissue, smooth muscle or metaplastic bone.

The epithelium is frequently arranged into lobules and numerous extralobular ducts which have a double layer of epithelium and myoepithelium, a feature easily appreciated with the pericanalicular pattern. With the intracanalicular pattern the epithelium is often stretched and thinned and the myoepithelial layer may be lost. The epithelium may become stratified sometimes forming papillary excrescences six or seven cells thick. Tangential sectioning of these areas may produce complex and worrisome patterns, but without cellular atypia. The epithelium and stroma may show fibrocystic change, sclerosing adenosis and apocrine metaplasia as is seen in benign breast disease. Secretory change, too, may be seen in pregnant or lactating women and also occasionally in the non pregnant or lactating state.

Although imprecisely understood, fibroadenomas may have a natural history with phases of proliferation, involution and even ultimate regression. Furnival *et al* (1983), Kern and Clark (1973) and Foster *et al* (1988) demonstrated declining stromal cellularity with age and many fibroadenomas in the post menopausal period are calcified and hyalinised in keeping with an advanced stage of involution. However, some have appeared histologically as cellular and active as tumours of younger women (Fechner, 1987).

It has been estimated that fibroadenomas take 6-12 months to double in size, growth usually ceasing when a 2 or 3 cm diameter is reached (Haagensen, 1971). In a small group of patients the tumours may reach very large sizes of between 12-15 cm and are termed giant fibroadenomas. Most, but not all, giant fibroadenomas occur in adolescence and grow rapidly to attain a size two to four times the size of the opposite breast with stretching of the overlying skin and displacement of the nipple.

Morphologic changes in fibroadenomas in relation to phase of the menstrual cycle have not been investigated. Such changes have, however, been described in normal breast tissue and it is possible that similar changes may occur in fibroadenomas. In the normal breast, with progression of the cycle, increasing acinar cell differentiation, acinar luminal diameter, myoepithelial cell vacuolisation, active secretion, stromal oedema and stromal lymphocytic infiltrate have been described (Vogel *et al* 1981, Longacre and Bartow, 1986).

Studies on normal breast have also shown changes in cell proliferation with the menstrual cycle with a peak in the second half of the cycle. Cell proliferation has also been shown to decline significantly with age (Meyer, 1977; Ferguson and Anderson, 1981; Anderson *et al*, 1982; Going *et al*, 1988; Potten *et al*, 1988 and Anderson *et al*,1989).

Fibroadenomas appear to be proliferative lesions, but this proliferation has not been comprehensively investigated. The effect of hormonal stimuli in relation to

pathogenesis, modification of cell proliferation and biological behaviour is unknown and the role of natural hormonal changes occurring during the menstrual cycle and exogenous hormones require assessment. The putative natural history of fibroadenomas also requires investigation to shed further light on the biological behaviour of these commonly encountered tumours.

The main objectives, then, of this study are:

(i) to test the hypothesis that both the morphology and cell proliferation of the fibroadenoma are responsive to hormonal stimulation,

(ii) to assess morphology and cell proliferation in relationship to patient age in order to demonstrate trends in the natural history of fibroadenomas.

MATERIALS AND METHODS

Patient Selection

The clinical data were originally part of a separate clinico-pathological study conducted by the Departments of Surgery and Anatomical Pathology of the University of Cape Town on 507 women with benign breast disease presenting to the breast clinic at Groote Schuur Hospital from 1985 to 1986. Included were 131 women with 139 histologically confirmed fibroadenomas. At the time of initial presentation, a detailed clinical history was taken which especially included the date of the last menstrual period, cycle length and regularity and hormonal contraceptive use. Knowing the date of the last menstrual period, the day of the menstrual cycle on the day of lump excision could be calculated assuming a regular 28 day cycle. 90 women had menstrual data regarded as suitable for this study. Essential requirements were that there was a regular peri 28 day cycle and that not more than two cycles had occurred from time of presentation to time of lump excision.

Morphologic Assessment

Histology

Tissues previously fixed in buffered formalin and embedded in paraffin wax were obtained from departmental archives. Sections were cut at 4μ from each tissue block and stained with haematoxylin and eosin. Sections were also stained with the van Gieson stain to assess collagen content.

All histologic assessments were performed without any knowledge of clinical and other data.

Stromal Nature

Each fibroadenoma was assigned to one of three categories: myxoid, fibrous or hyaline. The stroma is considered to have the least amount of collagen when myxoid (figure 1), the most when hyaline (figure 3) and intermediate when fibrous (figure 2). When there was a mixed stromal pattern, the assigned category was that of the predominant pattern.

Stromal Cellularity

Stromal cellularity was assessed semi-quantitatively according to the method of Kern and Clark (1973) and graded on a scale of I to III. Grade I was characterised by minimal cellularity, often with hyalinisation (figure 3). Grade II was reserved for an intermediate appearance (figure 4) and Grade III (figure 5) was characterised by considerable cellularity.

Epithelial Component

The epithelial component present within a fibroadenoma was expressed as a percentage of total surface area. This was assessed visually with the aid of a 10x10 square eyepiece grid, a 4x objective lens and a 10x eyepiece lens. In the estimation, allowance was made for spaces made up by glandular lumina. The result was expressed to the nearest 5%.

Stromal Inflammatory Cells

Sections stained with haematoxylin and eosin were examined for the presence of stromal lymphocytes and plasma cells. The Giemsa stain was used to detect stromal mast cells. The density of stromal infiltrate of each cell type was assessed and graded as follows: Grade I - mild density (scanty cells present), Grade II - moderate density and Grade III - marked density.

Immunocytochemistry

Myoepithelial cells were stained immunocytochemically using a mouse monoclonal antibody to human alpha-smooth muscle actin (Dako-Smooth Muscle Actin, 1A4). A separate series of slides was stained for S100 protein using a polyclonal rabbit anti-cow S100 antibody (Dako).

The staining for smooth muscle actin was performed by the avidin-biotin peroxidase complex method as follows. 4μm thick sections were cut and mounted onto slides coated with 3-aminopropyltriethoxysilane. The sections were incubated for 30 minutes at 60 °C to promote adherence to the slides. Digestion was performed by placing the slides into 0.1% trypsin (Sigma Laboratories) in TRIS buffer for 7 minutes at 37 °C. The sections were washed in running tap water for 5 minutes and then deparaffinised to 96% alcohol. To block endogenous peroxidase, the sections were immersed for 15 minutes in 1% hydrogen peroxide in methanol. The sections were washed in running tap water for 5 minutes and then rinsed in phosphate-buffered saline. The sections were covered with normal rabbit serum and allowed to react at room temperature for 10 minutes. They were then incubated with the primary antibody diluted 1:50 with phosphate-buffered saline for 30 minutes. The sections were rinsed with phosphate-buffered saline and incubated for 30 minutes with the link antibody, biotinylated monoclonal rabbit anti-mouse (Dako) made up as follows: 4ml rabbit anti-mouse, 40ml human serum, 956ml phosphate-buffered saline. After rinsing the sections in phosphate-buffered saline, they were incubated with avidin-biotin-peroxidase complex (Dako) at room temperature for 30 minutes. The sections were rinsed in phosphate-buffered saline. 0.03% diaminobenzidine was used for colour development followed by counterstaining with Mayer's haematoxylin. The sections were washed in running tap water for 5 minutes and then dehydrated, cleared and mounted.

The peroxidase anti-peroxidase method was used for S100 staining. 4μm thick sections were cut, mounted onto slides and deparaffinised to 96% alcohol.

Endogenous peroxidase activity was blocked by immersing the sections in 1% hydrogen peroxide in methanol for 15 minutes. The sections were washed in running tap water for 5 minutes. A digestion step was performed by placing the slides into 0.1% trypsin (Sigma Laboratories) in TRIS buffer for 5 minutes at 37 °C. The sections were washed in running tap water for 5 minutes. They were then allowed to react with normal swine serum (Dako) diluted 1/20 in phosphate buffered saline for 10 minutes at room temperature. The serum was tapped off and the excess wiped away. Overnight incubation with the primary antibody diluted 1/100 at 4 °C followed. The slides were washed in phosphate buffered saline for 20 minutes with a mechanical stirrer. They were then incubated with the link antibody, Dako swine anti-rabbit diluted 1/20 with phosphate buffered saline for 20 minutes at room temperature. The slides were washed for 20 minutes in phosphate buffered saline using a mechanical stirrer and then incubated with the peroxidase anti-peroxidase complex (Dako) for 30 minutes at room temperature. The slides were washed in phosphate buffered saline using a mechanical stirrer for 20 minutes. 0.03% diaminobenzidine was used for colour development. The slides were rinsed in phosphate buffered saline, washed with water, counterstained with Mayer's haematoxylin, dehydrated, cleared and mounted.

The degree of vacuolisation of the stained myoepithelial cell was graded on a scale of I to III. Grade I was characterised by little or no vacuolisation (figure 6), Grade II by an intermediate degree of vacuolisation (figure 7) and Grade III by marked vacuolisation (figure 8).

Assessment of Cell Proliferation

Immunocytochemistry

The state of cell proliferation of stromal and epithelial cells was assessed by immunocytochemical staining for proliferating cell nuclear antigen (PCNA) using monoclonal mouse anti-PCNA (Dako-PCNA, PC10).

The staining was performed by the avidin-biotin peroxidase complex method similar to that used for smooth muscle actin. There were, however, several modifications: heat was not used to promote adherence of the sections to the glass slides and, instead, the slides were left to air-dry overnight. A digestion step was not included. The primary antibody was diluted 1:150.

Case Selection

PCNA staining was performed on all patients who presented and underwent fibroadenoma excision within the same menstrual cycle. Patients were selected from the group that was in the second cycle following presentation in order to produce a reasonable spread throughout the cycle and as wide an age range as possible. Staining was also performed on all patients using an oral contraceptive (OC) or Depo-Provera.

As fixation times were unknown, and as staining can be abolished by prolonged fixation time, all cases showing weak or no staining were discarded. Cases that showed weak staining at the periphery of the lesion, but good staining more centrally, where fixative had presumably penetrated the least, were deemed suitable for cell counting purposes.

Overall, PCNA staining was performed on 37 fibroadenomas. Eight were on an OC and 9 were on Depo-Provera.

Counting Method

Cell counting was performed with the aid of a 10x10 square eyepiece grid, at high power magnification (10x eyepiece lens, 40x objective lens). All nuclei showing staining, (including weak staining) were counted as positive. Cell counts were recorded with a manually operated mechanical counter. All epithelial cells and all stromal cells were counted in each field until a total of at least 1000 of both cell types was reached. Stromal mononuclear inflammatory cells and endothelial cells were not included. Epithelial counts included myoepithelial cells. PCNA index was expressed as the percentage of positive cells for the total number of cells counted. Each case was selected randomly and counted with no knowledge of clinical or other data. Field selection was conducted moving progressively downwards and then across the slide. Areas at the periphery where staining was poor were avoided. Each fibroadenoma was counted once, although randomly selected fields were recounted (average of 3.4 fields per lesion) to assess reproducibility of the counting method (see appendix).

Statistical Analysis

Because of marked skewing, the PCNA data were log transformed. The relationship between variables was tested using Pearson's correlation coefficient with the Stat Graphics package (Statistical Graphics Corporation, 1988).

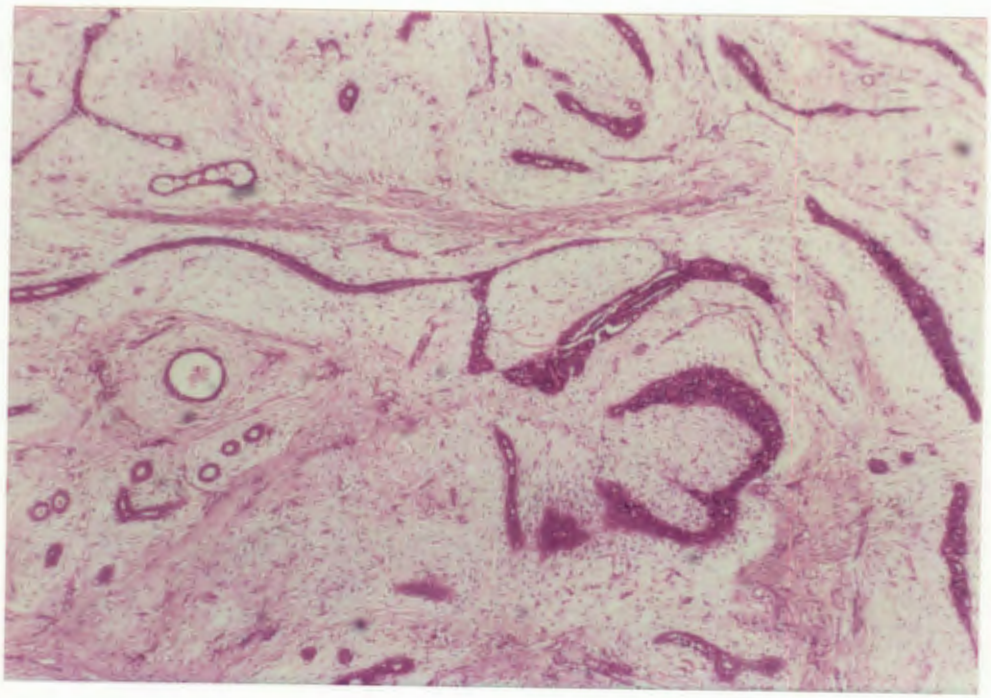


Figure 1. **Fibroadenoma with myxoid stroma (H+E x40).**

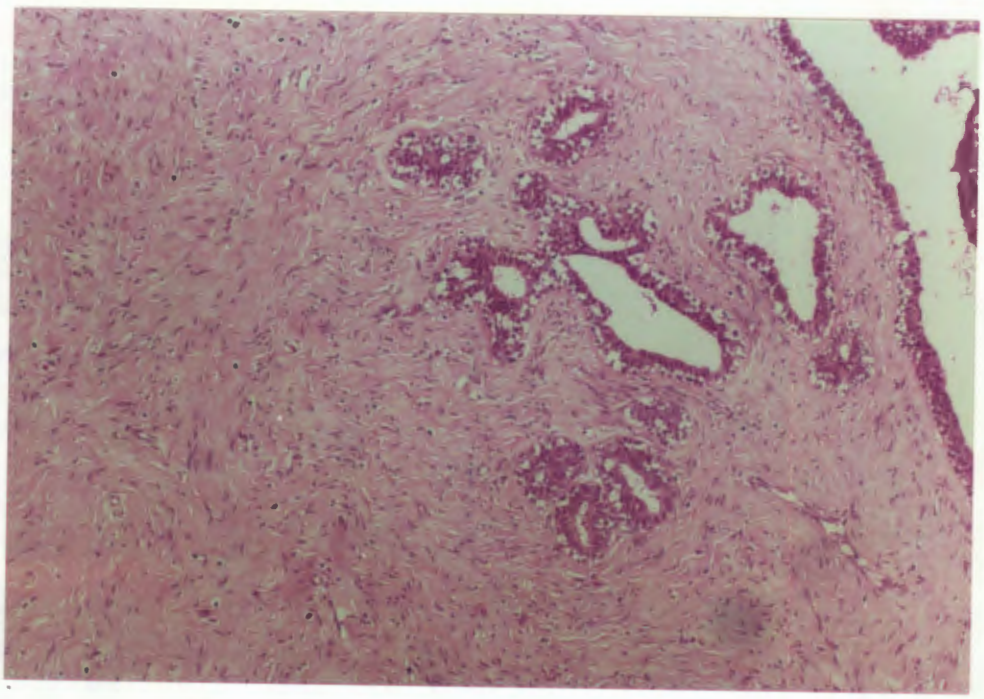


Figure 2. **Fibroadenoma with fibrous stroma (H+E x40).**

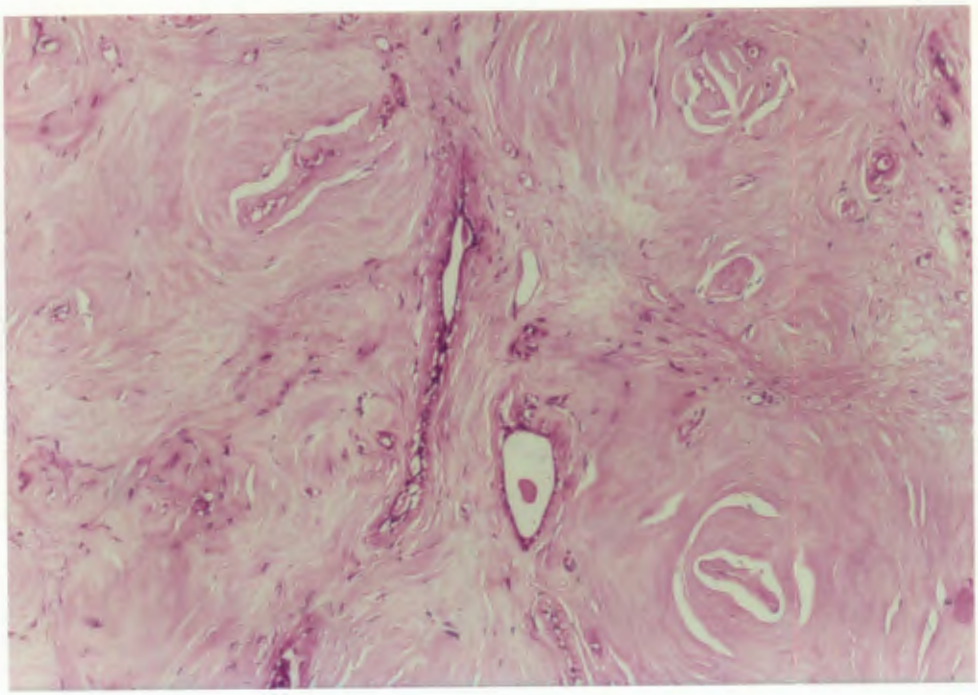


Figure 3. **Fibroadenoma with hyaline stroma showing minimal (Grade I) cellularity (H+E x40).**

