effectiveness of preventive measures, such as immunisation. Lambert showed that trends in the notifications of meningococcal infection corresponded well with those in hospital admission and in mortality data.⁴ Our data were insufficient to assess this correlation accurately. Nevertheless, they expose clearly the fallacy of concluding from the annual number of deaths from meningococcal infection and the number of notifications that "one patient in four still dies" from this illness.⁵

The third important use of notifications is to study the epidemiology, spread, and relative importance of different infections. The results of such studies will be misleading if notified cases are unrepresentative. In this study, 66% of notified cases of bacterial meningitis were meningococcal compared with 34% of all cases identified (table I); 30% of notified cases had been admitted to infectious disease hospitals compared with only 8% of all cases (table II); 3% of notified cases were in neonates compared with 10% of all cases (table III); and the case fatality rate was only 6% for notified cases compared with 11% for all cases (table IV). There were no notifications for children who died outside hospital. These findings suggest the possibility of serious bias in studies based solely on cases identified from notifications.

This situation, in which notifications of an important and dangerous group of infections such as bacterial meningitis are seriously deficient, is clearly unsatisfactory. It has been suggested that reports from microbiological laboratories would provide a more reliable source of surveillance data than clinical notification.⁶ But microbiological confirmation is unusual in some illnesses (such as measles) and variable in others (such as meningitis) and should not be the sole criterion for reporting the diagnosis. Responsibility for statutory notification of clinical disease probably rests most appropriately with the clinicians.⁷ Laboratory reports, however, may often provide com-

plementary data by identifying unnotified cases and carriers and add invaluable information by monitoring the types and properties of organisms. The most appropriate source of surveillance data varies with the nature of the infection, the purpose to be achieved by reporting it, and the urgency with which information is required. The requirements for each disease (or organism) should be defined and reviewed periodically.

The essential lesson of our findings is that infectious-disease surveillance should not be dependent on one source of information alone. Data from all available sources—especially statutory notifications and microbiology laboratories—need to be exchanged and integrated routinely at both local and national level to obtain a complete and accurate description of the facts.

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Immunoglobulin synthesis in the "resting" breast

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Summary

Nulliparous women have a greater risk of developing breast cancer than women who have borne children, but so far no functional differences in breast tissue have been reported between parous women and nulliparae. Macroscopically and histologically normal breast tissue was obtained from 74 women of reproductive age during biopsy of benign breast lesions and was examined for the

presence of plasma cells by immunofluorescence. Immunoglobulin synthesis was detected by an in-vitro culture technique.

Synthesis of IgA was detected in 81% of specimens, of IgG in 45%, and of IgM in 3%. IgA synthesis was much more intense than IgG or IgM synthesis. Plasma cells containing IgA were seen in 71% of the specimens examined, and 88% of specimens had deposits of IgA in the ductules. The findings were not significantly influenced by the nature of the condition necessitating biopsy or by oral contraception. Nulliparous women showed no cyclical changes, but among parous women IgA synthesis was more intense during the luteal phase of the menstrual cycle. This suggests that after the first pregnancy the breast is more sensitive to progesterone.

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Introduction

The term "resting breast" is often applied to the human mammary gland that is not secreting milk, implying that the gland is metabolically inactive. Small amounts of secretion can be aspirated from at least 75% of non-lactating breasts,¹ however,

and changes in breast volume during the menstrual cycle suggest that the non-lactating breast responds to fluctuating levels of ovarian hormones.²

Although milk-specific proteins and large amounts of immunoglobulin³ are secreted in milk, there is little information about the ability of the non-lactating breast to produce these proteins. The purpose of our study, which was part of a wider investigation of the effects of parity and the menstrual cycle on the breast, was to determine whether the resting breast produces a milk protein (IgA) and whether such synthesis varies with the stage of the menstrual cycle.

We therefore studied immunoglobulin production in the non-lactating human breast using an in-vitro organ culture technique and also examined the distribution of immunoglobulins by immunofluorescence.

Patients and methods

We studied 74 women of reproductive age (mean age 33 years; range 15-52) with regular menstrual cycles. They had been admitted to hospital for diagnostic biopsy of a breast lump or for reduction mammoplasty. Before surgery each patient was invited to participate in the study, and a full reproductive and menstrual history was taken. To ascertain the time of ovulation venous blood was taken at the time of surgery for progesterone assay, and the patient was asked to notify us of her next menstrual period. Patients whose biopsies showed malignant disease of the breast were excluded from the study.

BIOPSY TECHNIQUE

After the clinically abnormal lump was removed a piece of macroscopically normal breast tissue was removed through the same incision from a site as far away as possible from the abnormal area. Reduction mammoplasty specimens were randomly selected from the excised tissue. The tissues were transported in normal saline and prepared for culture and histological examination within one and a half hours. Adjacent blocks were fixed in 4% neutral buffered formaldehyde.

