Virchows Archiv A Pathol Anat (1988) 413:189–196

Virchows Archiv A
Pathological Anatomy and Histopathology
© Springer-Verlag 1988

Proliferative and secretory activity in the pregnant and lactating human breast

S. Battersby and T.J. Anderson
Department of Pathology, University of Edinburgh, Edinburgh, United Kingdom

Summary. In this study the proliferative and secretory activity of human pregnant and lactating breast has been examined. 17 cases of pregnant tissue were obtained, comprising 9 from first trimester, 6 from the second and 2 from the third, together with 3 cases of lactating breast. Proliferation, measured by 3H thymidine autoradiography, gave a constant Thymidine labelling index of approximately 6% during the first 20 weeks, with a reduction by 50% thereafter. Levels of 0.2% were seen during lactation. Morphological assessment showed a high frequency of both mitosis and apoptosis throughout gestation. Although mitotic frequency did reduce with time, values were more variable than thymidine labelling. The reduction in thymidine uptake halfway through pregnancy coincided with generalised morphological lactational differentiation and the major immunocytochemical expression of alpha-lactalbumin. However, cytoplasmic expression of the secretory component of IgA was generalised and marked even during the first trimester and was closely related to increased proliferation and general synthetic cellular activity. These and other observations support a complex relationship of autocrine and paracrine factors in addition to the customary endocrine ones for breast regulation.

Key words: Breast – Human – Pregnancy – Proliferation – Secretion

Introduction

In the light of recent documentation of the variability in breast epithelial proliferation in the normal "resting" state (Ferguson and Anderson 1981; Anderson et al. 1982; Meyer and Connor 1982; Going et al. 1988) it would be relevant to know in more detail the situation that exists during the development and full expression of physiological differentiation to lactation in this tissue. Previous reports of pregnant breast noted a much higher level of proliferation than normal (Salazar et al. 1975; Meyer 1977) and a study of eight cases of pregnant breast noted a high occurrence rate of both proliferation (mitotic frequency) and deletional cell death (apoptotic frequency) (Ferguson and Anderson 1983). The breast has a distinctive development that is related to sexual maturity and menstruation but without recourse to pregnancy, features which distinguish it from the equivalent glands of other mammals (Short and Drife 1977). This report documents the relationship of proliferation and secretory activity at different stages of pregnancy and during lactation, and considers these events firstly in relation to the situation during the menstrual cycle and secondly to the modulating factors.

Offprint requests to: T.J. Anderson

Materials and methods

Samples of breast tissue of normal appearance were obtained, over a 3 year period, from 17 pregnant and 3 lactating women, aged from 17 to 34 years. Both parous and nulliparous women were included (see Table 1). All were biopsied for clinical reasons usually a palpable mass giving rise to concern. In 13 cases, the normal breast tissue was taken from areas surrounding, but separate from a fibroadenoma. The remaining 7 samples were from specimens in which no pathological changes were evident.

Autoradiography. Thin slices of breast tissue were stained with methylene blue dye and groups of breast lobules microdissected, using iris scissors, as described previously (Going et al. 1988). The lobules were incubated in Modified Eagle's Medium (Gibco, Europe) with 5 μCi/ml 3[H]-methyl thymidine (Amersham International) (specific activity 1.5 Ci/mM) under hyperbaric oxygen conditions at 37° C for one h. The lobule strips

were fixed in Carson's buffered formalin (Carson et al. 1973), embedded in paraffin wax, 5 μm sections obtained and autoradiographs were prepared by dipping sections in K5 Nuclear Research Emulsion (Ilford) diluted 1:1 with 1% glycerol. Sections were exposed for 14 days at 4° C and the autoradiographs were then developed using D-19 developer (Kodak), fixed (Unifix-Kodak) and stained with haematoxylin and eosin.

Quantitation. Quantitation of labelled cells was performed as described previously (Going et al. 1988). The total number of cells in each terminal duct lobular unit (TDLU) was determined by estimating the number of cells in each ductule (and in certain cases the intralobular portion of the terminal duct) to the nearest 10 and multiplying by the number of ductules. The number of labelled nuclei were then assessed (identified as those showing at least 5 grains above background) and expressed as a percentage of the total cell number. Adequate penetration of thymidine was confirmed by labelling of nuclei at the centre of the lobule clusters. Sixteen lobules (TDLUS) were counted for each case (approximately 8000 cells) and the mean labelling index calculated to obtain the TLI value.