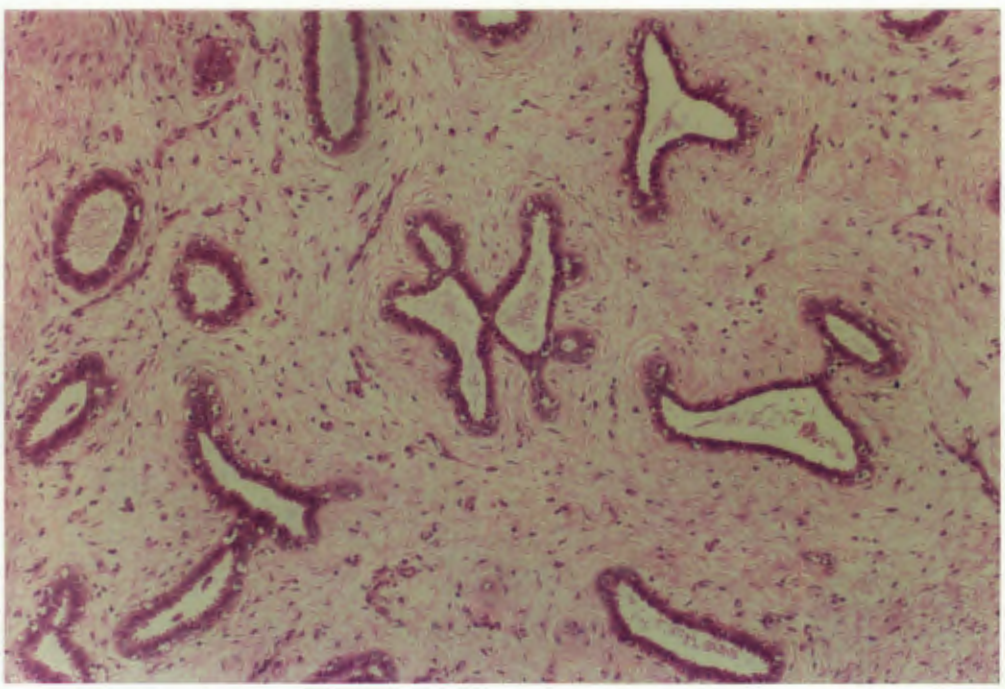


Figure 4. **Fibroadenoma showing intermediate (Grade II) stromal cellularity (H+E x100).**

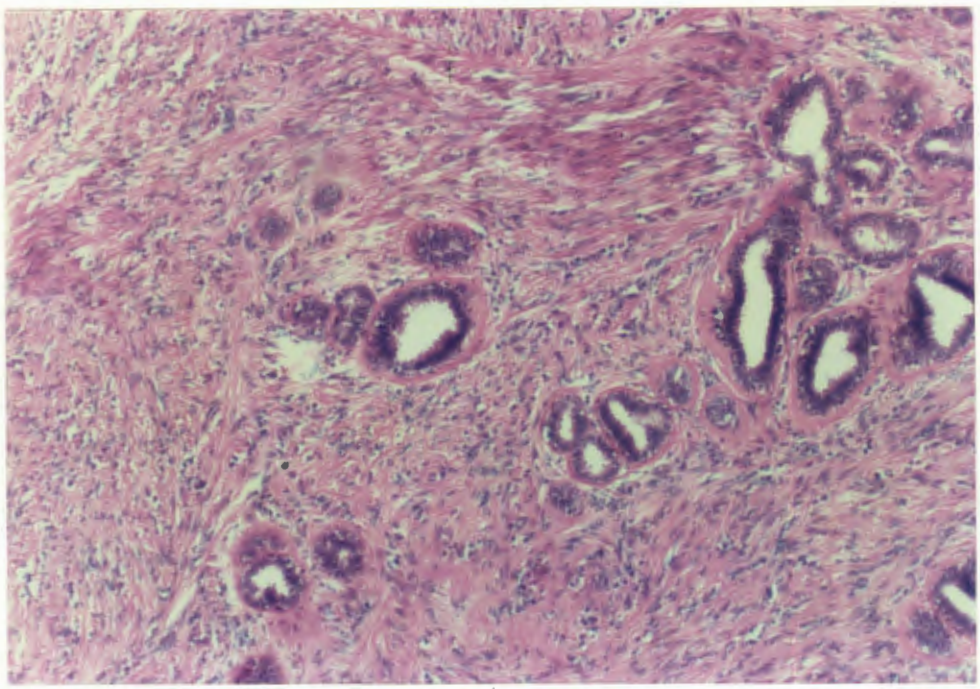


Figure 5. Fibroadenoma with considerable (Grade III) stromal cellularity (H+E x100).

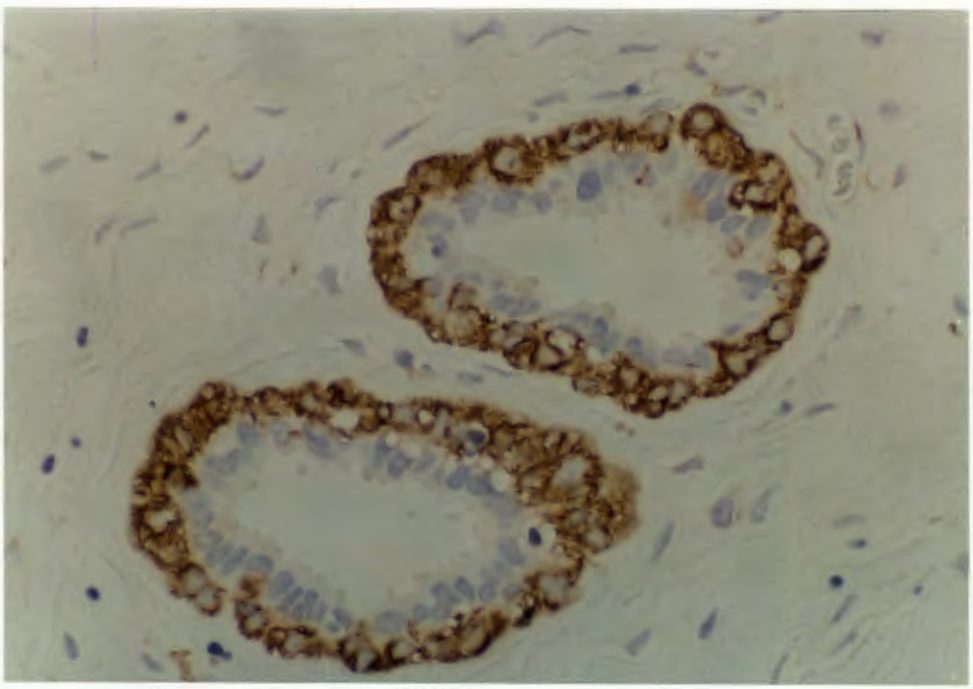


Figure 6. Myoepithelial cells stained using anti-human smooth muscle actin showing minimal (Grade I) vacuolisation (H+E x400).

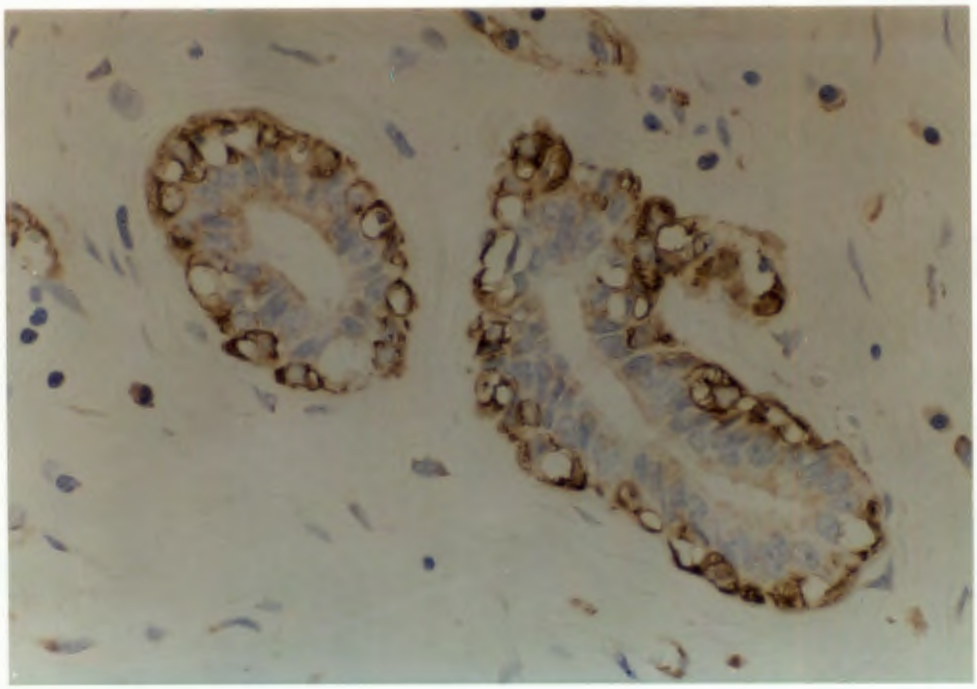


Figure 7. **Myoepithelial cells stained using anti-human smooth muscle actin showing intermediate (Grade II) vacuolisation (H+E x400).**

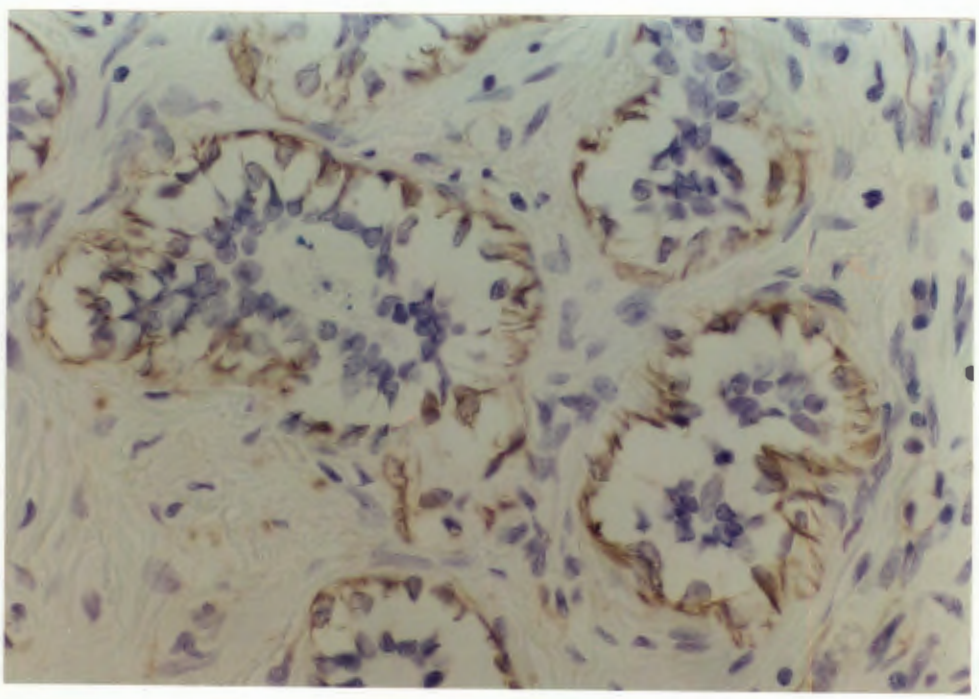


Figure 8. **Myoepithelial cells stained using anti-human smooth muscle actin showing marked (Grade III) vacuolisation (H+E x400).**

RESULTS

Age Distribution

The age distribution of fibroadenomas in this series is shown in figure 12. There was an age range of 15-60 years with a mean age incidence of 24.1 years and a peak incidence in the 20-24 year age group.

Fibroadenoma Size and Patient Age

The relationship of size and age is shown in figure 13. Fibroadenoma size ranged from 0.2 to 7.0cm with a mean size of 2.2cm. Most fibroadenomas greater than 3.5cm in diameter occurred at 20 years of age or younger. There was a tendency for fibroadenomas to decrease in size with increasing patient age ($r = -0.177$; $p = 0.0392$).

Stromal Character and Patient Age

As shown in figure 14, all fibroadenomas in the 15-19 year age group were either myxoid or fibrous with slightly more fibrous than myxoid tumours. In the 20-24 year age group, the great majority of fibroadenomas were fibrous; included was a small number of hyaline lesions. Cases over 25 years of age were relatively few with mixed proportions of myxoid, fibrous and hyaline lesions. Myxoid lesions were noted to occur up to the 45-49 year age group. A single fibroadenoma in the 60-64 year age group was hyaline.

Stromal Cellularity and Patient Age

Figure 15 shows that nearly all fibroadenomas in the 15-19 and 20-24 year age groups had Grade II or Grade III cellularity of the stroma with the latter predominating. In the 20-24 year age group, a small number of Grade I tumours was also present. In the 25-29 year age group there were more Grade II than Grade III lesions. From 30-39 years Grade II and III lesions occurred in roughly equal

proportions, along with the occasional Grade I tumour. Notably, there was a single Grade III lesion in the 45-49 year age group.

Epithelial Component and Age

The epithelial component, expressed as a percentage of total surface area, as illustrated in figure 16, showed a tendency to decline with age ($r=-0.1613$, $p=0.0596$). All values of 35% and greater occurred at 25 years of age or younger.

Stromal Inflammatory Cell Infiltrate

Stromal lymphocytes, plasma cells and mast cells were observed in most fibroadenomas in variable proportions. In the vast majority of cases, the density of infiltrate of each cell type was scored as Grade I. There was no observable correlation with any other variable examined. In some fibroadenomas stromal eosinophils were noted; in one case, the infiltrate was fairly prominent. Stromal neutrophils were also present in some cases.

Myoepithelial Cell Vacuolisation

As shown in figure 17, there was no observable change in myoepithelial cell vacuolisation in phase with the menstrual cycle. Grade I, Grade II and Grade III vacuolisation occurred in roughly equal proportions in all quarters of the cycle. Grade I vacuolisation predominated.

Smooth muscle actin 1A4 antibody gave stronger and better defined staining compared to S100 and was the better method for demonstrating myoepithelial cells.

PCNA Immunoreactivity

The intensity of PCNA immunoreactivity ranged from weak granular to strong diffuse nuclear staining; this variability was observed in most high power counting fields (figure 9). Some fibroadenomas showed strong diffuse nuclear staining within most nuclei (figures 10 and 11).

Generally, high PCNA indices were recorded for both epithelium (mean=64.9%) and stroma (mean=67.4%). There was also a marked degree of variability. PCNA indices ranged from 24.5% to 95.5% for epithelium and 18.6% to 96.1% for stroma.

PCNA indices for epithelium did not correlate with phase of the menstrual cycle (figure 18), ($r=-0.0721$; $p=0.7153$); neither did stroma (figure 19), ($r=0.2869$; $p=0.1389$). Of 28 recorded indices, 23 epithelial and 22 stromal indices were above 50% with no observable variation in phase with the cycle.