IMMUNOLOGICAL METHODS

In-vitro synthesis of immunoglobulins—Eighty specimens, each weighing 100-150 mg, were cultured for 48 hours in medium containing ¹⁴C-labelled lysine and isoleucine.⁴ The cultures were tested for the presence of newly synthesised immunoglobulins and secretory component by radioimmuno-electrophoresis as described.⁵ The degree of immunoglobulin synthesis was assessed semi-quantitatively by grading the intensity of the autoradiographic image from ± (line barely visible) to +++ (a strong black line on the autoradiograph) (see fig 1). The grading was done independently by two observers.

Immunofluorescent detection of immunoglobulin in tissues—Tissue blocks were processed by the method of Brandtzaeg,⁶ fixed in ethanol at 4°C, and embedded in paraffin. Sections were stained by direct immunofluorescence using fluorescein-conjugated rabbit antisera against human α-, γ-, and μ-chains (Hoechst Pharmaceuticals), whose specificity has been described.⁷ Control experiments were performed by blocking with unconjugated antisera and by using absorbed conjugated antisera. The sections were examined by incident ultraviolet illumination. Sections stained with fluorescent antibody to IgA, IgG, and IgM were graded from ± to +++ both for the number of fluorescent plasma cells and for the number and intensity of fluorescence of non-cellular deposits. A separate assessment was made of deposits within the lumina of ductules, deposits in the intralobular stroma, and those in the extralobular stroma.

HISTOLOGY OF TISSUE STUDIED

Sections cut serially with those used for immunofluorescence were stained with haematoxylin and eosin and examined by two observers, including a pathologist who had no knowledge of the patients. Any specimens in which pathological lesions were found were excluded from the study. Sections from the adjacent formalin-fixed blocks were also examined, and no frank pathological features were seen, though a few showed minor changes (atrophic changes in five speci-

mens, small cysts in two, ductular dilatation in seven, and features of fibrosing adenosis in three). No specimens showed epitheliosis.

HISTOPATHOLOGY OF PRIMARY CONDITION

Patients included in the study had either benign breast lesions (biopsy cases) or no disease (mammoplasty cases). The histological findings on the clinically abnormal tissue are shown in table I. Group 1 consisted of patients in whom the disease would be expected to be confined to the excised lump—for example, fibroadenoma—with the remainder of the breast consisting of normal tissue. In group 2 the diagnosis was fibrosing adenosis, which may not necessarily have been localised to the lump excised, even though the tissue used in our study was histologically normal.

TABLE I—Histopathological diagnosis of breast lesions in patients from whom normal breast tissue was obtained for in-vitro culture

	Diagnosis	No of specimens for in-vitro culture	No of specimens also studied by immunofluorescence
Group 1	Fibroadenoma*	20	8
	Mammoplasty*	10	4
	No abnormal tissue	7	3
	Lipoma	1	1
Group 2	Fibrosing adenosis with or without cyst formation*	42	18
	Total	80	34

*Some patients in these groups provided more than one specimen: 74 patients provided specimens for culture and 31 for immunofluorescence.

STEROID ASSAYS

Plasma progesterone levels were determined by a modification of the radioimmunoassay developed by Neal *et al.*,⁸ and oestradiol-17β levels by a modification of the radioimmunoassay of Cameron *et al.*⁹

Results

IN-VITRO SYNTHESIS OF IMMUNOGLOBULINS

Synthesis of IgA was detected in 81% of the 80 specimens (fig 1) and synthesis of IgG in 45% (table II). IgA synthesis was strong (++ or +++) in 42% of the positive specimens, whereas 94% of the IgG-positive specimens showed only weak or barely detectable synthesis (+ or ±). IgM synthesis was seen in two specimens and was barely detectable in both.

FIG 1—Immunoglobulin synthesis in vitro detected by radioimmuno-electrophoresis. Above: stained immunoelectrophoresis pattern developed with anti-IgA serum showing IgA arc. Carrier is human milk. Below: autoradiograph showing labelling of IgA arc.

Influence of diagnosis—The number of positive specimens and the pattern of synthesis was similar in groups 1 and 2. The proportion of positive specimens was greater in group 2 (see table II), but the difference was not significant by the χ^2 test ($0.5 > P > 0.1$).