Mitosis and apoptosis. Blocks of normal tissue from 12 of the cases used for thymidine labelling studies were fixed in Carson's fluid, dehydrated and embedded in glycol methacrylate (GMA). Sections, 2 μm thick, were cut and stained using the Feulgen method, counterstained with fast green. Mitotic figures and apoptotic bodies were identified (Ferguson and Anderson 1981) and their frequency per lobule for 50 lobules calculated, although in two cases only 20 lobules were available for assessment. The number of ductules present per lobule was also counted, to allow expression of mitosis and apoptosis on a per ductule basis.

Immunocytochemistry. Slices of normal breast tissue, in each of the 20 cases examined, were fixed in Carson's fluid or methacarn fixative (6:3:1, methanol: chloroform: glacial acetic acid) and embedded in paraffin wax. Paraffin sections (5 μm thick) were cut, dewaxed, rehydrated and treated with 1% hydrogen peroxide in methanol to abolish endogenous peroxidase activity. Sections fixed in Carson's fixative were also pretreated with 0.1% trypsin (Difco 1:250) in 0.1% calcium chloride for 15 minutes at 37° C. A peroxidase-anti-peroxidase (PAP) method (Sternberger 1986) was then employed for localisation of alpha-lactalbumin (LA) and the secretory component (SC) of immunoglobulin A(IgA). The polyclonal primary antibodies to LA and SC (Nordic) were both used at a dilution of 1:400. Omission of the primary reagent was used as a negative control.

Results

A. Proliferative activity

Thymidine labelling. Proliferative activity in the human breast, measured by 3[H] thymidine incorporation into nuclei, was consistently high throughout the first half of pregnancy (Table 1), but showed a marked reduction during the second 20 weeks. The thymidine labelling index (TLI) was estimated for the TDLU as a whole, but, where the intralobular terminal duct could be identified separately it also showed a high TLI (4.11 for the first half of pregnancy and 2.75 for the second half). Labelled cells were seen in most ductules in a majority of lobules (Fig. 1) but were not situated in any particular region or orientation within the ductule. Labelling activity did not appear to be related to history of previous pregnancy or duration of lactation (Table 1), although the lowest values seen in the first half of pregnancy were in tissue from 2 nulliparous women. There was also frequent 3[H] thymidine accumulation in both intralobular stromal cells and in the cells lining the walls of vessels (Fig. 2).

In the lactational breast there was little proliferative activity (Fig. 3) although morphologically non-lactational lobules, seen occasionally within an area of lactating breast, had a consistently higher TLI (Table 1).

Mitosis and apoptosis. The number of mitotic figures per lobule noted during pregnancy was high (Table 2) and consistent with the high TLI. However, mitotic frequency showed greater variability than thymidine labelling when used as a method of measuring proliferative activity, whether expressed per lobule or per ductule (Table 2). How-

Table 1. 3[H] thymidine accumulation in pregnant and lactating breast

Length of pregnancy (weeks)	Thymidine labelling index (TLI) for TDLU	Parity	Time since last pregnancy	Length of previous lactation
6	10.99	2+1	4 years	0
6	6.6	1+0	3.5 years	8/12
8	6.4	1+0	10 years	N/K
9	6.3	1+0	2 years	3/12
9	**6.8} 6.8}	1+0	2 years	15/12
10	3.6	0+0	–	–
10	7.7	1+0	22/12	6/12
11	6.7	1+0	17/12	12/12
13	5.9	0+0	–	–
14	**6.1} 5.9}	1+0	6 years	1/12
15	6.6	0+0	–	–
17	6.5	0+0	–	–
19	6.6	0+0	–	–
21	3.3	0+0	–	–
21	3.7	1+1	1 year	6/12
30	3.3	1+1	4 years	N/K
32	2.9	0+0	–	–
Lactating	0.2 (NLL=1.8)	2+0	2/12	Current
Lactating	0.17 (NLL=2.7)	2+0	3/12*	Current
Lactating	0.23	2+0	6/12*	Current

* Currently using continuous progestin oral contraceptive
** Bilateral specimens
N/K = not known
NLL = non-lactational lobules