No correlation of stromal and epithelial indices in phase with the cycle was observed when comparing patients using the OC ($n=8$) and patients not using any exogenous hormone ($n=20$) (figures 18 and 19). Patients using Depo-Provera ($n=9$) were assumed not to be in cycle. PCNA indices for patients using Depo-Provera ranged from 36.3% to 91.5% for epithelium and 55.7% to 82.2% for stroma with means of 68.0% and 72.2% respectively. These values are not observably different when compared to those women using an OC or those cycling naturally. One patient on Depo-Provera had two fibroadenomas; the indices of the two showed some similarity and were 74.9% and 74.2% for epithelium and 70.7% and 82.2% for stroma respectively.

Age did not correlate with PCNA index for epithelium (figure 20), ($r=-0.2291$; $p=0.1665$) or stroma (figure 21), ($r=0.0088$; $p=0.9580$). The lowest indices for epithelium and stroma were from a patient aged 43 years; however, indices in other patients older than 35, although few in number, were mostly as high as those of younger patients.

The PCNA epithelial/stromal ratio did not correlate with phase of menstrual cycle (figure 22), ($r=0.01$) or with age (figure 23), ($r=-0.11$). The epithelial stromal ratio in the majority of cases ranged between 0.6 and 1.4, indicating that PCNA immunoreactivity for epithelium and stroma did not vary greatly. Stroma

immediately adjacent to epithelium showed increased staining when compared to more distant stroma.

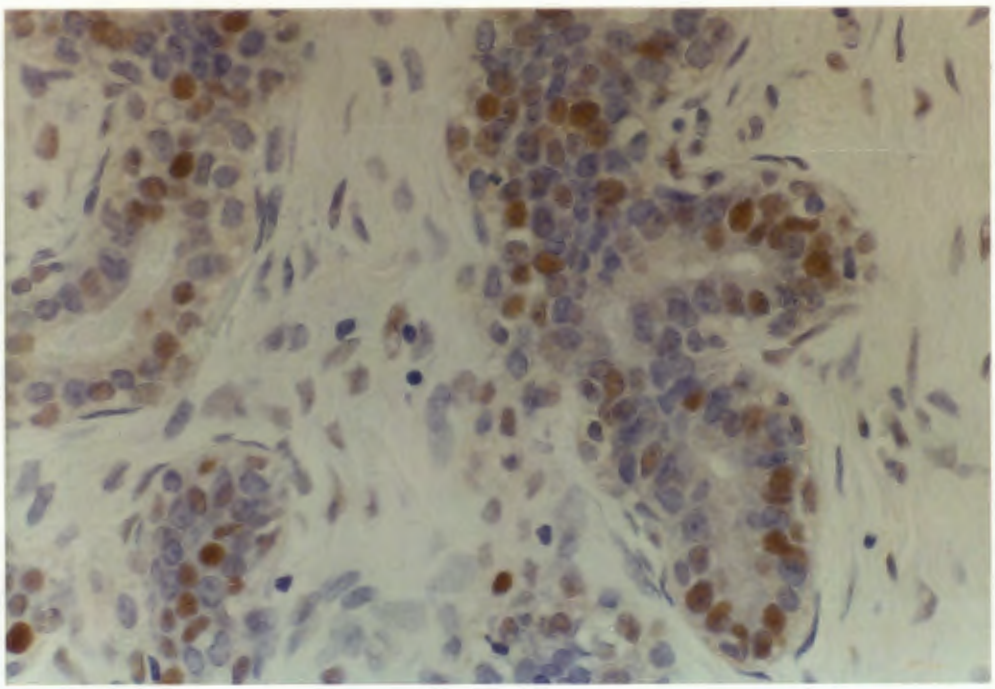


Figure 9. Fibroadenoma showing epithelial and stromal PCNA immunoreactivity. Nuclei show a variable intensity of staining (H+E x400).

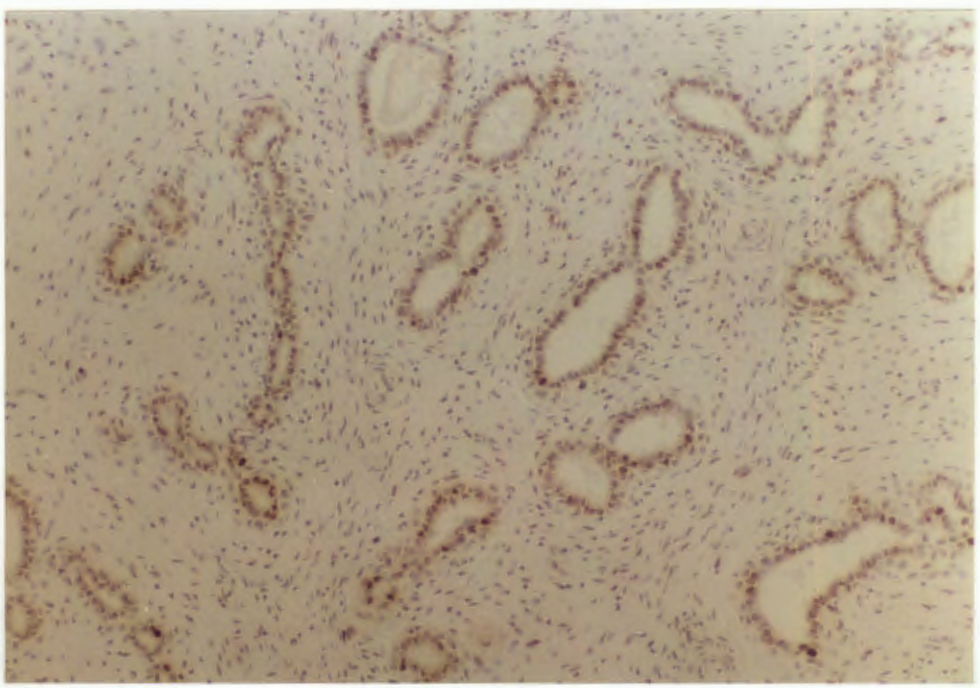


Figure 10. Fibroadenoma showing a high degree of epithelial and stromal PCNA immunoreactivity (H+E x100).

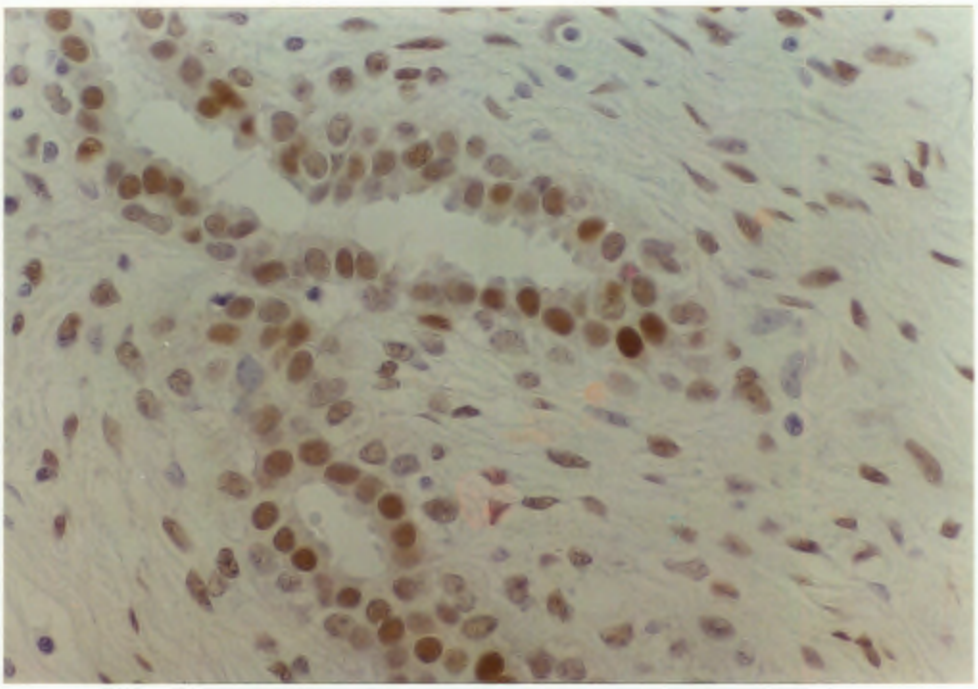


Figure 11. **High power view of fibroadenoma in Figure 10 showing strong intensity of nuclear staining (H+E x400).**

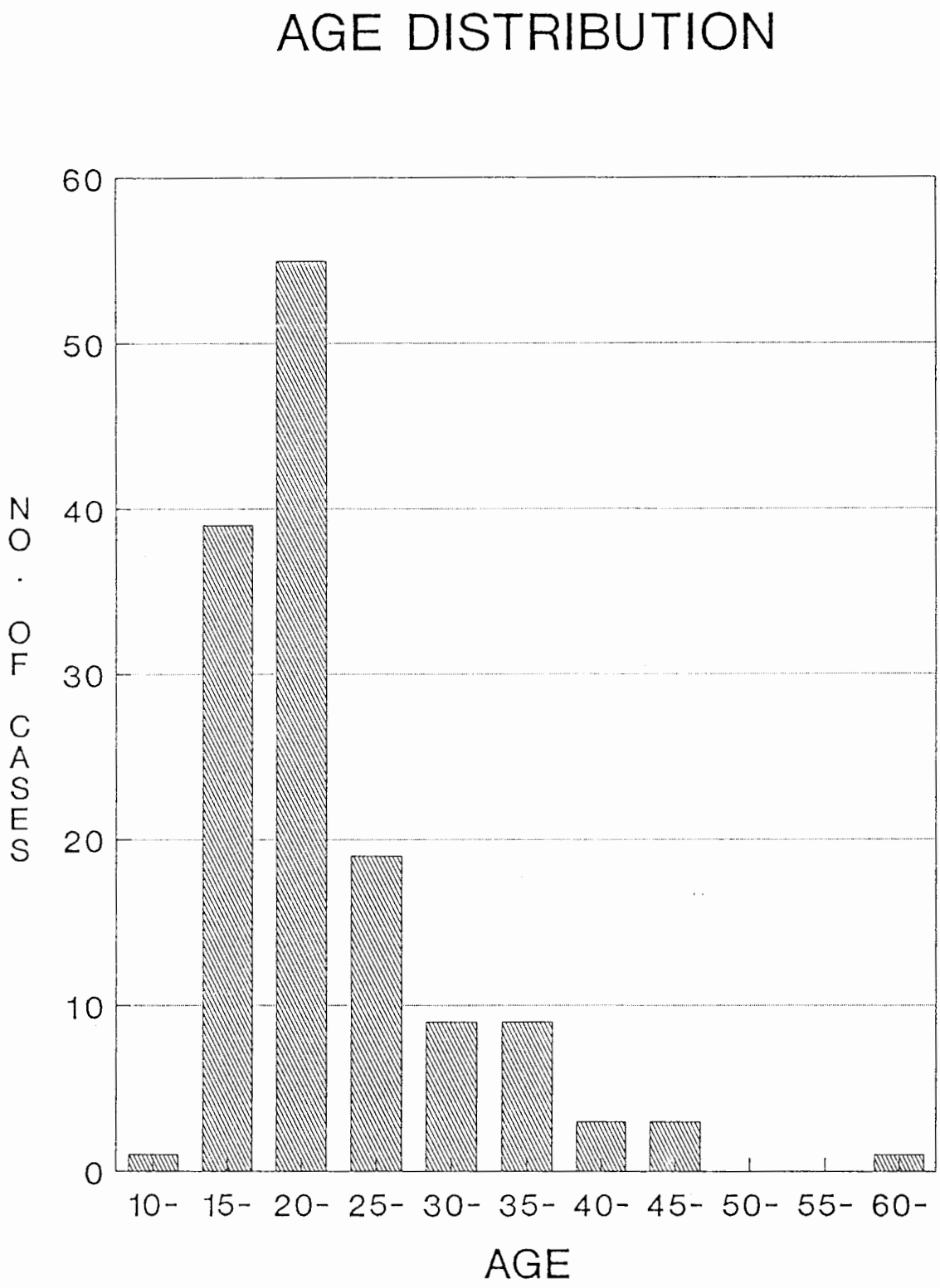


Figure 12. Age distribution of fibroadenomas showing ages at 5 year intervals.

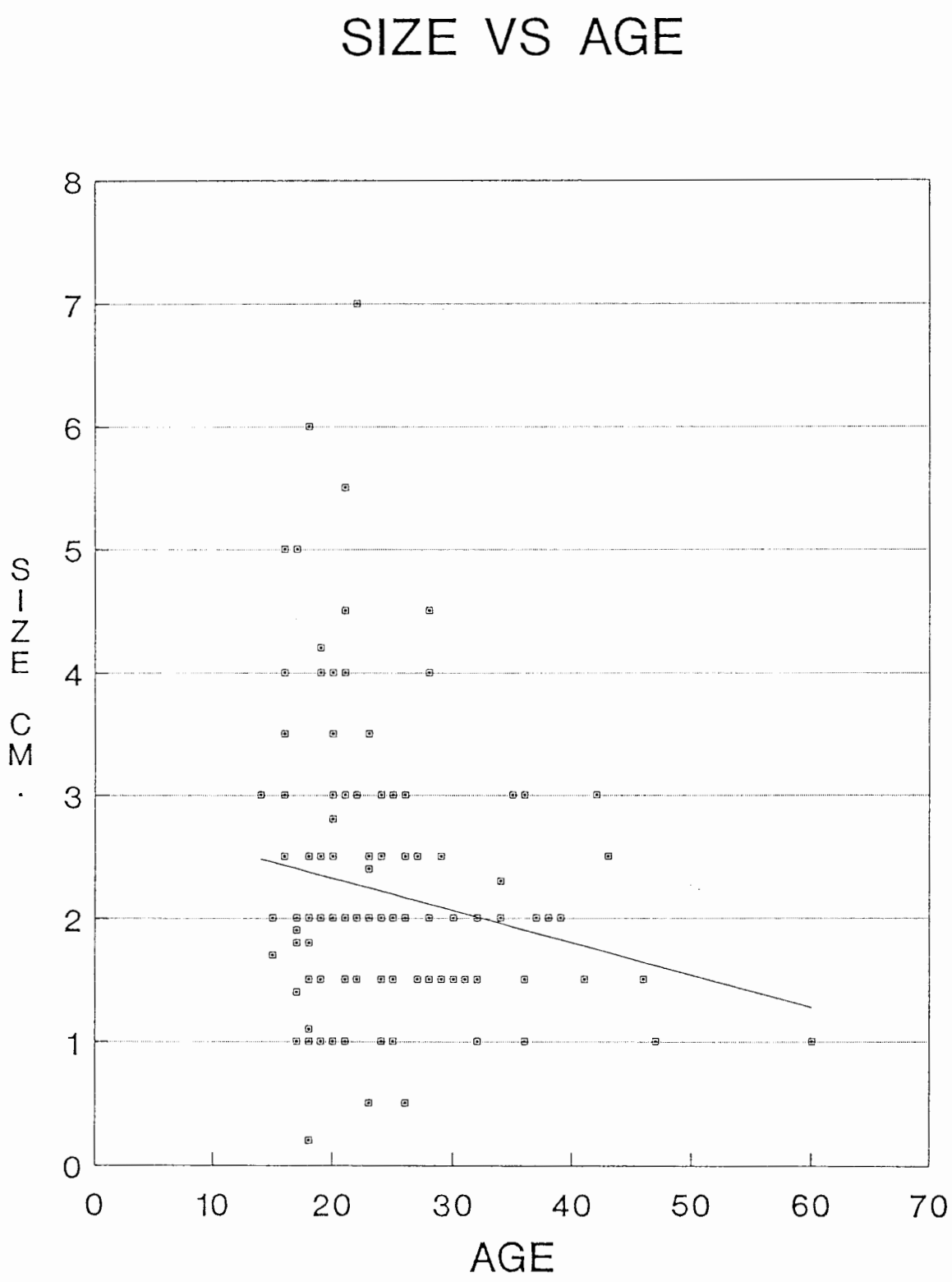


Figure 13. Distribution of fibroadenoma size in centimetres with patient age.

