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Immunoglobulin-containing cells in pig mammary gland

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INTRODUCTION

The mammary gland of the sow is of great importance to the baby pig both as a source of food and as a source of immunoglobulin. With respect to the latter role it not only provides a rich source of immunoglobulins in colostrum for absorption by the piglet, but also a continuous supply of antibody in milk throughout the suckling period. Milk antibodies are not absorbed by the piglet but play an important role in intestinal defences (Kohler & Bohl, 1966; Wilson & Svendsen, 1971).

Immunoglobulins A, G and M are found in both colostrum and milk. These two body fluids differ greatly, however, in their immunoglobulin content (Porter, 1969; Porter, Noakes & Allen, 1970*a*; Curtis & Bourne, 1971) and in the proportions of the immunoglobulins which are locally secreted or derived from serum (Bourne & Curtis, 1973). As part of a study of the distribution of immunoglobulins and immunoglobulin-containing cells in pig tissues the immunocyte populations in pig mammary gland at different stages of lactation were investigated.

MATERIALS AND METHODS

Preparation of antisera. The isolation of pig IgA, IgG and IgM and the preparation of antisera specific to these immunoglobulins were carried out as described by Bourne (1969) and Curtis & Bourne (1971).

Preparation of peroxidase-conjugates. Specific antisera were conjugated with horseradish peroxidase enzyme (Grade VI, Sigma Chemical Co.) according to the method of Avrameas (1969). Conjugates were stored at 4 °C and diluted to 1 in 40 with phosphate buffered saline before use.

Preparation of tissues for microscopy. Biopsy specimens were fixed in methanol and processed according to the method of Sainte-Marie (1962); 4 μm sections were cut and, after removal of wax, endogenous peroxidase activity was inhibited by the use of sodium nitroferricyanide (Straus, 1971). Incubation with specific labelled antisera and demonstration of peroxidase activity with diaminobenzidene was carried out as described elsewhere (Brown, Bourne & Steel, 1974).

Experimental animals. Biopsies were taken from mammary gland tissues of pregnant Large White pigs. Table 1 gives details of the ages of animals and the stages of gestation or lactation when biopsies were taken; on each occasion tissue was taken from two different glands. Piglets were weaned at 4–5 weeks of age.

Table 1. *Ages of animals and stages of gestation or lactation at the time of mammary biopsy*

		Stage of gestation or lactation at time of biopsy									
		Weeks pre-farrowing				Weeks post-farrowing					Weeks post-weaning
Animal	Age at farrowing (years)	3	2	1		1	2	3	4		1
8050	1	+	+	−		−	−	−	−		−
AHD	1½	−	−	+	Farrowing	+	+	+	+	Weaning	+
J6	2	−	−	−		+	+	−	−		+
AH2	1½	−	−	−		−	+	+	−		+

+, Biopsy obtained.
−, No sample taken.

Table 2. *Changes in immunoglobulin-containing cell populations in pig mammary gland*

	Stage of gestation or lactation										
	Weeks pre-farrowing				Weeks post-farrowing						Weeks post-weaning
Immuno-globulin class	3	2	1		1	2	3	4			1
IgA	+/−	+	+		++	+++	++++	++++			+
IgM	+/−	+/−	+	Farrowing	+	++	+++	+++	Weaning		+
IgG	+/−	+/−	+/−		+	+/+	+++	+++			+

+/−, Cells present, but in small numbers only.
+, Cells present, in groups of up to 5 cells.
++, Cells present, in groups of up to 10 cells.
+++, Cells present, in groups of up to 15 cells.
++++, Cells present, in groups of up to or greater than 15 cells.

RESULTS

The results presented represent combined observations from each of the samples examined. A synopsis of the quantitative changes is shown in Table 2.

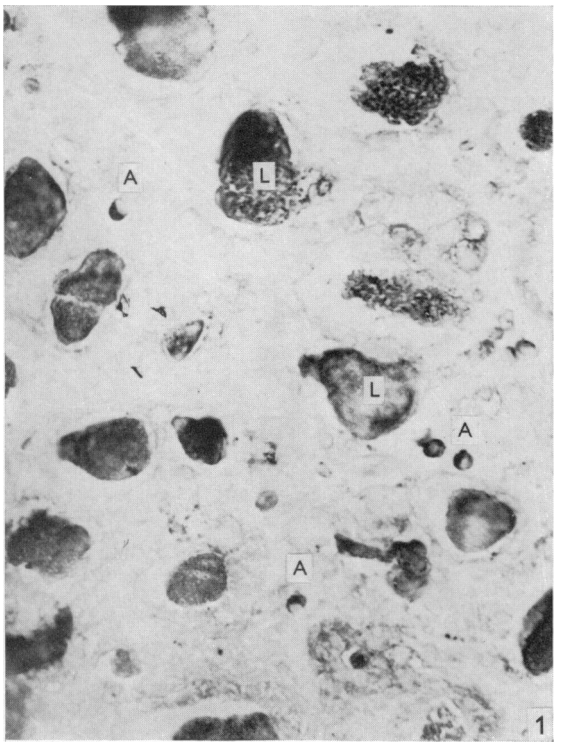
Pre-farrowing (Fig. 1). Specific cell staining was demonstrated three weeks before farrowing. Immunoglobulin-containing cells were distributed throughout the tissue and occurred individually or in small groups, although many areas contained very

Fig. 1. Mammary gland biopsy 1 week before farrowing. IgA staining is present in the cytoplasm of plasma cells (A) and in luminal contents (L).