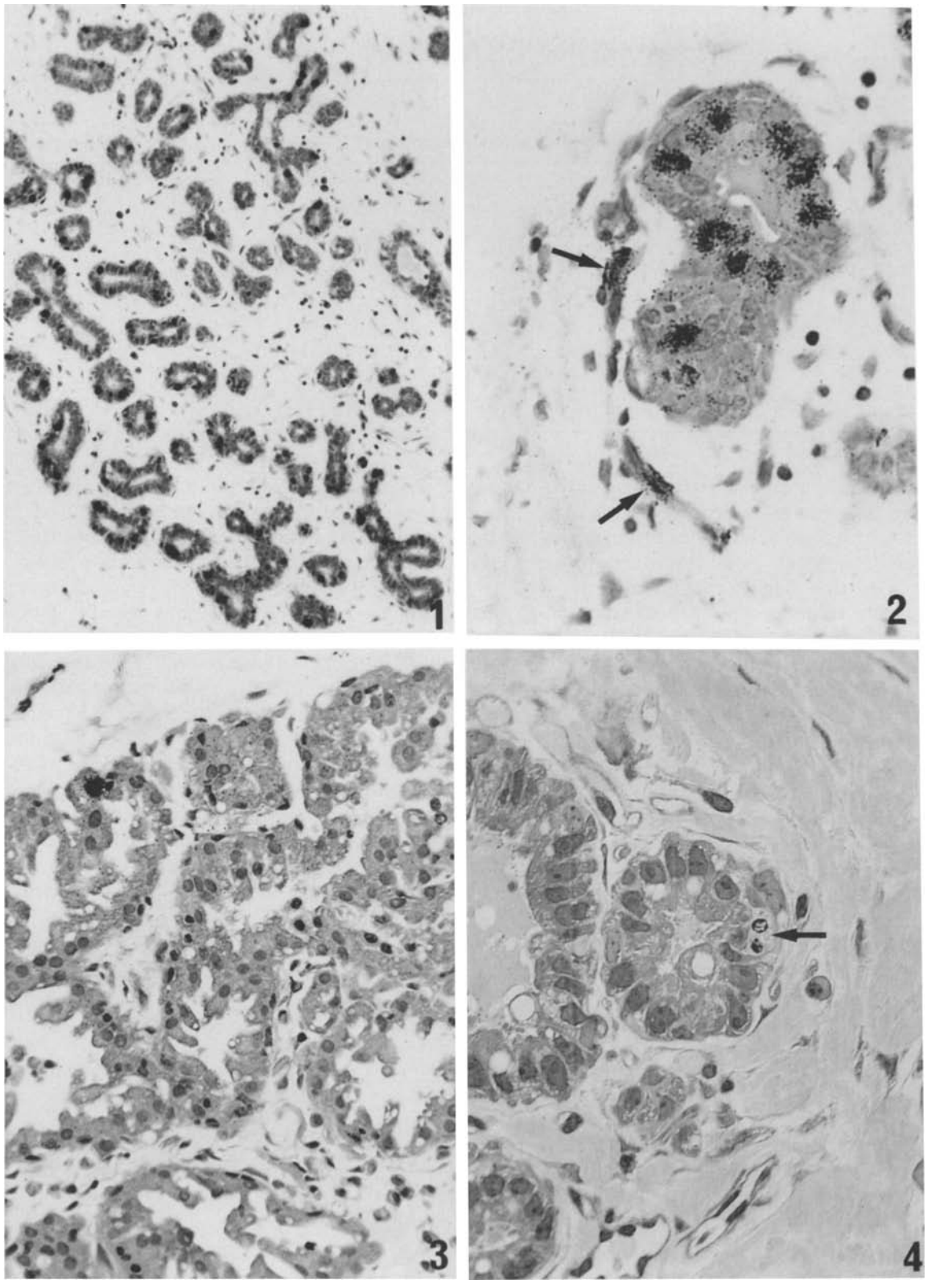


Fig. 1. Six week pregnant breast tissue. Nuclei heavily labelled with 3H thymidine are seen throughout lobule × 160

Fig. 2. Vessel in stroma surrounding ductule shows two nuclei (*arrowed*) overlain with grains × 500

Fig. 3. Autoradiograph of lactating breast. One labelled nucleus is seen × 320

Fig. 4. Apoptotic bodies (*arrowed*) are present in epithelium of 21 week pregnant breast tissue × 500