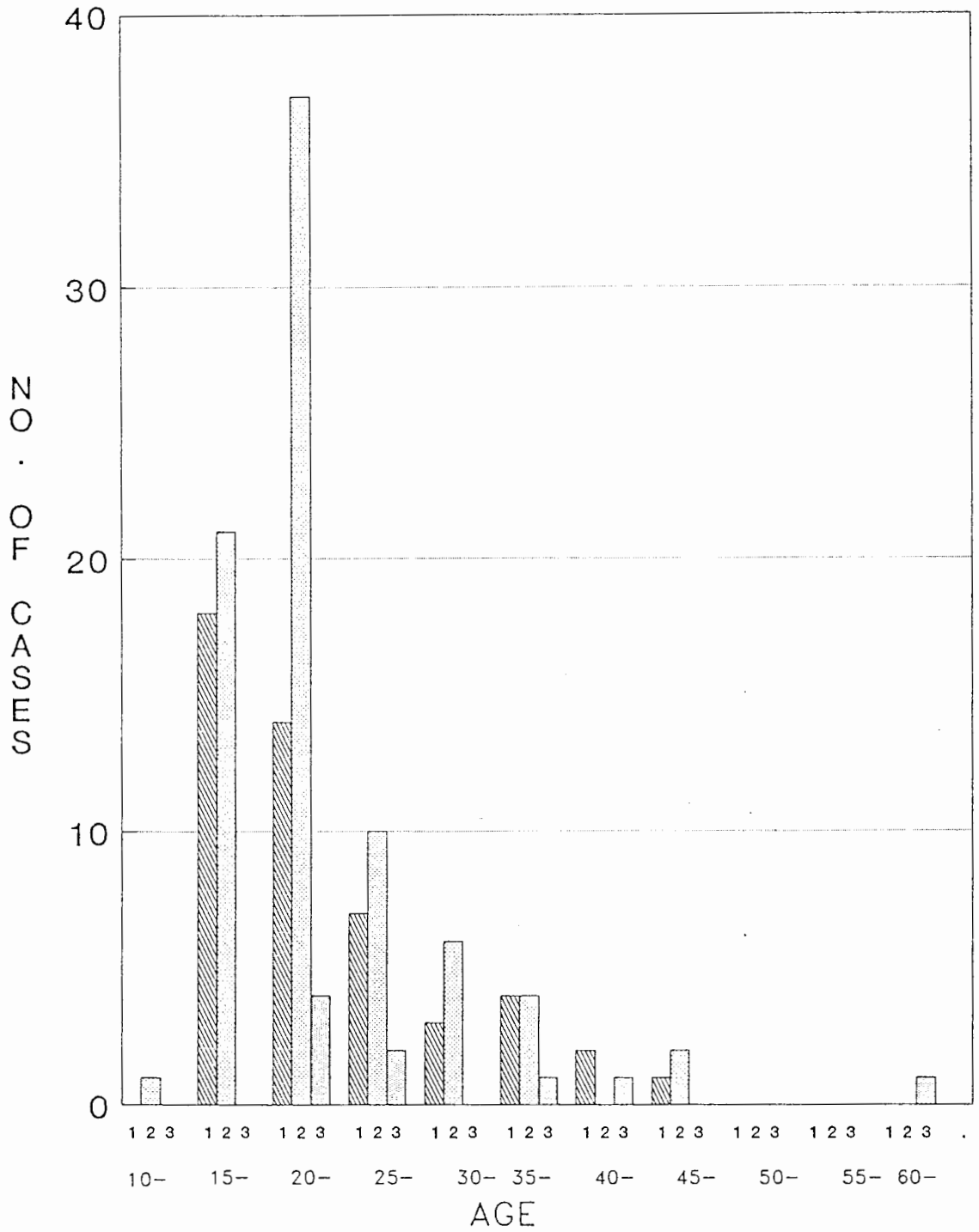


Figure 14. Distribution of stromal character with age. In each 5 year interval, 1 = myxoid, 2 = fibrous, 3 = hyaline.

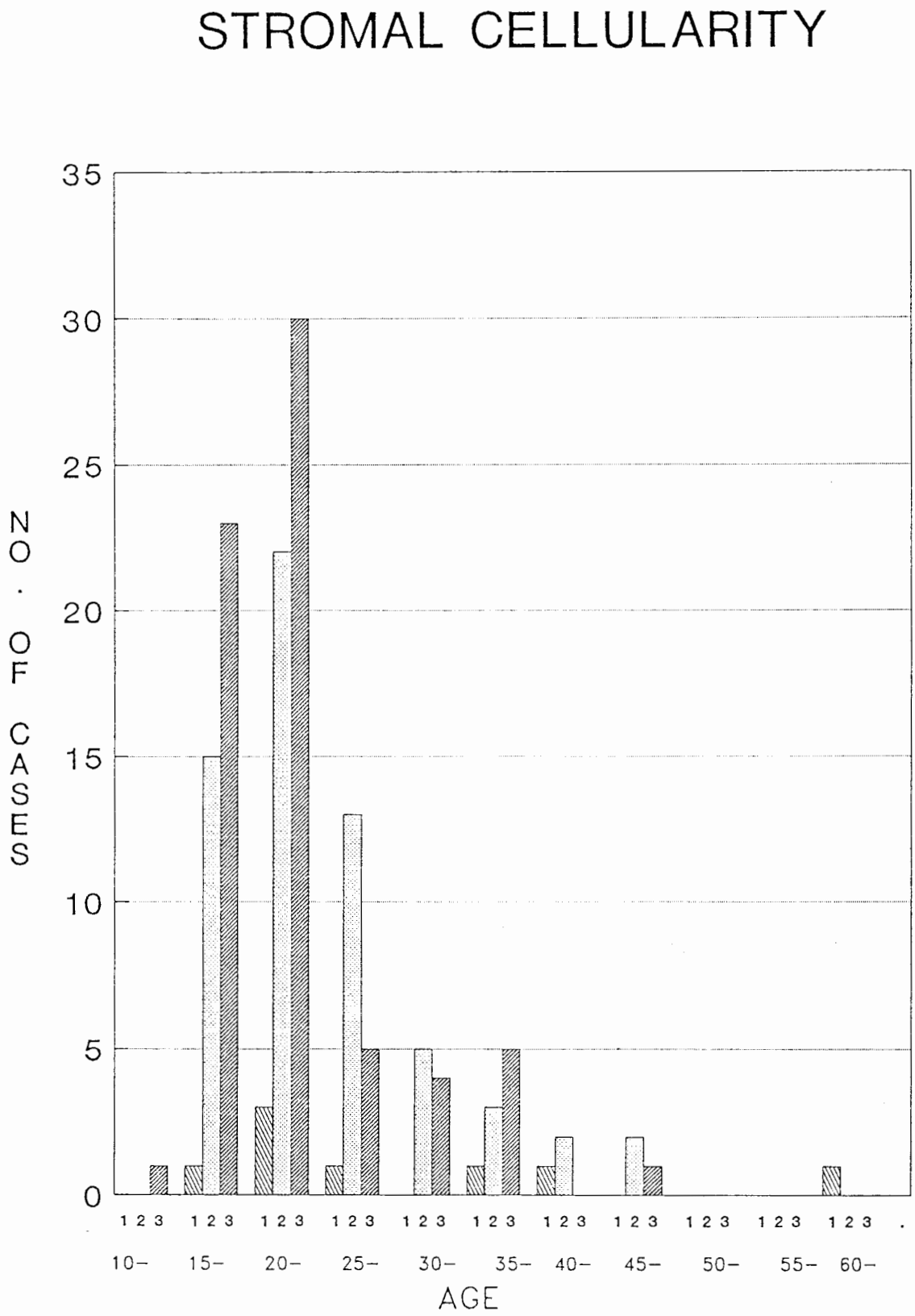


Figure 15. Distribution of stromal cellularity with age. In each 5 year interval, 1 = Grade I cellularity, 2 = Grade II cellularity, 3 = Grade III cellularity.

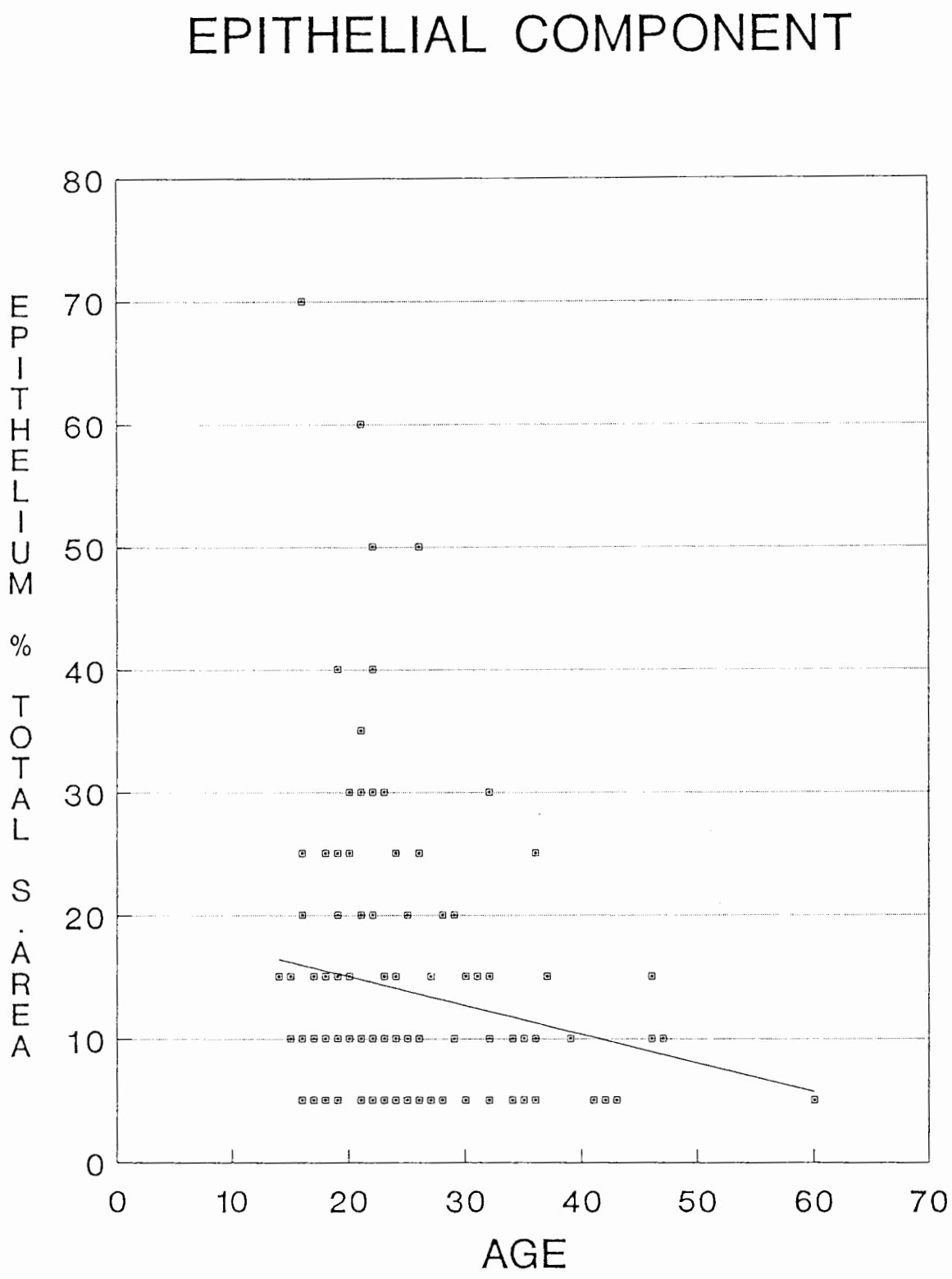


Figure 16. Distribution of epithelial component, expressed as a percentage of total surface area, with patient age.

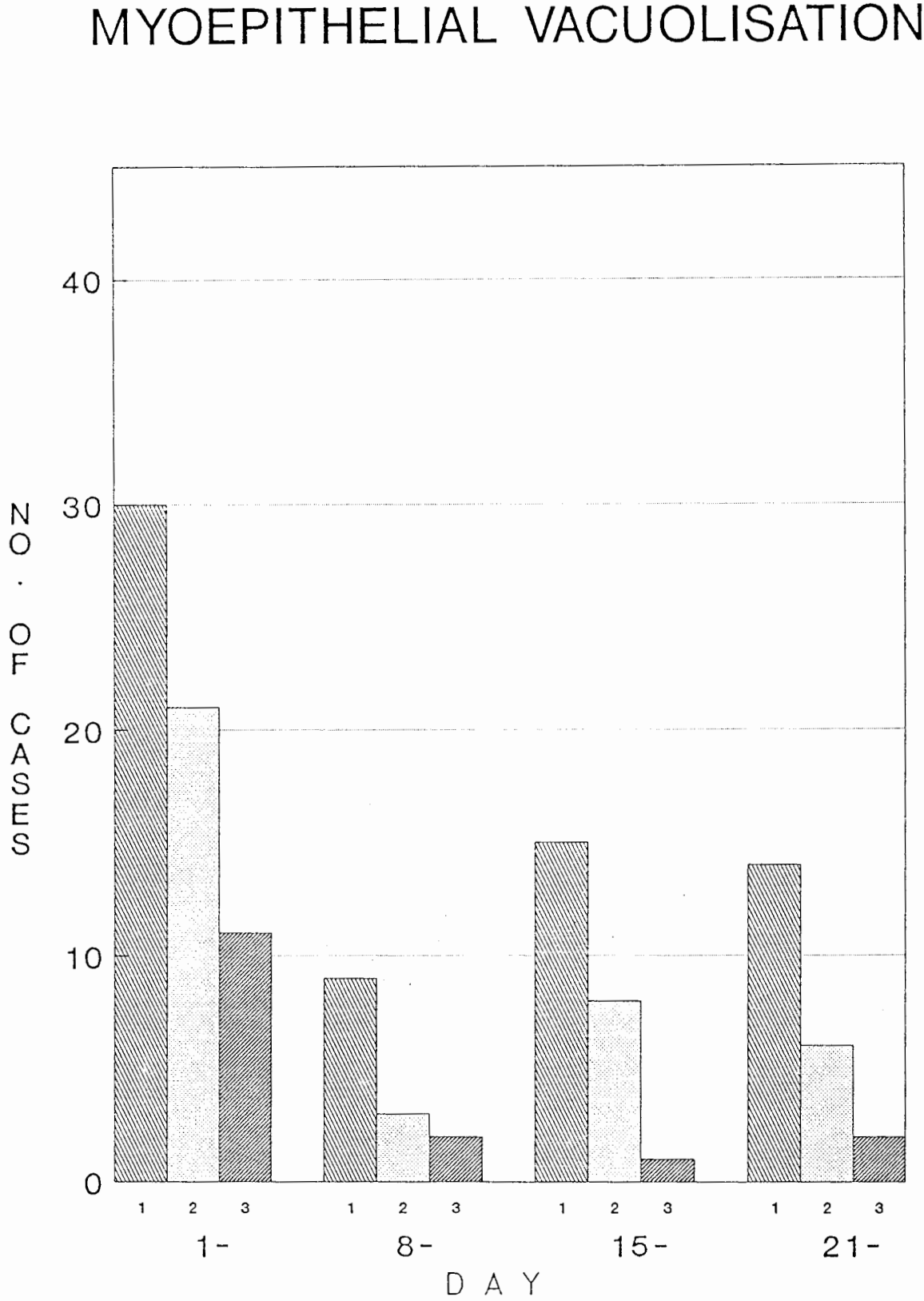


Figure 17. Distribution of myoepithelial vacuolisation with phase of menstrual cycle in quarters. Within each quarter of a cycle, 1=Grade I vacuolisation, 2=Grade II vacuolisation, 3=Grade III vacuolisation.