Influence of parity and stage of menstrual cycle—Similar comparisons were made between the parous and nulliparous patients, and the proportions of positive specimens (81% and 77% respectively) were not significantly different. There was no significant difference between tissue removed during the luteal phase of the cycle (as shown by a plasma progesterone concentration greater than 3.2 nmol/l (1 ng/ml)),

TABLE II—Synthesis of immunoglobulins by breast tissue cultured in vitro

	No of positive specimens (and % of total)	Mean intensity of labelling of autoradiographs (No of specimens)			
		+++	++	+	±
<i>All specimens (n = 80)</i>					
IgA	65 (81)	9	18	24	14
IgG	36 (45)		2	14	20
IgM	2 (3)				2
<i>Histologically normal tissue from group 1 (n = 38)</i>					
IgA	27 (71)	5	9	10	3
IgG	16 (42)		2	6	8
IgM	1 (3)				1
<i>Histologically normal tissue from group 2 (n = 42)</i>					
IgA	38 (91)	4	9	14	11
IgG	20 (48)			8	12
IgM	1 (2)				1

and the rest of the cycle. When the influence of the stage of the cycle was examined in parous and nulliparous women separately, however, cyclical changes were seen in the parous group but not in the nulliparae (table III). Sixteen parous women showed strong (++ or +++) synthesis in the luteal phase, compared with three in the proliferative phase ($P < 0.05$). Among nulliparae there was no significant difference between the phases of the cycle. When patients in the luteal phase were examined synthesis was significantly greater among parous women than among nulliparae ($P < 0.05$). In neither parous nor nulliparous women did the amount of IgA synthesis correlate with plasma oestradiol levels. There was no significant difference between the groups of parous and nulliparous women in average age, time since menarche, or plasma progesterone or oestradiol levels.

TABLE III—Influence of parity and stage of menstrual cycle on in-vitro synthesis of IgA

	Phase of menstrual cycle	No of positive specimens (and % of total)	Mean intensity of labelling of autoradiographs (No of specimens)			
			+++	++	+	±
Nulliparous patients (n = 22)	Proliferative (n = 12)	11 (92)	2	2	7	
	Luteal (n = 10)	6 (60)		1	4	1
Parous patients (n = 43)	Proliferative (n = 17)	12 (71)	1	2	5	4
	Luteal (n = 26)	23 (89)	6	10	3	4

Influence of oral contraceptives—Thirteen patients were taking oral contraceptives, and IgA synthesis was detected in 12 of them. This proportion (92%) was not significantly different from the overall total.

IMMUNOFLOUORESCENT IDENTIFICATION OF IMMUNOGLOBULIN

IgA—Table IV shows the numbers of specimens that were positive when sections were examined after staining with fluorescent antibody to IgA. Plasma cells were seen in 71% of the sections and the number of cells was moderate or large in 14 of the 24 positive specimens. In all but two of the sections the cells were seen in greater numbers in the intralobular stroma than in the extralobular stroma. Fig 2 shows a section graded as +++ for plasma cells. Deposits of IgA were seen in the lumina of ductules in 88% of the specimens and as non-cellular foci in the intralobular stroma in 53%. In only five specimens (15%) were deposits seen outside the lobules.

FIG 2—Section of breast lobule stained with FITC conjugated anti-IgA showing fluorescing plasma cells. ($\times 1910$.)

TABLE IV—Detection of IgA in 34 tissue specimens by immunofluorescence

	No of positive specimens (and % of total)	Mean intensity of labelling of positive specimens (No of specimens)			
		+++	++	+	±
Plasma cells ..	24 (71)	4	10	5	5
Non-cellular material { Duct lumen ..	30 (88)	6	13	7	4
Lobule ..	18 (53)		2	13	3
Stroma ..	5 (15)	1		2	2

IgG—IgG-containing cells were seen in only three sections. Non-cellular deposits of IgG were seen in 10 sections (29%), and all were weakly fluorescent or barely visible (+ or ±). More deposits of IgG were seen in the extralobular stroma than in the lobules or the lumina of the ductules.

IgM—Fluorescent IgM-containing plasma cells were seen in one specimen and non-cellular deposits of IgM in three. The IgM deposits showed only barely detectable fluorescence.

Comparison of results classified according to parity, stage of cycle, contraceptive use, and diagnosis showed no significant differences between groups in terms of numbers of positive sections or pattern of fluorescence. In those patients in the luteal phase of the cycle, more IgA-positive specimens were again found in the parous group (70%) than among the nulliparae (50%), but the difference was not significant.

The total number of specimens examined by immunofluorescence was limited to 34 because of technical problems or shortage of tissue. Also sections in which no lobular tissue could be identified were excluded. Despite this selection there was good overall agreement in the proportion of specimens found to be positive by the fluorescence and organ culture methods.

REPEATABILITY OF RESULTS

Four specimens were taken from different parts of a single breast at mastectomy and coded as separate specimens. None of the observers knew which specimens came from the same patient. After organ culture IgA synthesis was graded as +++ in two and ++ in the other two; IgG synthesis as ++ in two and + in two; and IgM negative in all four. Immunofluorescence was done on three of these specimens. IgA-containing plasma cells were seen in two and graded as + and ±; non-cellular deposits of IgA in the intralobular stroma were graded as + in all three; and IgA deposits within the lumina of ductules were + in two and ± in one. Neither cells nor deposits of IgA were seen in the extralobular stroma in any of the three specimens. No cells or deposits were seen in the sections stained for IgG or IgM.