Table 2. Mitotic and apoptotic frequency during pregnancy and lactation

Weeks of pregnancy	Mitotic frequency				Apoptotic frequency			
	Per lobule	Mean value	Per ductule	Mean value	Per lobule	Mean value	Per ductule	Mean value
6	1.14	1.37	0.02	0.05	1.06	0.66	0.02	0.02
8	0.86		0.05		0.4		0.02	
9	0.29		0.02		0.14		0.008	
10	3.2		0.12		1.02		0.04	
13	1.21	1.23	0.06	0.05	0.58	0.41	0.02	0.02
17	1.54		0.06		0.16		0.06	
19	0.95		0.02		0.5		0.03	
21	0.42	1.43	0.01	0.03	0.64	1.13	0.02	0.03
21	2.64		0.06		2.36		0.05	
30	1.24		0.03		0.38		0.009	
lactating	0.02	0.02	0.0003	0.001	0.06	0.04	0.001	0.001
lactating	0.02		0.001		0.02		0.001	

ever when measured on a per ductule basis, introduced to take account of increasing lobule size, mean mitotic frequency decreased with increasing length of gestation. High numbers of apoptotic bodies were seen throughout pregnancy (Fig. 4) but the apoptotic frequency per lobule was consistently less than the mitotic frequency (Table 2) and remained constant even when expressed on a per ductule basis. Both mitotic figures and apoptotic bodies were seen only rarely in the lactating breast.

B. Immunocytochemistry

Alpha-lactalbumin. Early in pregnancy (6–12 weeks), alpha-lactalbumin (LA) staining was noted in very few scattered epithelial cells of a few lobules as a predominantly diffuse cytoplasmic pattern (Fig. 5). Although it was present in most lobules at 13–20 weeks the milk protein was localised to relatively few cells and many ductules were negative; staining was often seen both diffusely in the cytoplasm and towards the luminal border of the epithelium (Fig. 6). From week 21 of pregnancy, positivity of staining was greater with many cells showing reactivity. This was usually in the apical region of the epithelium, where it was frequently of a granular pattern, and in luminal contents (Fig. 7). The intensity and location of staining for LA was similar from 21 up to 32 weeks of pregnancy. In the lactating breast, localisation of LA resembled late pregnancy with the cytoplasm and luminal contents (where present) showing intense staining. Non-lactational lobules within lactating breast tissue showed only sporadic cytoplasmic reactivity for alpha-lactalbumin in a few epithelial cells, similar to weeks 6–12 of pregnancy.

Secretory component of immunoglobulin A. In early pregnancy (6–12 weeks) the secretory component

Fig. 5. Alpha-lactalbumin is localised to cytoplasm of occasional epithelial cells in six week pregnant breast tissue. Peroxidase-anti-peroxidase method. Haematoxylin counterstain × 160

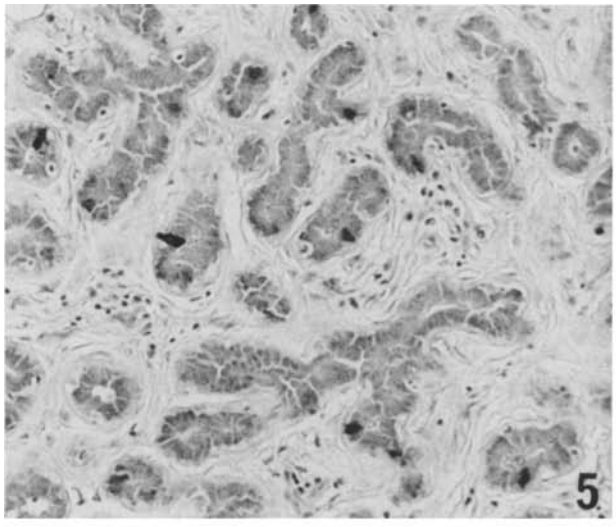
Fig. 6. Sporadic staining for alpha-lactalbumin is seen in the cytoplasm and lumina of the epithelium of 13 week pregnant breast. Peroxidase-anti-peroxidase method. Haematoxylin counterstain × 160

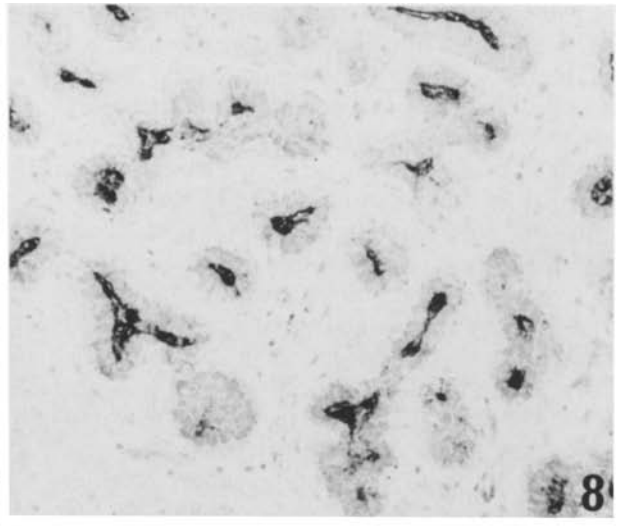
Fig. 7. 30 week pregnant breast tissue shows typical intense alpha-lactalbumin localisation in apical cytoplasm and lumina. Peroxidase-anti-peroxidase method. Haematoxylin counterstain × 160