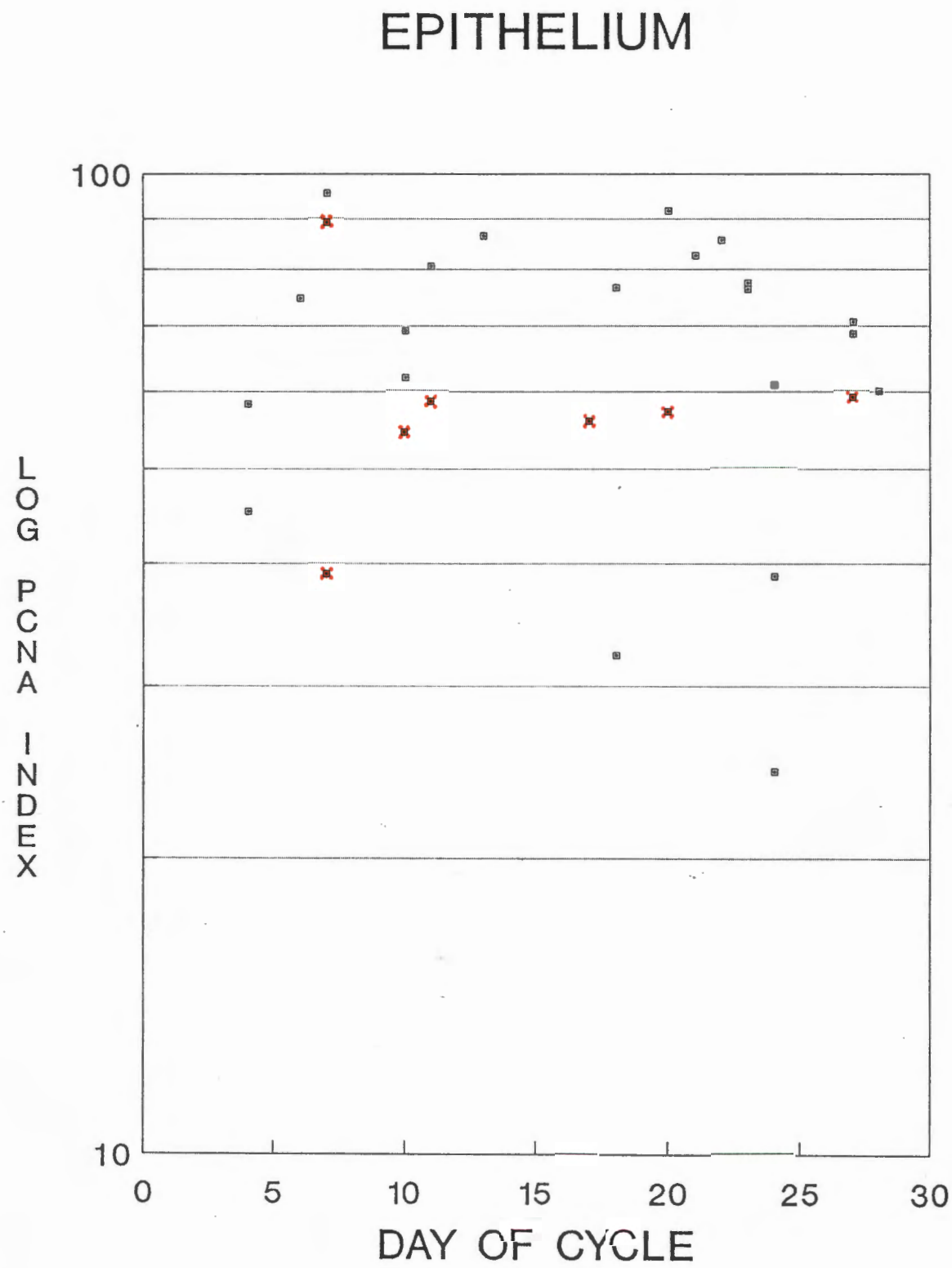


Figure 18. **Distribution of log transformed PCNA indices of epithelium with day of the menstrual cycle. Points in red represent patients on an OC.**

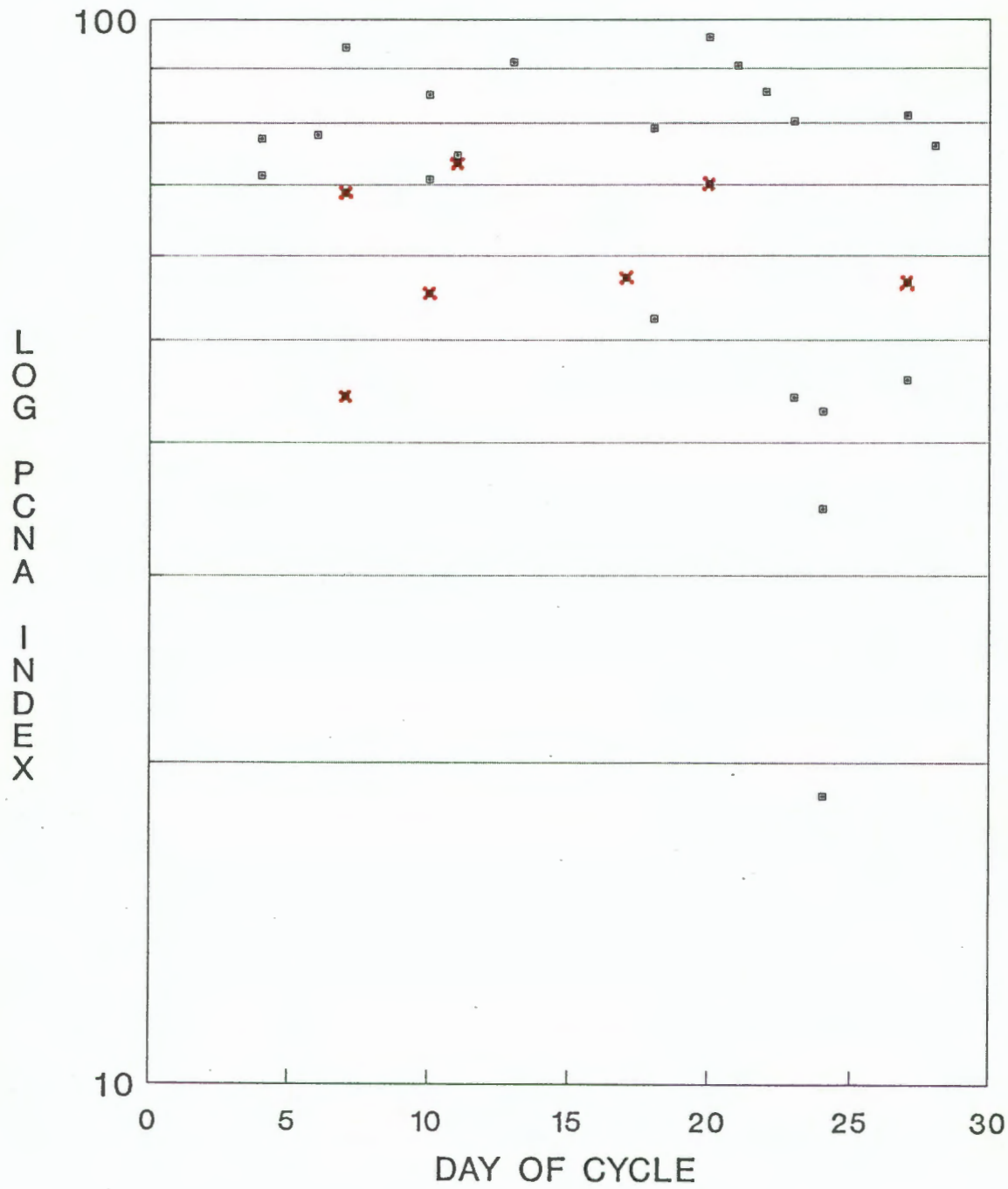


Figure 19. Distribution of log transformed PCNA indices of stroma with day of the menstrual cycle. Points marked in red represent patients on an OC.

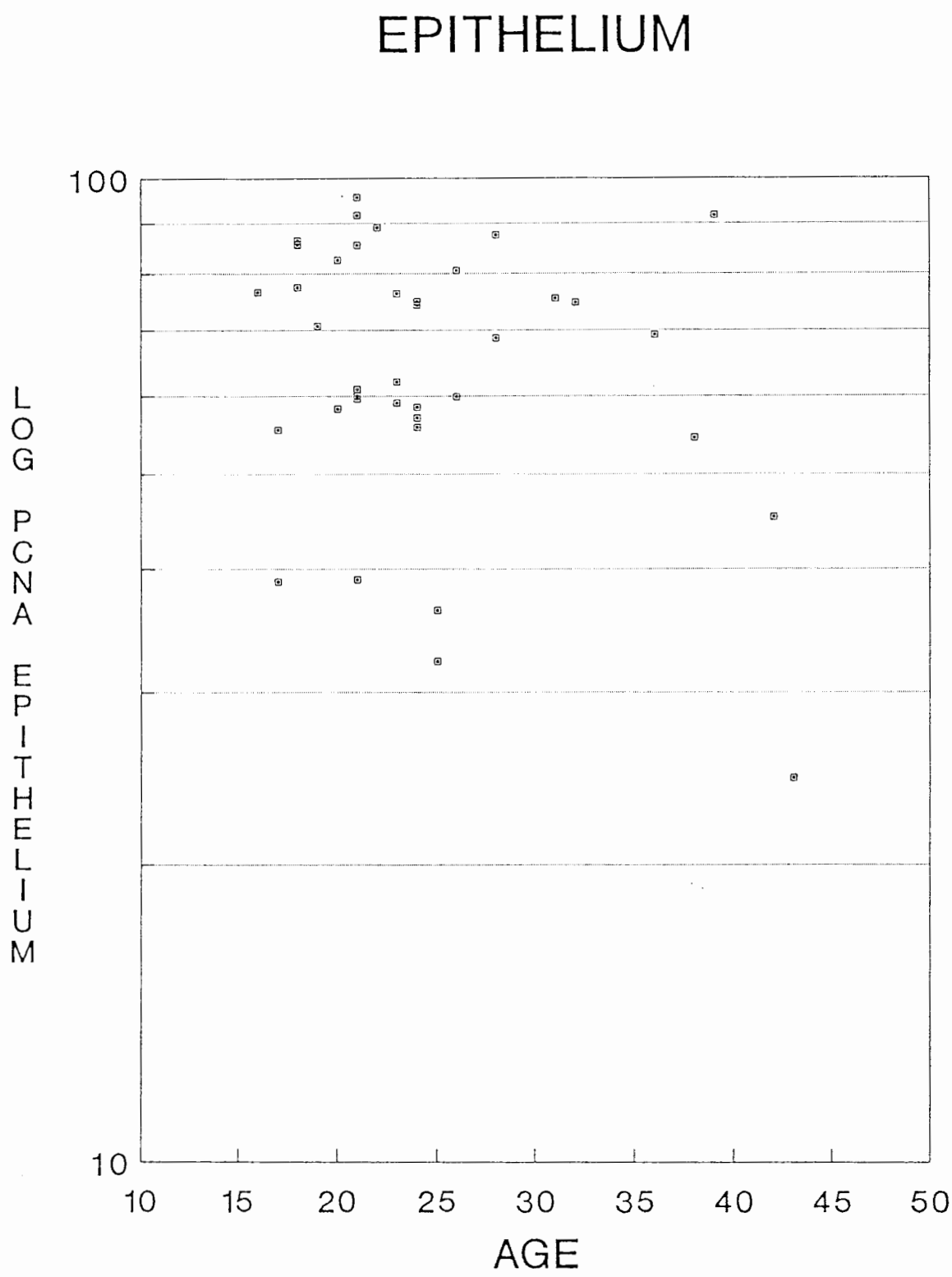


Figure 20. Distribution of log transformed PCNA indices of epithelium with patient age.

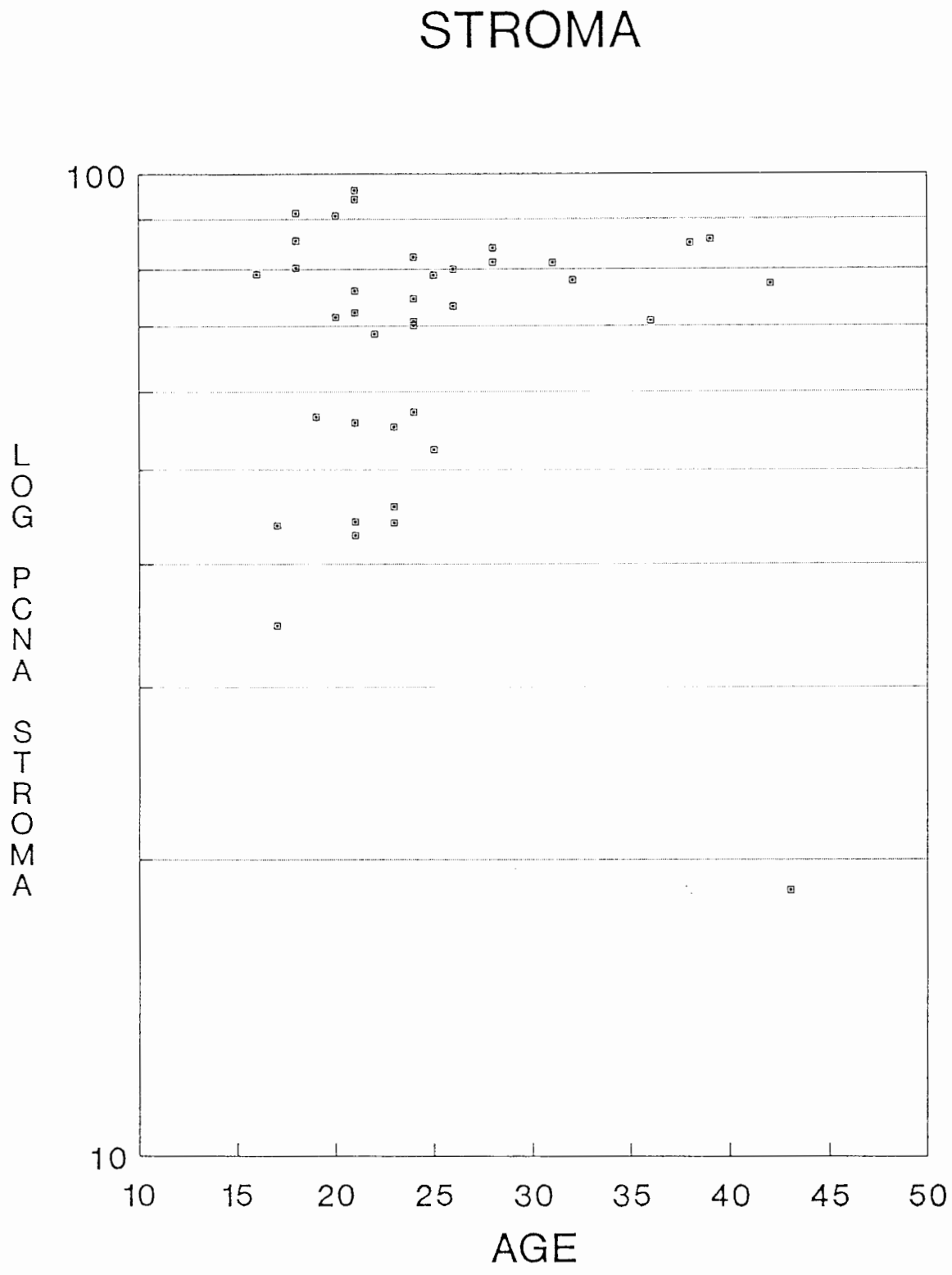


Figure 21. Distribution of log transformed PCNA indices of stroma with patient age.