Fig. 2. Mammary gland biopsy 2 weeks after farrowing. IgA staining is present in the cytoplasm of several groups of plasma cells (A) and in the luminal contents (L) of a different area of the same section.

Fig. 3. Mammary gland biopsy 2 weeks after farrowing. IgG staining is present in the cytoplasm of a small number of plasma cells (G).

Fig. 4. Mammary gland biopsy 2 weeks after farrowing. IgM staining is present in the cytoplasm of a small group of plasma cells (M).


A
L
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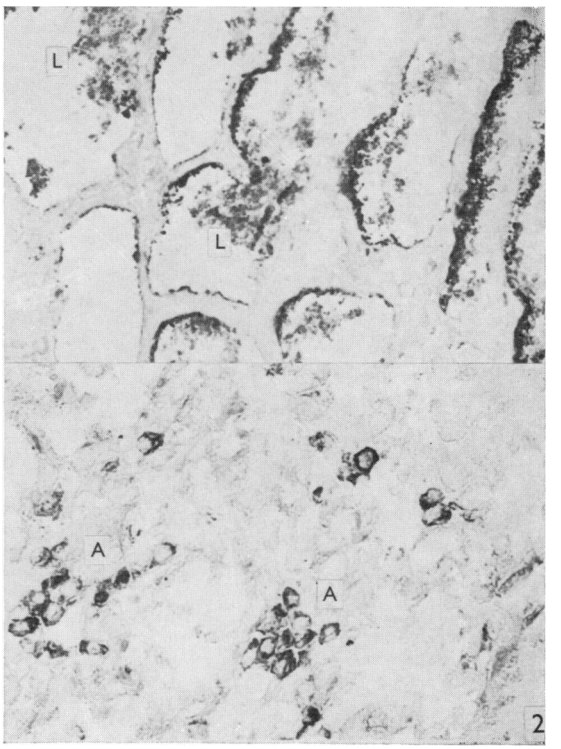
1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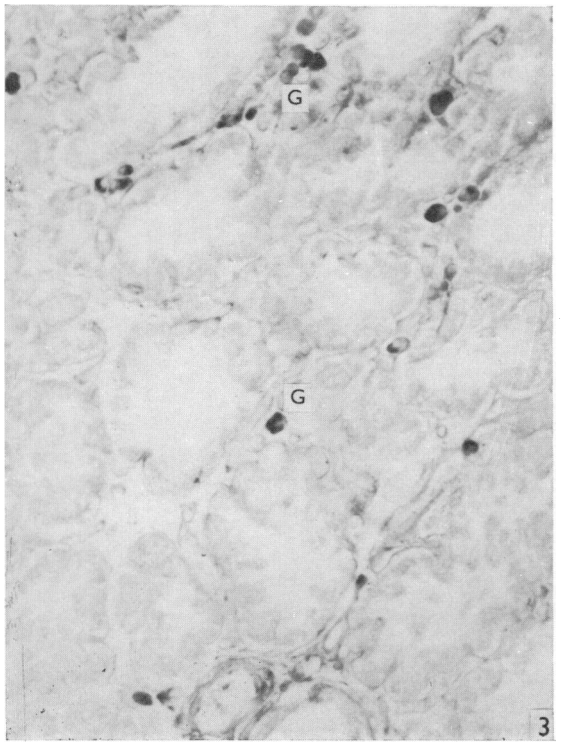
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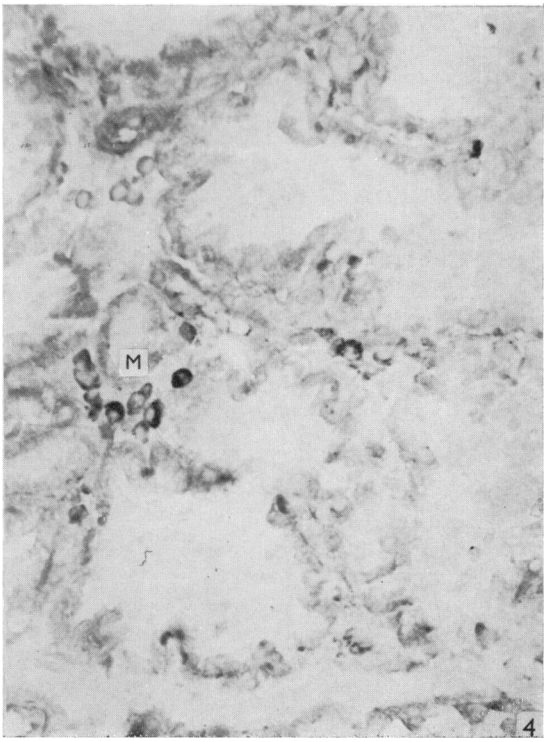
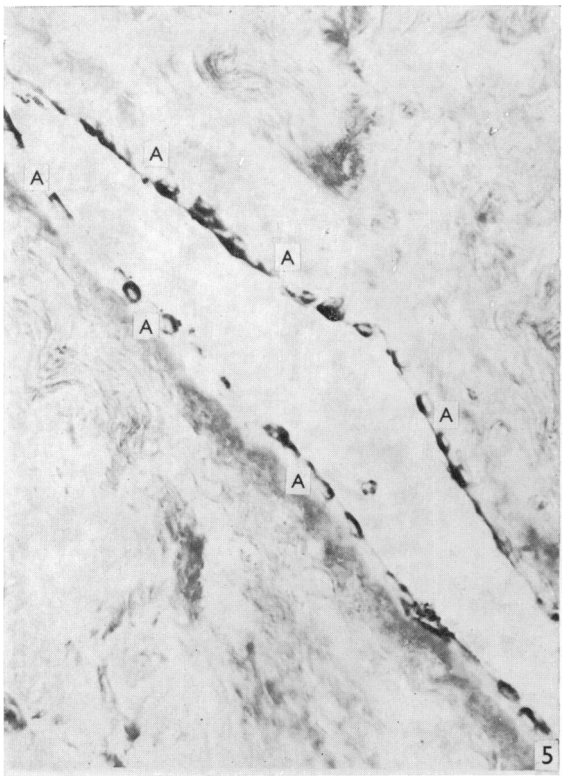
G
3