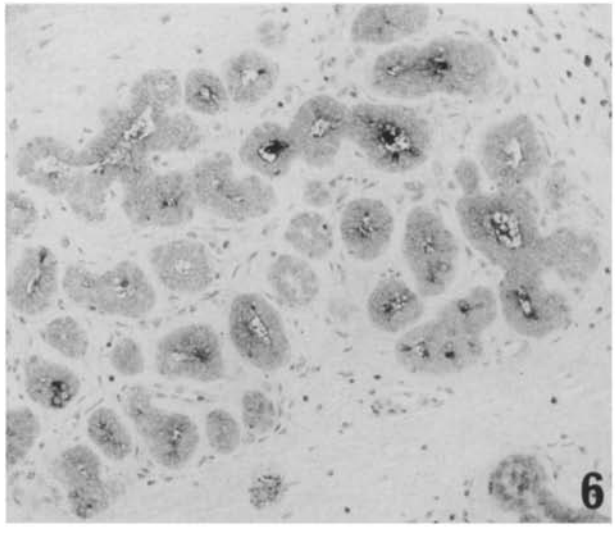
Fig. 8. Luminal staining for the secretory component of IgA is seen in the six week pregnant breast. Peroxidase-anti-peroxidase method. Haematoxylin counterstain × 160

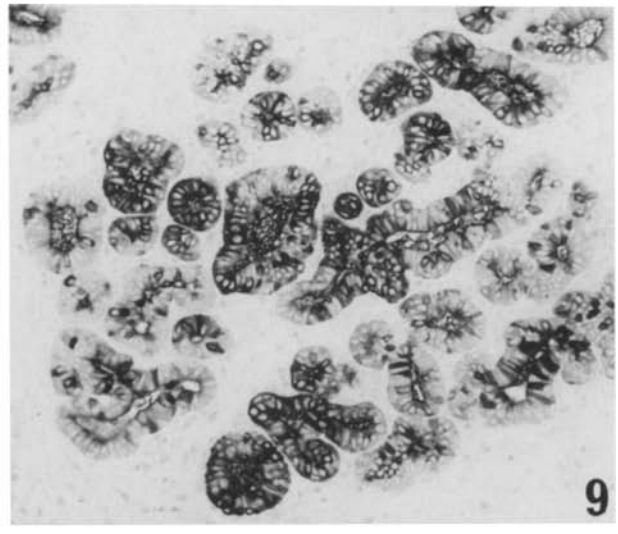
Fig. 9. Breast tissue at 17 weeks of pregnancy. Variable cytoplasmic expression of secretory component within lobule gives a mosaic staining pattern. Compare with staining for alpha-lactalbumin at similar stage of gestation (Fig. 6). Peroxidase-anti-peroxidase method. Haematoxylin counterstain × 160

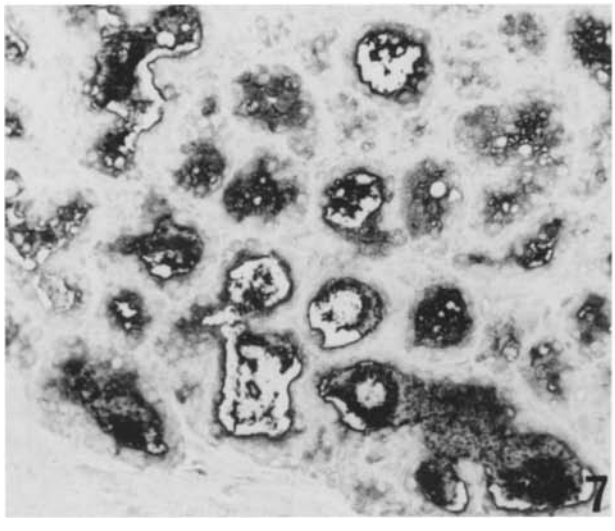
Fig. 10. Predominantly luminal localisation of secretory component is present in lactating breast. Peroxidase-anti-peroxidase method. Haematoxylin counterstain × 160