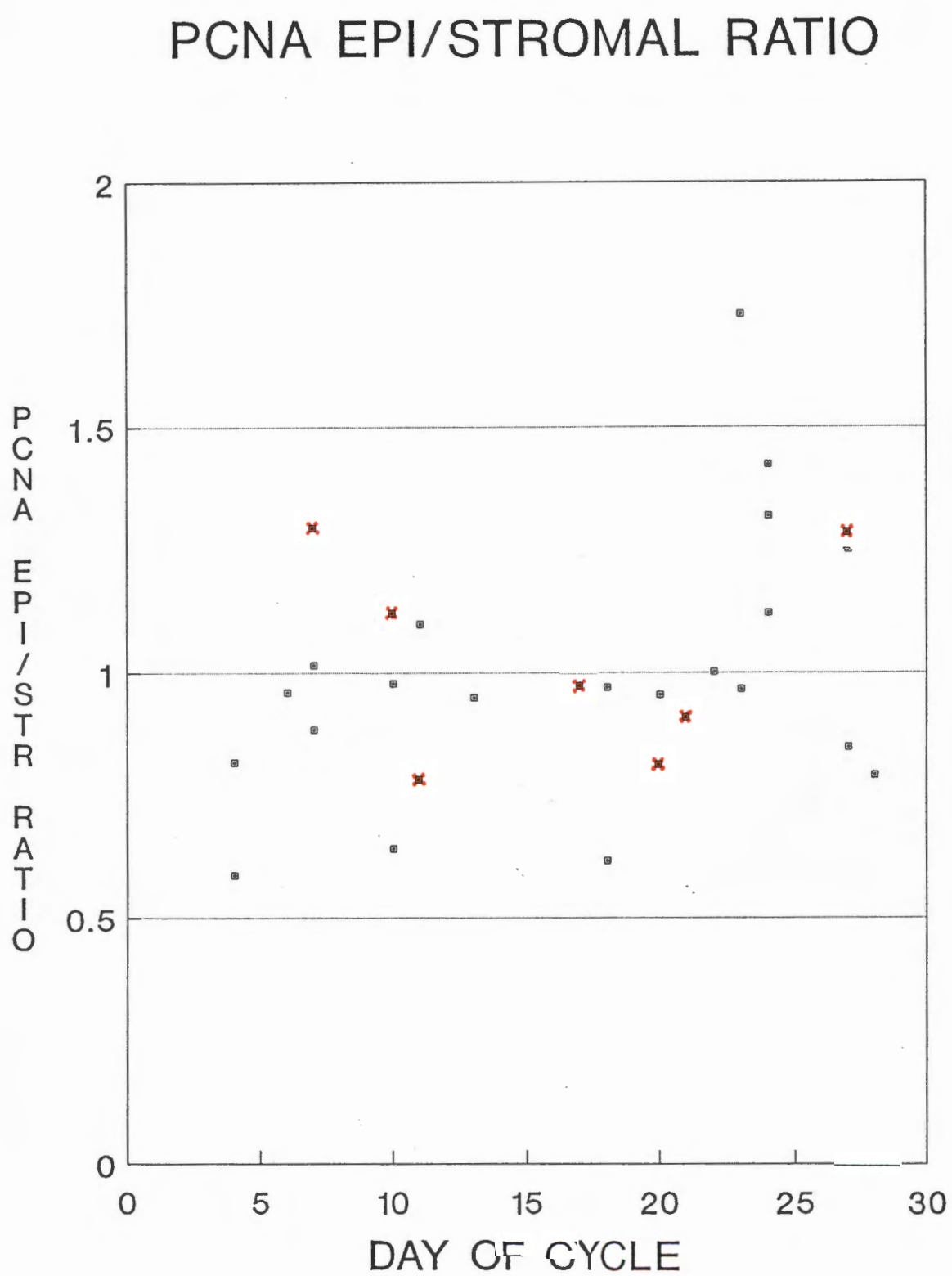


Figure 22. **Distribution of the ratio of epithelial and stromal PCNA indices with day of the menstrual cycle. Points in red represent patients on an OC.**

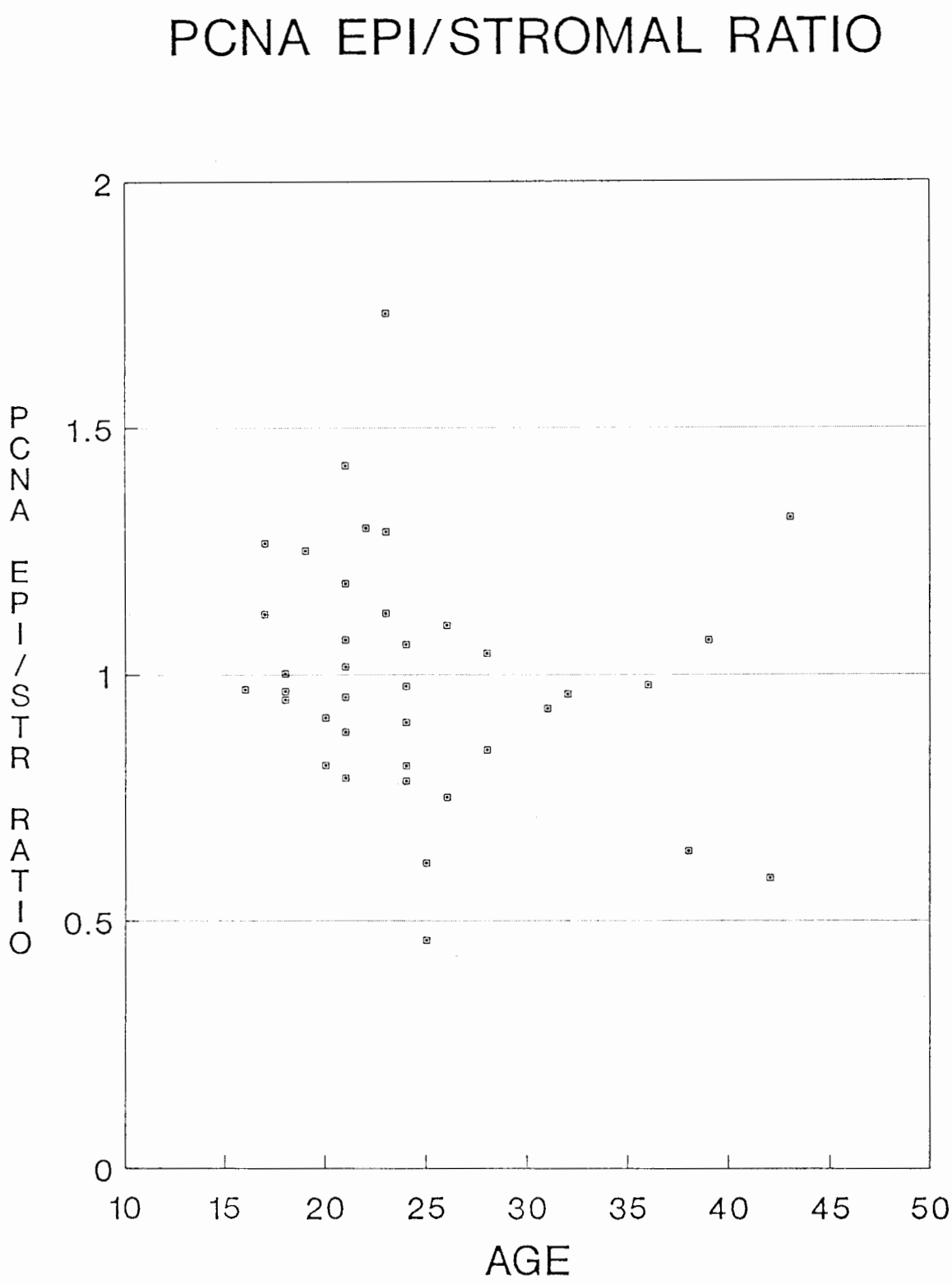


Figure 23. Distribution of the ratio of epithelial and stromal PCNA indices with patient age.

DISCUSSION

In this series there was a peak incidence of fibroadenomas in the third decade, which is in keeping with the findings of others (Foster *et al*, 1988). While it is probable that a proportion of fibroadenomas are never excised, these age incidences show that they are rare in post menopausal women. It has also been reported that fibroadenomas are seldom encountered in carefully examined mastectomy specimens from post menopausal women (Kern and Clark, 1973). It is possible, therefore, that fibroadenomas begin in young women as "active" cellular tumours and become hyalinised fibrous lesions in later decades. Some may even regress. However, the overall assessment of stromal collagen content and stromal cellularity in this study did not support the hypothesis that fibroadenomas have such a natural history in keeping with breast age. The finding of hyalinised lesions in younger age groups suggests that fibroadenomas may have a relatively short natural history and not all myxoid or cellular lesions are confined to younger individuals, which may indicate that fibroadenomas have the potential to occur *ab initio* in older women.

On the other hand, data of this study indicate that fibroadenomas have a tendency to be smaller with increasing age and that the epithelial content assessed as a percentage of total surface area showed a tendency to decline with age. These factors suggest that fibroadenomas are more atrophic with increasing age. This may be related to age-related vascular changes. The cell proliferation data, however, do not support this and show that PCNA indices of fibroadenomas of older women can be just as high as those of younger women, although the numbers were relatively few and the sample may not be entirely representative.

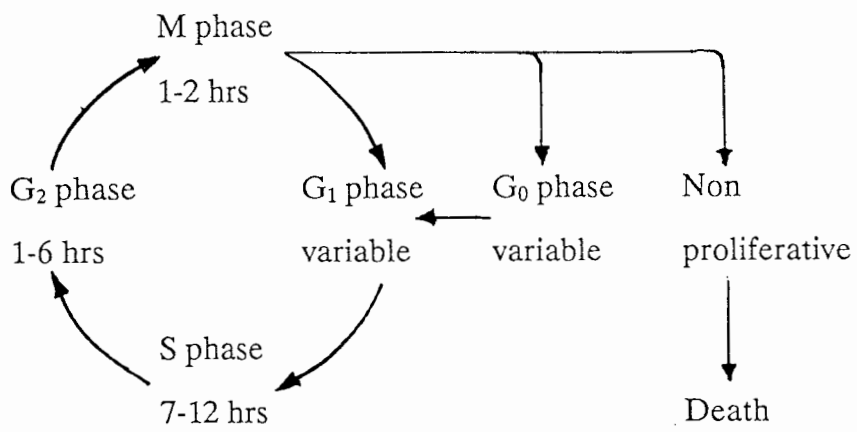
In this study, myoepithelial cell vacuolisation and density of stromal cell infiltrate were criteria used for assessing morphologic changes in phase with the menstrual cycle. Unlike studies on normal breast, neither criterion showed any cyclic variation. The morphologic data, therefore, indicate that fibroadenomas differ in biologic behaviour from normal breast.

Cell Proliferation

There have been several recent publications reviewing methodology for assessment of cell proliferation (Hall and Levison, 1990; Quinn and Wright, 1990; Linden *et al*, 1992).

Fundamental to the understanding of cell proliferation is the concept of the cell cycle.

The Cell Cycle



During the synthetic or S phase, DNA is synthesized and the genome doubled. The S phase is separated from the previous mitosis (M phase) by a variable period known as the first gap or G_1 phase. Following DNA synthesis there is a second gap or G_2 phase which precedes the next mitosis. Interphase is made up of successive G_1, S and G_2 phases and forms the largest part of the cell cycle. Although still controversial, a G_0 phase has been proposed in which there is a non cycling population of cells that can, after suitable stimuli, rejoin the cycling population. In any tissue there will always be cycling and non cycling cell compartments. The proliferation fraction of any cell population can be defined as the ratio of cycling cells to cycling plus non cycling cells.

Mitosis Counting

Counting mitotic figures is the traditional, oldest and most easily performed method to assess cell proliferation. The M phase is the only part of the cell cycle that is recognized by this simple morphological examination. Because of its shortness, the M phase accounts for the smallest portion of the proliferating cell population. Major criticisms are lack of standardization and reproducibility. Variables to be considered include high power field area and counting of random high power fields or the most mitotically active areas. Delay in tissue fixation allowing mitoses to go to completion, and section thickness can lead to variation in measurement, too. Observer experience is important and pyknotic nuclei and suspicious cells should not be counted. Mitotic rates can be expressed as the number of mitoses per number of high power fields, mitoses per square millimeter or mitoses per number of cells counted (mitotic index).

Thymidine Labelling

The incorporation of a labelled DNA precursor, tritiated thymidine, during DNA synthesis in the S phase permits direct morphologic assessment of proliferating cells. The method, however, requires viable cells in culture as only actively proliferating cells will take up the radiolabelled thymidine. Freshly excised tissue is incubated with tritiated thymidine for 1-2 hours and is then fixed and routinely processed. Autoradiographs are prepared and then developed after one week of exposure. The thymidine labelling index (TLI) is determined as the number of positive cells per total number of cells counted.

Main disadvantages are the need to incubate fresh tissue and delay in finished product making this procedure unsuitable for routine practical use in histopathology.

Bromodeoxyuridine Incorporation

5-Bromodeoxyuridine (BrdU) is a thymidine analogue and shares many of the technical requirements and disadvantages of thymidine labelling. The BrdU assay, however, does not require radiolabelling or autoradiography. Monoclonal antibodies to BrdU have been developed and cells in the S phase can be identified by immunocytochemistry, immunofluorescence or flow cytometry and this reduces delay. Studies comparing thymidine labelling and BrdU incorporation have shown identical results (Meyer *et al*, 1989).

Immunocytochemical Methods

Antibodies have become an important means of assessing cell proliferation. In addition to their use in the bromodeoxyuridine assay, they can detect a variety of cell proliferation-related proteins. These antigens are not necessarily related to the same component of the cell cycle. A major advantage is that, because tissue sections are being used, the spacial orientation of the proliferating cell fractions can be assessed. This can be applied to different phenotypic cell populations, either by simple morphologic means or by double-staining immunocytochemical methods. These methods are static in that they measure the "state" rather than the "rate" of cell proliferation.

Ki-67

This is perhaps the best known antibody recognising proliferating cells. It is a mouse monoclonal antibody raised against a crude nuclear fraction of a Hodgkin's disease derived cell line by Gerdes *et al*, (1983) in Kiel. It detects an unidentified antigenic epitope of a proliferation-associated nuclear protein. Expression is from Mid-G_1 phase and throughout S, G_2, and M phases. A major disadvantage for routine use has been the requirement of fresh tissue for cryostat preparations. However, a monoclonal antibody, MIB1 has recently been developed that is suitable for use in formalin-fixed paraffin-embedded tissue (Gerdes *et al*, 1992).

Proliferating Cell Nuclear Antigen (PCNA)

PCNA (also known as cyclin) is a stable 36kD nuclear protein that has been identified independently by several groups and given different names. Miyachi *et al*, (1978) identified autoantibodies reactive with nuclear antigens in proliferating cells in the sera of patients with systemic lupus erythematosus. Celis *et al*, (1984) characterised a nuclear protein, cyclin, by two dimensional gel electrophoresis in proliferating and quiescent cells. Mathews *et al*, (1984) found that PCNA and cyclin are identical nuclear proteins.

PCNA functions as a cofactor for DNA polymerase delta. Lee and Hurwitz, (1990) have demonstrated that in the presence of single-stranded-DNA-binding protein, the elongation of primed DNA templates by DNA polymerase delta is dependent upon ATP and two protein factors, activator 1 (A1) and proliferating cell nuclear antigen. A1, PCNA and DNA polymerase delta form a stable complex in the presence of ATP that can be isolated by gel filtration. The binding of PCNA to the A1 DNA polymerase complex is ATP dependent.

It is stated that PCNA levels increase rapidly in mid-G1, remain elevated throughout S phase, and begin to decrease from G2/M to G1 (Linden *et al*, 1992). Morris and Mathews (1989), however, assayed PCNA in synchronised HeLa cells by two dimensional gel electrophoresis. They demonstrated an increase in the rate of PCNA synthesis with a peak in early S phase, but the magnitude of the increase was only 2-3 fold. Throughout the cell cycle, the ratio of PCNA to total cell protein remained constant. They also observed that up to one third of total PCNA is tightly associated with the nucleus, presumably in replication complexes at the peak of the cell cycle. They concluded that cyclic synthesis of PCNA is in excess of the amount involved directly in DNA replication and that the amount of protein neither fluctuates significantly with the cell cycle nor is limiting for DNA synthesis.

Recently, several commercially available antibodies to PCNA have become available for formalin-fixed, paraffin embedded tissue sections. This has obvious advantages and recently numerous PCNA-related investigations have been published in the literature. PCNA staining is confined almost entirely to the nucleus with a diffuse or granular pattern, or with a mixture of both. During mitosis, there is diffuse cytoplasmic staining attributable to loss of nuclear membrane. Some mitotic figures, however, have been noted to stain negatively (Hall *et al*, 1990).

PCNA has a half life of about 20 hours (Bravo *et al*, 1987) and, therefore, may be detectable in cells that have recently left the cell cycle. PCNA immunoreactivity is markedly reduced if heat is used to promote adherence of sections to glass slides (Hall *et al*, 1990). PCNA immunoreactivity is present in tissues fixed in a wide range of solutions including formalin, Bouin's reagent and methacarn. Fixation time is of importance. Hall *et al*, (1990) have observed that staining is greatly reduced after 48 hours and is virtually abolished after 72 hours. They also state that protease digestion is not necessary to unmask antigen and may even reduce immunoreactivity.

Hall *et al* (1990) examined PCNA staining in a variety of non neoplastic tissues. They found positive staining in tissues known to be actively proliferating with a spacial relationship as would be expected. Examples included staining in germinal centres of lymphoid tissue, staining in the basal layer of stratified squamous epithelium and staining in the crypt epithelium of gastrointestinal mucosa. PCNA staining was minimal in tissues known to be non-proliferative, such as peripheral and central nervous system and smooth, skeletal and cardiac muscle.