Discussion

IgA is the principal immunoglobulin in most human external secretions, including colostrum and milk. IgA in salivary and

intestinal secretions is known to be synthesised locally by subepithelial plasma cells.¹⁰ Many plasma cells are seen in the lactating human breast,³ and this has led to the assumption that the IgA in human milk is also locally synthesised. Local synthesis of immunoglobulin by mammary tissue has been shown in several animal species,^{11,12} but there is only one brief report of such a study in the human breast.¹³

Our observation that the non-lactating breast produces IgA rather than other immunoglobulins suggests that this synthesis is not a non-specific inflammatory response but a process associated with secretion. This interpretation is supported by the localisation of IgA in plasma cells associated with the lobules. Deposits of IgA are also concentrated in the lobules and particularly in the lumina of ductules, whereas IgG-positive cells are randomly distributed throughout the lobules and stroma.

Many of our biopsy specimens were taken from women who had breast nodules and were therefore not truly "normal." Mammoplasty specimens gave similar results, however, and we therefore conclude that IgA synthesis is not a reaction to benign breast disease but a property of normal breast tissue.

The secretion of IgA into the ductules may represent a form of defence against the entry of infection, but it seems more likely that the IgA secretion is simply a basal level of activity in an organ whose primary function is full lactation. In our primitive ancestors the breast was probably lactating almost continuously during the reproductive years,¹⁴ and it is unlikely that mechanisms adapted specifically for the non-lactating state have had time to evolve.

IgA synthesis occurs in both parous and nulliparous patients, but the observation that cyclical changes in IgA synthesis occur only in parous women implies some functional difference in the breast after the first pregnancy. The increase in IgA synthesis in the luteal phase of the cycle among parous women suggests that after the first pregnancy the breast is more sensitive to progesterone. A study of DNA synthesis by human breast epithelium *in vitro*¹⁵ has also shown cyclical variation in tissue from parous women and no cyclical variation among nulliparae.

The nulliparous breast is more susceptible to cancer, and the risk of subsequent breast cancer increases with increasing time elapsed between menarche and first pregnancy.^{16,17} The suggestion in our results that the nulliparous breast is less responsive to progesterone than the parous breast may simply be a reflection of the "immaturity" of the nulliparous breast, but the difference in hormonal sensitivity of breast tissue may have a direct bearing on its susceptibility to malignant change. It has been suggested that "unopposed" oestrogen stimulation

can lead to neoplastic changes¹⁸: if the nulliparous breast is relatively insensitive to progesterone this might mean that oestrogen stimulation during the menstrual cycle is inadequately "opposed" by progesterone.

We have no evidence that oral contraceptives produce abnormal stimulation of breast tissue. Our results suggest that the nulliparous breast will be relatively insensitive to the progestogen component of the combined oral contraceptive; the effect of the oestrogen component remains a matter for conjecture.

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SHORT REPORTS

Simplified ECG monitoring with an electrode mat

After myocardial infarction continuous ECG monitoring allows early recognition of arrhythmias, and impending ventricular fibrillation and asystole.¹ In sick neonates progressive slowing of cardiac rate gives early warning of hypoxia and potential cardiac arrest.² Conventional ECG electrodes take time to apply correctly, tend to become disconnected, and in neonates are prone to cause maceration of the skin. A newly available electrode mat* incorporates three electrodes of thin metal foil on to a single card (34 × 23 cm) and in some circumstances has definite advantages over conventional electrodes. The electrode mats are often used during surgery but have other applications: in casualty, after cardiac arrest, for intensive care, or during transit by ambulance. We report the successful monitoring of ECG and cardiac rate in 26 neonates.

The electrode mat

The mats are disposable, x-ray translucent, require no conductive gel, and can be positioned in seconds with minimum patient disturbance; infants can be laid across the whole electrode. For small neonates (< 1.75 kg) the mat should be trimmed to one-third of its original length and inserted halfway under the infant (see figure), to give direct skin contact with each of the three foil strips. ECG tracings are comparable with those from conventional electrodes and heart rate can be metered audibly. In neonates any material interferes with signal clarity and, in particular, "silver-swadlers" obliterate meaningful recording. The electrode is connected to the monitor by a three contact clamp and single lead, and any mains-powered monitor can be used.

Infants studied

Twenty-six neonates weighing from 770 to 4700 g were monitored, two while in transport incubators. A pneumatic apnoea alarm³ may be used simultaneously if the electrode is placed directly between the infant and the apnoea mattress. With infants weighing < 1.75 kg the apnoea mattress may