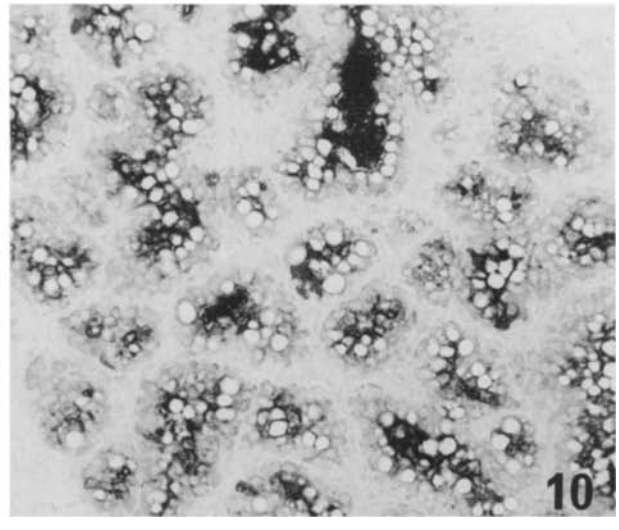

5


8


6


9


7


10

of IgA (SC) was localised to a thin rim at the luminal border and to the luminal contents of most breast ductules (Fig. 8). However in one sample (8 weeks) the staining pattern was similar to that seen at 13–20 weeks of pregnancy. Here there was intense apical and general cytoplasmic staining in the majority of ductules in addition to the luminal contents (Fig. 9). This pattern was also particularly noted in the intralobular terminal ducts. Generally, it was prominent that only a proportion of cells in any ductule were stained, giving a mosaic pattern. From week 21 of pregnancy and in lactation, secretory component was localised to the apical aspect of the epithelium and to the luminal contents (Fig. 10). Although most cells showed reactivity, it was often of lower intensity than the staining seen during the first half of gestation.

Discussion

This study has clearly demonstrated a distinct biphasic pattern in the proliferative response of the breast during pregnancy. The first half is characterised by a constant, high rate, with a fall in proliferation in the second half at the time morphological and functional differentiation is expressed. In view of the limited data available, it is important to consider the particular relationship of these features of proliferative activity and functional differentiation in the human.

The levels of proliferation are comparable with those reported previously for 7 (4.45%) and 18 (6.20%) week pregnant human tissue (Meyer 1977), but contrast with the variable activity in the normal "resting" premenopausal breast, which has reported ranges of 0–8.84% (Meyer and Connor 1982) and 0.04–5.69% (Going et al. 1988). Several factors contribute to this variability, but the highest values are characteristic of the late "luteal" phase. That similar levels are sustained throughout early pregnancy is consistent with a stable proportion of the cell population being active in the growth cycle. Moreover, the reduction by 50% in the TLI values during the second half of pregnancy (Table 1) indicates a change in this stability. From a theoretical point of view the three most likely ways of achieving this are through (I) alteration in the length of the S phase of the cell cycle, (II) increase in the length of the most variable phase of the cycle, G1, or, (III) a decrease in the number of cells in the proliferating compartment (Pardee et al. 1985; Calaf et al. 1986). The present study has no direct evidence on any of these aspects, but the evidence of secretory differentiation (see later) is in favour of either (II) or (III), or both.

The proliferative response measured by mitosis was more variable, as noted previously (Ferguson and Anderson 1983). This is consistent with the comment by Meyer (1984) of reduced accuracy of mitotic index compared with thymidine labelling as a measure of proliferation. Nevertheless, a major benefit of morphological assessment is that it also allows evaluation of deletional cell death (apoptosis). This study has confirmed a previous report (Ferguson and Anderson 1983) of a high frequency of apoptosis during pregnancy. The occurrence of this event is perhaps surprising in view of the major increase in cell number required. However the factors initiating apoptosis and its significance may be different from the resting breast, where it has been suggested that a balance is maintained between cellular proliferation and cell death, with apoptotic cells frequently derived from recently mitotic cells (Anderson et al. 1982; Mukherji 1983). In pregnancy, as in many developmental situations (Wyllie et al. 1980), apoptosis is more likely to be involved in tissue shaping and may therefore be required for orderly development and expansion of the lobular units.