Reports in the literature indicate that assessment of PCNA immunoreactivity is a valid method that has correlated well with other methods for assessing cell proliferation (Garcia *et al* 1989; Woods *et al* 1990; Kamel *et al* 1991 and Dervan *et al* 1992).

Battersby and Anderson (1990), in 47 cases of normal resting breast tissue, found a highly significant correlation between PCNA staining with antibody 19A2 and tritiated thymidine labelling, (tau = 0.748, p = 0.00003). They concluded that PCNA localisation provided a useful index of DNA synthesis and cell proliferation in breast.

Hall *et al* (1990), using a double labelling method, demonstrated a general concordance between PCNA immunoreactivity and tritiated thymidine labelling in cultured human keratinocytes and in 16 non Hodgkin's lymphomas. There was a linear relationship between Ki-67 and PCNA (PC10) staining, (r = 0.91).

Some studies, however, have shown poor correlation of PCNA with other methods for assessing cell proliferation. Jain *et al*, (1991) found a poor correlation between PCNA index and flow cytometric evaluation of S phase in 93 cases of gastric cancer. Yu *et al* (1991), in 42 cases of haemangiopericytoma, also found no correlation between PCNA index and S phase fraction. Leonardi *et al*, (1992) in a series of 106 breast carcinomas found that PCNA (PC10) index did not correlate with Ki-67 index or any other variable examined. Hall *et al* (1990) suggest that poor correlation between S phase fraction and PCNA staining may be due, in part, to the long half life of PCNA. Hall *et al* (1990) also suggest that, in certain neoplasms, there is deregulation of PCNA gene expression by autocrine or paracrine secretion of growth factors, such as platelet derived growth factor. Increased PCNA staining has been noted in normal tissue surrounding some breast and pancreatic tumours, possibly due to the paracrine influence of growth factors elaborated by the tumour. These growth mechanisms may increase messenger RNA half-life with a resultant increase in PCNA levels (Hall *et al* 1990). Injection of human carcinoma cell lines *in vivo* into the liver and kidney of nude mice and infusions of growth factors into rats have given rise to an increase in PCNA (PC10) immunoreactivity with no change in the number of S phase cells. PCNA immunoreactivity can occur, therefore, without

cell proliferation in association with neoplasia, and this may be mediated by growth factors *in vivo* (Hall *et al*, 1992).

In establishing a method of evaluating and scoring immunostaining, several problems arise. In many tissues, immunostaining is heterogeneously distributed. There is no clarity whether one should restrict counting to histological fields showing the most positive staining, or whether there should be random selection. PCNA staining is variable in intensity and there is no clarity either whether one should count all positive cells, including weakly positive cells, or only strongly positive cells. To date there is no universally accepted standardised method for histological field selection and quantitation of immunostaining. Results from different centres are, therefore, often not comparable (Linden *et al*, 1992). M^{c}Gurrin *et al* (1987) using Ki-67 immunostaining in breast carcinomas reported a mean index of 22% (per 1000 cells) counting areas with the most dense immunostaining. In contrast, Wrba *et al* (1989) counted Ki-67 immunolabelling in breast carcinomas using random field selection and reported a mean index of 7.2% (per 1000 cells).

Battersby and Anderson (1990) compared thymidine labelling index and PCNA immunoreactivity in 47 cases of normal resting breast tissue and commented on variability in strength of PCNA nuclear staining. When only strongly reactive nuclei were counted, values very similar to tritiated thymidine labelling were obtained, but if weakly staining nuclei were counted as well, values were approximately 30% higher.

In this study, all stained nuclei, including those weakly stained, were counted. Although the resulting PCNA indices were higher, this method reduced the level of uncertainty in deciding between positive and negative nuclei. This also allowed for consistency in comparing fibroadenomas within the same study and no attempt was made to compare the results of other studies. Fields for cell counting were not selected for any particular density of stained nuclei. A minimum of 1000 stromal and epithelial cells were counted. Using binomial parameters it has been calculated that

if 1000 cells are counted for a 95% confidence level, the margin of error would be 3% (Linden *et al* 1992). In this study, repeat counting indicated a high level of reproducibility of the cell counting method.

Generally the stromal and epithelial cells of the fibroadenoma exhibited a high degree of PCNA immunoreactivity. In some cases the indices were of the order of 90%. These values are far greater than TLI and PCNA indices encountered in studies on normal resting human breast. Unfortunately, in this study, the amount of normal breast tissue surrounding the fibroadenomas was inadequate for an internal control. Counting all stained nuclei is almost certainly a contributing factor to this high index. As illustrated, however, (figures 10 and 11) some fibroadenomas showed a very high index of strong nuclear staining, even in the face of relatively few mitotic figures. Several explanations are possible:

(i) PCNA has a long half life and may still be present in cells that have left the cell cycle.

(ii) Morris and Mathews (1989) have shown that PCNA is present throughout the cell cycle and PCNA is in excess of requirements for DNA synthesis.

(iii) Fibroadenomas are in fact actively proliferating lesions with most cells forming the cycling cell compartment.

(iv) The cells may have a long cell cycle with a prolonged G_1 phase. Few cells are in G_0.

(v) Hall *et al* (1992) have shown that non-cycling cells can express PCNA, possibly as a result of growth factor stimulation.

Tritiated thymidine labelling has been used by several groups to assess cell proliferation in normal lobular epithelium (Meyer 1977; Anderson *et al* 1982; Going *et al* 1988; Potten *et al* 1988). All investigations demonstrated a significant cyclic variation of TLI with a peak late in the second half of the menstrual cycle. Most

studies found no effect of OC on cell proliferation in breast epithelium (Meyer 1977; Anderson *et al* 1982; Going *et al* 1988; Potten *et al* 1988). Anderson *et al* (1989) found significantly higher mean TLI values in late proliferative and late secretory phases of women with OC regulated cycles when compared to women with natural cycles.

In these studies a significant decline of TLI with age was also observed. In assessing the effect of breast age (duration between menarche and time of biopsy) it was found that breast epithelium from very young women (less than 5 years breast age) was more proliferative than that of older women (Anderson *et al* 1989).

However, there remains a marked paucity of existing understanding of normal breast development and the numerous factors that influence the proliferation of normal breast epithelium. In the past, it has been suggested that oestrogen stimulates proliferation of breast epithelium and that progestins are associated with differentiation and secretion in keeping with the misconception that regulation of breast proliferation and differentiation is similar to that occurring in the endometrium. The demonstration of increased proliferation of breast epithelium fairly late in the second half of the cycle contradicts this. It is possible that the differences between breast and endometrial proliferation reflect differences in receptor status. Anderson *et al* (1989) state that oestrogen is a mitogen for endometrium, but not for breast. Oestrogen may facilitate progestin action by increasing progesterone receptor levels. Proliferation and development of breast epithelium is obviously a complex process and hormones may alter the effect of a host of "growth factors", either by influencing growth factor secretion or by changing receptor activity (McCarty, 1989). The interactions of these effectors of development have barely been characterised. The secretion of a number of growth factors, including insulin-like growth factor and transforming growth factor-alpha can be modified by oestrogens (McCarty, 1989).

Unlike the various cell proliferation studies on normal human breast, the cell proliferation data of this study, expressed as PCNA immunoreactivity, show no correlation with the menstrual cycle. Exogenous hormones, in the form of OC agents or injectable progestogen, do not appear to exert any effect either. This study does indicate that fibroadenoma is a highly proliferative lesion. This is in keeping with its fairly rapid growth and increase in size.

The high level of proliferative activity of fibroadenomas and their apparent lack of response to hormonal stimuli indicates that these lesions exhibit some degree of autonomous biological behaviour. This may be due to a localised paracrine or autocrine influence, possibly related to changes in receptor or growth factor status. Wilkinson and Forrest (1985) state that the fibroadenoma represents a group of hyperplastic breast lobules, but the lack of response to hormones demonstrated in this study may rather indicate a neoplasm. Autocrine influences such as epithelial-stromal interactions may play an important role. In this study, the stroma generally exhibited as high a degree of PCNA immunoreactivity as the epithelium. Although not quantitated, the stromal cells immediately adjacent to epithelium were observed to have a higher degree of immunoreactivity than those stromal cells further away. This suggests some kind of interaction at the stromal-epithelial interface, possibly mediated by growth factors.

Unlike declining proliferative indices observed in normal breast with increasing age, PCNA immunoreactivity did not correlate with patient age. High PCNA indices occurred in fibroadenomas of older women, although one did show low indices. This is in keeping with the finding of myxoid or cellular "active" appearing lesions in older age groups. Fibroadenomas, therefore, appear to retain their ability to proliferate beyond the third decade, although in terms of age incidence, this ability becomes increasingly limited. High PCNA indices of fibroadenomas in older women are in contradiction to the morphologic data that show a tendency for fibroadenomas to decrease in size and epithelial component with age. This may be due to skewing of

PCNA data produced by the small PCNA sample number. Falsely high PCNA indices for the various reasons already mentioned may also be of importance.

CONCLUSION

The hypotheses initially stated are not supported by this study. There are no morphologic changes in fibroadenomas related to hormonal stimulation in terms of the menstrual cycle or hormonal contraceptive.

Cell proliferation in fibroadenomas shows no relationship to steroid stimulation, but there may be limitations to the methodology used as discussed by Hall *et al* (1992). A true alternative method would be to perform *in vitro* studies on cells from the fibroadenoma in culture and assess the effect of steroids on tritiated thymidine labelling.

A strong age-related incidence of fibroadenomas has been demonstrated. Although cell proliferation showed no relationship to age, fibroadenoma size and epithelial component both showed a tendency to decline with age that was not statistically significant. Fibrous lesions are commoner in younger age groups. However, if one presumes that young lesions are myxoid and hyaline lesions are old, then this study indicates that young fibroadenomas can occur in older women. The only true way to show a natural history would be to design a study in which fibroadenomas are left *in situ* within the breast for varying periods of time (even possibly years), but this would probably not be ethically permissible.

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APPENDIX

PCNA Index Assessment:

Each line represents a counting of a single 10x10 square grid at high power with positive and negative epithelial and stromal cells. Numbers in brackets are recounts of the number immediately above.

PATIENT SD NO.	EPITHELIUM			STROMA		
	+VE	-VE	TOTAL	+VE	-VE	TOTAL
11684/86	91	53	144	64	111	175
	134	80	357	76	79	320
				(72)	(75)	
	146	106	610	67	64	451
	170	109	889	79	97	627
				(84)	(98)	
	81	48	1018	84	112	818
	142	94	1254	110	181	1109
11717/85	157	84	241	31	36	67
	184	98	523	64	74	205
	107	85	715	99	92	396
	109	104	928	77	101	574
	113	64	1105	69	81	724
	79	81	1265	84	121	929
	(80)	(88)				
	129	93	1487	65	73	1067
15213/86 1	64	139	203	21	130	151
	97	130	430	49	66	266
	101	129	660	40	92	398
	50	84	794	43	64	505
	67	107	968	40	71	616
	78	115	1161	28	64	708
	(74)	(99)				
	57	112	1330	49	55	812
				(57)	(58)	
	23	68	1421	51	73	936
	56	50	1527	42	71	1049

14922/86	41	111	152	94	35	129
	63	92	307	112	33	274
	57	70	434	133	31	438
	35	65	534	78	44	560
				(83)	(39)	
	55	92	681	96	30	686
	48	116	845	101	15	802
	37	63	945	74	14	900
	54	75	1074	117	15	1022
16108/85	127	103	230	54	40	94
	66	46	342	76	59	229
	92	46	480	70	44	343
				(67)	(49)	
	104	81	665	70	45	458
	(108)	(92)				
	95	81	841	82	47	587
	73	70	984	67	39	693
	(69)	(67)				
	74	64	1122	46	40	779
	72	71	1265	63	48	890
	79	60	1404	67	85	1042
				(70)	(84)	
3713/86	164	25	189	122	16	138
	190	42	421			
	(206)	(27)				
	185	55	661	84	27	359
	127	25	813	147	37	543
	100	17	930	141	19	543
	162	9	1101	130	13	846
	142	11	1254	125	6	977
	147	22	1423	129	24	1130
9562/85	94	74	168	115	50	165
	141	137	446	83	30	278
	127	116	689	63	36	377
	169	133	991	41	34	412
	137	76	1204	69	47	528
	68	72	1344	76	34	638
	145	72	1561	61	36	735
	(167)	(89)				
	96	62	1719	103	43	881
	(88)	(56)		(94)	(48)	
	57	39	1815	121	72	1074
1075/86	127	26	153	113	15	128
	79	80	312	114	90	332
	157	48	517	103	21	456
	(175)	(47)				
	186	48	751	81	11	548
	172	31	954	120	18	686
	96	19	1193	129	16	978
	127	31	1351	71	34	1083

12292/85	105	85	190	82	31	113
	153	41	384	88	32	233
	81	31	496	72	53	358
	229	97	822	68	43	469
	156	139	1117	45	44	558
	(154)	(148)				
	161	117	1395	51	57	666
	40	51	1486	58	57	781
	(40)	(47)				
	64	38	1588	33	76	890
	(61)	(43)				
	55	42	1685	49	28	967
				(47)	(31)	
	93	55	1833	47	60	1074
	(86)	(56)				
10224/85	120	49	169	54	80	134
	99	29	297	105	63	302
	129	74	500	65	51	418
	97	26	623	88	69	575
	116	46	785	66	51	692
	(106)	(50)				
	131	69	985	97	56	845
	85	31	1101	112	82	1039
18377/85	88	32	120	67	25	92
	115	46	281	105	26	223
	(117)	(50)				
	140	42	463	77	17	317
	128	49	640	88	18	423
	92	39	771	92	38	553
	119	53	943	107	16	676
	106	38	1087	96	12	784
	78	11	1176	106	18	908
	91	22	1289	112	14	1034
2268/86	95	95	190	59	34	93
	61	71	322	73	78	244
	93	72	487	52	63	359
	88	75	650	69	97	525
	(88)	(69)				
	82	22	754	75	38	638
	98	45	897	75	28	741
	(95)	(41)				
	88	28	1013	89	27	857
	94	62	1169	54	58	969
				(59)	(57)	
	74	54	1297	66	62	1097

18377/85 L	92	28	120	11	4	15
	64	22	206	23	9	47
	7	10	223	37	21	105
	17	6	246	34	35	174
	59	26	331	32	23	229
	64	24	419	56	25	310
	(68)	(20)				
	47	23	489	62	23	395
	82	54	625	8	22	425
	100	31	756	31	12	468
	28	4	788	83	16	567
	61	8	857	72	11	650
	93	33	983	54	23	727
	(92)	(39)				
	93	20	1096	65	9	801
	49	17	1162	69	11	881
	78	20	1260	47	18	946
	(74)	(19)				
	49	4	1313	30	14	990
8605/85	72	26	98	66	55	121
	69	118	285	45	87	253
	107	63	455	40	89	382
	172	137	764	46	56	484
	(175)	(148)				
	98	68	930	44	71	599
	119	116	1165	73	67	739
	(113)	(116)				
	105	103	1373	48	51	838
	100	69	1542	47	57	942
	(92)	(77)				
	78	42	1662	57	64	1063
5194/86	89	53	142	54	39	93
	158	80	380	85	51	229
	102	53	535	113	53	395
				120	54	
	112	51	698	77	69	541
				(71)	(63)	
	129	20	847	138	16	695
	109	52	1008	92	40	827
12045/86	153	72	225	44	89	133
	154	36	415	52	68	253
	82	44	541	66	129	448
	124	37	702	87	88	623
	146	37	885	82	66	771
	103	23	1011	62	98	931
	152	35	1198	94	80	1105

8442/86	117	55	172	117	30	146
	93	33	298	152	36	335
	114	51	463	99	14	448
	153	48	664	117	26	591
	181	48	893	114	44	749
	(186)	(38)				
	108	24	1025	136	38	923
	101	24	1150	162	21	1106
6728/86	59	52	111	93	35	128
	80	59	250	100	18	246
	69	51	370	101	22	369
	50	43	463	70	20	459
	110	45	618	105	35	599
	(103)	(47)				
	88	54	760	79	44	722
	(96)	(50)				
	60	43	863	71	36	829
	60	58	981	89	24	942
	37	32	1050	70	30	1042
4987/85	152	93	245	61	25	86
	156	93	494	75	15	176
	54	39	587	108	15	299
	136	93	816	81	18	398
	108	84	1008	76	27	501
	69	76	1153	72	36	609
	118	85	1356	79	19	707
	80	55	1491	101	17	825
	37	27	1555	92	16	933
	99	30	1684	96	23	1052
10286/86	167	13	180	107	20	127
	100	4	284	138	26	291
	170	13	467	99	25	415
	150	21	638	86	29	530
	121	10	769	166	38	734
	120	4	893	155	18	907
	113	17	1023	166	13	1086
	123	17	1163	158	12	1256
2268/86 R	151	41	192	93	83	176
	151	16	359	135	56	367
	(150)	(15)				
	225	60	644	99	47	513
	145	11	800	123	26	662
	178	16	994	109	36	807
	173	32	1199	92	20	919
	117	19	1335	128	31	1078
	(124)	(24)				

16852/85	32	111	143	63	95	158
	62	119	324	64	60	282
	76	97	497	75	85	442
	62	70	629	62	100	604
	(66)	(68)		(60)	(107)	
	55	81	765	84	118	806
	61	116	942	58	87	951
	94	98	1134	64	47	1062
13125/86	39	80	119	4	83	87
	42	113	294	16	60	163
	48	78	420	18	67	248
	29	166	615	8	76	332
				(9)	(78)	
	32	130	777	6	83	421
	23	50	850	5	72	498
	(25)	(50)				
	30	76	956	41	70	609
				(36)	(70)	
	76	148	1180	46	54	709
	(76)	(157)				
	15	99	1294	10	79	798
				(12)	(79)	
	19	112	1425	8	68	874
	32	99	1556	13	73	960
	21	76	1653	20	66	1046
14630/86	103	216	319	55	65	120
	39	92	450	90	106	316
	51	167	668	107	100	523
	70	199	937	131	65	719
	85	101	1123	81	88	888
	83	132	1338	84	75	1047
18005/85	101	112	213	57	9	66
	64	86	363	52	23	141
	40	71	473	55	21	217
	77	55	605	48	10	275
	75	45	725	40	10	325
	115	40	880	42	32	399
	83	21	984	42	5	446
	73	54	1111	75	17	539
	51	57	1219	54	23	616
	91	60	1370	35	16	666
	139	63	1572	50	15	731
	49	16	1637	72	28	831
	83	26	1746	64	20	915
	52	25	1823	86	15	1016

8681/85	260	86	346	44	9	53
	154	53	553	62	21	136
	127	55	735	45	14	195
	106	45	886	61	16	272
	155	39	1080	69	18	354
	187	52	1319	49	12	420
	151	29	1499	67	13	500
	167	59	1725	53	12	565
	182	78	1985	49	17	631
	192	32	2209	57	11	699
	145	47	2401	61	20	780
	97	31	2529	50	11	841
	87	27	2643	64	24	929
	153	33	2829	60	12	1001
7722/86	174	39	213	71	31	102
	95	27	335	58	21	181
	86	12	433	54	24	259
	115	13	561	74	20	353
	117	18	696	80	27	460
	99	27	822	55	11	526
	105	19	946	53	31	610
	(111)	(18)				
	127	68	1141	86	41	737
	82	41	1264	78	29	844
	193	35	1492	54	9	907
	147	21	1660	70	20	997
	52	17	1729	50	21	1068
8072/86	76	68	144	55	29	84
	113	67	324	35	38	157
	103	67	495	46	7	210
	108	68	643	44	10	264
	90	29	762	65	15	344
	(95)	(30)				
	58	15	835	53	10	407
	107	49	991	37	13	457
	51	14	1056	46	13	516
	55	6	1117	56	10	586
	58	15	1190	61	3	646
	71	38	1299	37	8	691
	73	37	1409	32	4	727
	46	37	1492	42	8	777
	(48)	(33)				
	37	21	1550	40	1	818
	43	25	1618	45	6	869
	85	28	1731	39	6	914
	70	25	1826	35	5	954
	82	22	1930	50	3	1007

3235/86	190	34	224	112	9	121
	107	65	396	84	10	215
	197	12	605	49	21	285
	89	21	715	130	12	427
	213	54	982	85	8	520
	142	26	1150	149	15	684
	(144)	(30)				
	148	23	1321	85	9	778
	134	24	1479	116	9	903
	81	16	1576	137	6	1046
14569/86	92	20	112	57	14	71
	83	21	216	55	24	150
	90	34	340	57	20	227
	72	38	450	67	14	308
	101	36	587	46	17	371
	86	21	694	61	18	450
	75	27	796	66	21	537
	97	18	911	86	18	641
	(96)	(21)				
	112	49	1072	59	12	712
	(112)	(49)				
	96	51	1219	66	24	802
	64	18	1301	115	26	943
	47	10	1358	67	19	1029
1338/86	183	23	206	25	28	53
	141	22	369	30	31	114
	126	22	517	44	28	186
	103	17	637	49	20	255
	91	10	738	29	24	308
	47	5	790	37	14	359
	137	22	949	41	15	415
	131	14	1094	31	18	464
	65	5	1164	49	32	545
	84	12	1260	41	18	604
	79	16	1355	40	10	656
	101	12	1468	45	25	724
	104	11	1583	58	13	797
	92	6	1681	63	10	868
	141	12	1840	49	13	930
	182	19	2041	41	15	986
	75	5	2121	43	12	1041
4692/86	140	12	152	138	8	146
	119	19	290	101	5	252
	130	8	428	157	10	419
	120	20	568	112	2	533
	106	1	675	106	4	643
	141	10	826	142	1	786
	188	20	1034	166	8	960
	(189)	(22)				
	147	9	1190	121	4	1085
				(121)	(4)	

17962/85	143	20	163	50	8	58
	190	21	374	69	13	140
	120	21	515	65	5	210
	109	6	630	97	10	317
	183	32	845	99	36	452
	122	23	990	72	18	542
	159	21	1170	65	9	616
	(153)	(22)				
	106	11	1287	54	14	684
	122	24	1433	84	12	780
14631/86	48	61	109	43	17	60
	35	59	203	21	14	95
	75	97	375	14	17	126
	43	36	454	42	5	173
	41	56	551	20	16	209
	40	53	644	49	6	264
	37	39	720	32	9	305
	(38)	(37)				
	54	59	833	35	7	347
	44	48	925	32	8	387
	38	55	1018	37	10	434
	(38)	(54)				
	60	71	1149	38	5	477
	48	40	1237	35	3	515
	41	45	1323	35	10	540
	(44)	(43)				
	57	58	1438	47	12	619
	(52)	(56)				
	37	62	1537	57	15	691
	29	34	1600	32	14	737
	52	72	1724	37	11	785
12893/86	71	62	133	85	47	132
	181	159	473	97	25	254
	135	128	736	112	48	414
	158	66	960	143	31	588
	96	78	1134	81	80	749
	125	34	1293	107	33	889
	(131)	(30)				
	92	89	1474	129	38	1056
5691/86	98	9	107	78	10	88
	133	8	248	99	7	194
	148	29	425	81	4	279
	64	36	525	103	11	393
	(68)	(36)		(101)	(11)	
	101	25	651	92	24	509
	(97)	(30)				
	116	13	780	126	11	646
	132	12	924	93	6	745
	112	17	1053	87	11	843
	177	21	1251	170	6	1019

14047/85	111	3	114	152	9	161
	107	9	230	103	18	282
	153	6	389	145	8	435
	148	7	544	90	5	530
	103	2	649	128	6	664
	161	6	816	110	8	782
	146	8	970	93	3	882
	176	9	1155	95	2	979
	88	6	1249	65	2	1046
	(83)	(9)				
955/86	106	64	170	56	26	82
	(109)	(60)				
	201	108	479	49	17	148
	175	105	759	40	8	196
	110	155	1024	25	25	246
	64	99	1187	51	15	312
	53	53	1293	89	5	406
	45	91	1429	72	9	487
	69	87	1585	122	7	616
	57	17	1659	91	2	709
	80	24	1763	97	9	815

PATIENT SD NO.	AGE	CONTRA-CEPTIVE	CYCLE DAY	MYOEP. VACUOL.	STROMAL CHARACT.
1833/86	20		21	1	M
17606/85	17		5	1	M
13653/85	35		28	1	M
7432/86	29		18	1	F
4987/85	26	DEPOT		1	M
819/86	32		10	1	M
10224/85	19		27	1	F
15851/86	30		18	1	F
14629/85	21		25	2	F
17962/85	28	OC		2	F
2848/86	23		20	2	F
14569/86	32		6	1	M
17976/86	60			1	H
6071/86	23		24	1	F
925/86	18	OC	16	1	F
7003/86i	21		22	1	F
7003/86ii	21		22	1	F
16392/85	19		19	1	M
1338/86	22	OC	7	3	F
11062/85	23		13	1	M
12292/85	23	OC	10	1	M
15607/85	20		17	1	F
16247/85	21		12	2	H
7722/86	26		11	1	F
18377/85L	24	DEPOT		2	H
18377/85R	24			1	F
17580/86	19				F
2654/87	27	DEPOT		2	H
15470/86	36		5	2	M
5935/86	22			3	F
8072/86	28		27	2	M
955/86	38		10	3	H
14976/85	18		11	1	M
10257/86	17			2	M
10668/85	22		6	3	F
16875/85	23		4	2	F
18115/86i	15		7	1	F
18115/86ii	15		7	1	M
2092/86i	19		2		F
12145/86	21		8	2	F
5135/86	25		17	2	M
8681/85	16		18	3	F
14630/86	25		18	1	F
4692/86	21		20	1	M
3713/86	18		22	3	F
8605/85	17	DEPOT		2	M
1293/86	34			3	F
11523/86	17		18	1	M
14631/86	42		4	1	M
7403/86	17			1	M

7404/86	24		22	2	F
13125/86	43		24	1	H
1685/86	26		23	1	F
11684/86i	21		24		F
11684/86ii	21		24	2	M
10589/89	23		20	2	M
17932/86	25		18	1	M
1492/86	19		20	1	M
328/86	36			1	M
13129/86	21		24	1	F
2069/86	25	OC	28	1	H
9756/85	46	DEPOT		2	M
17722/86	17			1	M
5691/86	18		13	1	M
11446/85	19		1	2	F
957/86	34		25	1	F
16997/86	29		1	1	F
11967/85	20		18	2	F
1316/86	19	OC		2	F
15294/86	21			1	F
13827/85i	22			1	F
13827/85ii	22				F
8832/85	16		22	3	F
10414/85	16		8	1	F
17871/86	24		3	2	F
9562/85	24	OC	20	2	F
13131/85	21				M
6927/86	47		10	1	F
17588/86	19			1	F
8442/86	31	DEPOT		3	F
16106/86	20		15	2	M
12151/85i	18			1	M
12151/85ii	18			1	F
2415/87	22			3	M
3482/86	16		27		F
3296/87	24	DEPOT		2	M
3716/86	26		7	1	M
10395/86	19			1	F
9558/85	27			1	M
11717/85	23	OC	27	2	F
14515/85	36		10	1	F
18243/86	37		18	2	M
1222/86	32	DEPOT		1	M
3235/86	20		21	1	F
1709/86i	16			1	M
1709/86ii	16			2	M
14922/86	25	DEPOT		1	M
4136/86	18		3	1	M
4166/86	18			1	F
1075/86	18		23	1	F
2268/86R	21	DEPOT		2	F
2268/86L	21	DEPOT		1	F
188/86	21			2	F
18005/85	21		28	1	H
1319/86	24	DEPOT		1	H
859/86	28		8		F

12045/86	23		23		F
1741/87	21		16	1	M
12893/86	20		4		F
9912/86	19		2		F
1145/87	35			3	F
17530/86	18		7	2	M
18246/86	18			3	F
9200/86	30		28	1	F
15875/85	14			3	F
5194/86	36		10	3	F
8163/86	28				F
4806/86	46		25		F
15213/85	17		24		F
1980/87	20			1	F
5543/86	26			1	F
11297/85	23	OC	11		F
8855/86	19		25	1	F
10286/86	39	DEPOT		2	F
6728/86	24	OC	11		F
14919/85	23		17	1	M
14610/85	22		24		F
17828/86	20			2	F
16852/85	21	OC	7	2	F
14047/85	21		7	2	F
17253/85	26		20	2	F
5917/86i	20				M
5917/86ii	20			3	M
11063/85	16		6	2	M
12896/86	17		11	2	M
11918/86	41			3	M
17021/85	32			1	F
17610/85	23		22	2	F
16108/85	24	OC	17	1	F

AVERAGE 24.

PATIENT	EPITHELIAL COMPONENT%	SIZE cm	EPITH INDEX	STROM INDEX
1833/86	30	1		
17606/85	5	1		
13653/85	5	2		
7432/86	20	2		
4987/85	50		59.9	79.9
819/86	30	1		
10224/85	25		70.6	56.5
15851/86	15			
14629/85	20	2		
17962/85	20		87.5	84
2848/86	10			
14569/86	5		74.7	77.9
17976/86	5	1		
6071/86	30	2		

925/86	25	1		
7003/86i	10			
7003/86ii	5			
16392/85	10			
1338/86	40	7	89	68.7
11062/85	10	1		
12292/85	15	0.5	62	55.2
15607/85	10	0.5		
16247/85	5	2		
7722/86	25	1	80.5	73.3
18377/85L	5		74.9	70.7
18377/85R	10		74.2	82.2
17580/86	10	1		
2654/87	5	2		
15470/86	5	1		
5935/86	10			
8072/86		5	68.7	81.2
955/86		0.5	54.4	84.9
14976/85	10	3		
10257/86	10	2		
10668/85	50	2		
16875/85	15	3		
18115/86i	15			
18115/86ii	10			
2092/86i	40			
12145/86	30	2		
5135/86	10	1		
8681/85	25	2	76.5	79
14630/86	10		32.2	52.3
4692/86	5	0.5	91.6	96.1
3713/86	10	10	85.5	85.4
8605/85	10	1	55.4	43.8
1293/86	10			
11523/86	15			
14631/86	5	3	45.2	77.2
7403/86	5	2		
7404/86	5	1		
13125/86	5	1	24.5	18.6
1685/86	10	0.5		
11684/86i	35	2	60.9	42.8
11684/86ii	5	2		
10589/89	5			
17932/86	10	1		
1492/86	10			
328/86	5	1		
13129/86	10	5		
2069/86	20	1		
9756/85	10	0.5		
17722/86	10			
5691/86	10	0.5	86.4	91.1
11446/85	10	1		
957/86	5	2		
16997/86	10			

11967/85	10	2		
1316/86	20	0.5		
15294/86	5			
13827/85i	5			
13827/85ii	20			
8832/85	5	5		
10414/85	10	3		
17871/86	25			
9562/85	25	4	57	70
13131/85	20			
6927/86	10	1		
17588/86	5	2		
8442/86	15	3	75.4	81.1
16106/86	25	1		
12151/85i	15			
12151/85ii	15			
2415/87	5	2		
3482/86	70			
3296/87	15	1		
3716/86	10	3		
10395/86	15	4		
9558/85	15	1		
11717/85	15	1	59	45.8
14515/85	10	2		
18243/86	15	0.5		
1222/86	15	1		
3235/86	15	2	82.5	90.5
1709/86i	25			
1709/86ii	20			
14922/86	5		36.3	78.8
4136/86	5			
4166/86	15	1		
1075/86	10	1	77.4	80.2
2268/86R	20		85.4	72.2
2268/86L	5		59.6	55.7
188/86	60	5		
18005/85	5	2	60	76
1319/86	5	3		
859/86	20			
12045/86	10	0.5	76.3	44.1
1741/87	5	1		
12893/86	15	2	58.2	71.4
9912/86	15			
1145/87	10	3		
17530/86	10			
18246/86	5	1		
9200/86	5			
15875/85	15	2		
5194/86	25	4	69.2	70.8
8163/86	5	1		
4806/86	15	0.5		
15213/85	15	1	38.8	34.6
1980/87	10	3		
5543/86	5	1		
11297/85	15	1		
8855/86	5			

10286/86	10	2	91.5	85.6
6728/86	5		58.4	74.6
14919/85	5	3		
14610/85	30			
17828/86	15	2		
16852/85	10	3	39	44.2
14047/85	10	1	95.5	94.1
17253/85	25	2		
5917/86i	10			
5917/86ii	15			
11063/85	20	3		
12896/86	5	2		
11918/86	5	1		
17021/85	10	1		
17610/85	10	2		
16108/85	10		55.7	57.1