The most notable feature of secretory activity during pregnancy was the contrast between the staining patterns for LA and SC. SC was found more uniformly and intensely in the first trimester. The positive relationship to proliferation, seen previously in the resting breast (Going et al. 1988) was accentuated. It must be noted that SC is also present in a range of non-breast epithelial tissues, where it acts as a surface receptor for dimeric IgA (Brantzaeg 1974; Kuhn and Kraehenbuhl 1979). It is thus not a measure of breast differentiation but is rather an indicator of the generally increased synthetic activity of the epithelium during pregnancy. In contrast LA reactivity was similar to previous descriptions (Bailey et al. 1982; Clayton et al. 1982) and localisation of expression throughout the first trimester was at a low level similar to the resting breast (Going et al. 1988). The increase in detection thereafter closely correlated with the appearance of morphological differentiation of the breast, which is pronounced only late in the second trimester (Ferguson and Anderson 1983). This is consistent with its function in milk production as a "specifier" protein for the lactose synthetase system (Brew et al. 1968). The functional differentiation occurs at the same time as diminished proliferation, for which a prolonged G1 phase or a decreased growth fraction seem the most plausible explanations.

When considering the factors responsible for breast stimulation it is important to emphasize that

the shift from a proliferating to a differentiated state does not occur until pregnancy is well advanced. In respect of the humoral factors promoting mitosis and differentiation, the steroid hormones, oestradiol and progesterone are generally considered to stimulate the former, with a variable requirement also for insulin, thyroxine, hydrocortisone and placental lactogen (McManus and Welsch 1984; Calaf et al. 1986). Differentiation is promoted by prolactin (Wilson et al. 1980), with some competitive blocking activities attributed to progesterone and oestrogens (Tyson et al. 1975). In support of this the appearance of LA is coincident with the major rise in serum levels of prolactin after the twentieth week (Tyson et al. 1972; Rigg et al. 1977), although it must be noted that the drop in proliferation at that time contrasts with the continuously rising levels of the steroid hormones (Tulchinsky et al. 1972). Despite these informative observations, the difficulties encountered in inducing pregnancy like growth of human breast tissue in vitro (McManus and Welsch 1984) encourages re-examination of the various factors promoting modulation. It is now becoming evident that the different stimulant factors do not operate independently in the full expression of the mammary phenotype. Indeed, some authors have suggested that epidermal growth factor (EGF) is crucial for this expression (Tonelli and Sorof 1980; Imagawa et al. 1986; Stewart et al. 1987), although it must be noted that alterations in serum levels of EGF during pregnancy have not been consistently reported (Ances 1972; Hirata et al. 1980). Enthusiasm for a single factor playing the predominant role would therefore appear to be unjustified. Indeed, the present finding of actively proliferating, morphologically "resting" lobules in fully lactating breast tissue suggests that systemic hormones are not the only factors regulating the functional activity of the mammary gland and that autocrine and paracrine regulation of growth (Oka and Yoshimura 1986) must also be considered.

Acknowledgements. The continued co-operation of the Clinical Staff of the Surgical Unit, Longmore Hospital is gratefully acknowledged. We thank Dr. J.J. Going for his assistance with some of the incubation procedures and Mrs L. Ferrigan for technical help. This study was funded by grants from the Medical Research Council and the Imperial Cancer Research Fund.

References

Ances IG (1972) Serum concentrations of epidermal growth factor in human pregnancy. Am J Obstet Gynecol 115:357–362

Anderson TJ, Ferguson DJP, Raab GM (1982) Cell turnover in the 'resting' human breast: the influence of parity, contraceptive pill, age and laterality. Br J Cancer 46:376–382

Bailey AJ, Sloane JP, Trickey BS, Ormerod MG (1982) An immunocytochemical study of alphalactalbumin in human breast tissue. J Pathol 137:12–23

Brantzaeg P (1974) Mucosal and glandular distribution of immunoglobulin components: Differential localization of free and bound SC in secretory epithelial cells. J Immunol 112:1553–1559

Brew K, Vanaman TC, Hill RL (1968) The role of alpha-lactalbumin and the A protein in lactose synthetase: a unique mechanism for the control of a biological reaction. Proc Natl Acad Sci USA 59:491–497

Calaf G, Russo IH, Roi LD, Russo J (1986) Effects of peptides and steriod hormones on cell kinetic parameters of normal human breast tissue in organ culture. In Vitro Cell Dev Biol 3:135–140

Carson FL, Martin JH, Lynn JA (1973) Formalin fixation for electron microscopy. A re-evaluation. Am J Clin Pathol 59:365–373

Clayton F, Ordonez NG, Hanssen GM, Hanssen H (1982) Immunoperoxidase localization of lactalbumin in malignant breast neoplasms. Arch Pathol Lab Med 106:268–270

Ferguson DJP, Anderson TJ (1981) Morphological evaluation of cell turnover in relation to the menstrual cycle in the 'resting' human breast. Br J Cancer 44:177–181

Ferguson DJP, Anderson TJ (1983) A morphological study of the changes which occur during pregnancy in the human breast. Virchows Arch (Pathol Anat) 401:163–175

Going JJ, Anderson TJ, Battersby S, MacIntyre C (1988) Proliferation and secretory activity in human breast during natural and artificial menstrual cycles. Am J Pathol 130:193–204

Hirata Y, Moore GW, Bertagna C, Orth DN (1980) Plasma concentrations of immunoreactive human epidermal growth factor (Urogastrone) in man. J Clin Endoc Metab 50:440–444

Imagawa W, Tomooka Y, Hamamoto S, Nandi S (1985) Stimulation of mammary epithelial cell growth in vitro: interaction of epidermal growth factor and mammogenic hormones. Endocrinology 116:1514–1524

Kuhn LC, Kraehenbuhl J-P (1979) Role of secretory component, a secreted glycoprotein, in the specific uptake of IgA dimer by epithelial cells. J Biol Chem 254:11072–11081

McManus MJ, Welsch CW (1984) The effect of estrogen, progesterone, thyroxine and human placental lactogen on DNA synthesis of human breast ductal epithelium maintained in athymic nude mice. Cancer 54:1920–1927

Meyer JS (1977) Cell proliferation in normal human breast ducts, fibroadenomas and other ductal hyperplasias measured by nuclear labelling with tritiated thymidine. Hum Pathol 8:67–81

Meyer JS (1984) Practical breast carcinoma cell kinetics: review and update. Breast Cancer Res Treatment 4:79–88

Meyer JS, Connor RE (1982) Cell proliferation in fibrocystic disease and postmenopausal breast ducts measured by thymidine labeling. Cancer 50:746–751

Mukherji MJ (1983) The relationship between mitosis and apoptosis in the normal 'resting' human breast. B.Sc. Hons Thesis. University of Edinburgh

Oka T, Yoshimura M (1986) Paracrine regulation of mammary gland growth. Clin Endoc Metab 15:79–97

Pardee A, Campisi J, Gray HE, Dean M, Sonenshein G (1985) Cellular oncogenes, growth factors and cellular growth control. In: Ford RJ, Maizel AL (eds) Mediators in cell growth and differentiation. Raven Press, New York pp 21–29

Rigg LA, Lein A, Yen SSC (1977) Pattern of increase in circulating prolactin levels during human lactation. Am J Obstet Gynecol 129:454–456

Salazar H, Tobon H, Josimovich JB (1975) Developmental ges-

tational and postgestational modifications of the human breast. Clinical Obstet Gynecol 18:113–137

Short RV, Drife JO (1977) The aetiology of mammary cancer in man and animals. Symp Zool Soc Lond 41:211–230

Sternberger LA (1986) Immunocytochemistry. John Wiley & Sons, New York, 3rd Ed, p 90

Stewart HJ, Edwards PE, Foster V, Perusinghe N, O'Hara MJ, Gusterson BA (1987) Maintenance of normal human breast organoids within rat mammary fat pads in organ culture. Virchows Arch A (Pathol Anat) 410:495–500

Tonelli QJ, Sorof S (1980) Epidermal growth factor requirement for development of cultured mammary gland. Nature 285:250–252

Tulchinsky D, Hobel CJ, Yeager E, Marshall JR (1972) Plasma estrone, estradiol, estriol, progesterone and 17-hydroxyprogesterone in human pregnancy. Am J Obstet Gynecol 122:1095–1100

Tyson JE, Hwang P, Guyda H, Friesen HG (1972) Studies of prolactin secretion in human pregnancy. Am J Obstet Gynecol 113:14–20

Tyson JE, Khojandi M, Huth J, Andreassen B (1975) The influence of prolactin secretion on human lactation. J Clin Endocrinol Metab 40:764–773

Wilson GD, Woods KL, Walker RA, Howell A (1980) Effect of prolactin on lactalbumin production by normal and malignant human breast tissue in organ culture. Cancer Res 40:486–489

Wyllie AH, Kerr JFR, Currie AR (1980) Cell death: the significance of apoptosis. Int Rev Cytol 68:251–306

Accepted April 18, 1988