M
4



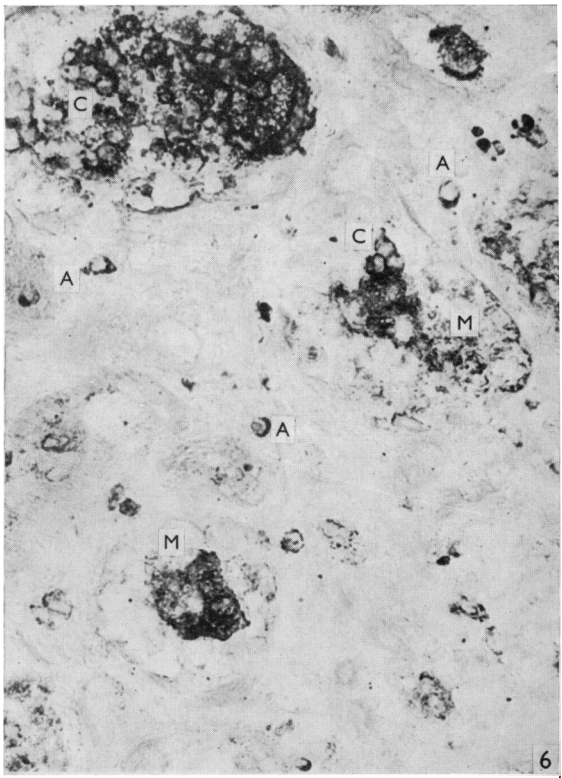


Fig. 5. Mammary gland biopsy 2 weeks after farrowing. IgA staining is present in the cytoplasm of some cuboidal epithelial cells (A), particularly towards the lumen of the duct.

Fig. 6. Mammary gland biopsy 1 week after weaning. IgA staining occurs in the cytoplasm of plasma cells (A) and in the luminal contents; the latter includes finely granular material (M) and some structures with a cellular appearance (C).

few stained cells. The numbers of cells and the extent of cytoplasmic staining increased in the weeks prior to parturition. At all stages IgA cells were more numerous than IgM or IgG cells, the latter occurring in only small numbers. In the week prior to parturition IgA cells occurred in groups of up to five cells.

Staining of the luminal contents of the gland occurred with IgA, IgG and IgM antisera. In some cases the homogeneous or finely granular secretion appeared to be condensed around the periphery of the lumen, closely associated with the glandular epithelium.

Post-farrowing (Figs. 2–4). At the end of the first week of lactation the mammary gland contained large numbers of plasma cells of all three classes, and these increased as lactation progressed. Plasma cells were found in the connective tissue spaces between and within the gland lobules, and frequently occurred in groups. The sizes of these groups varied at different stages of lactation and with the classes of immunoglobulin, but at all stages IgA cells were more numerous than IgG or IgM cells. Thus, 2 weeks after farrowing, IgA cells were present in groups of up to 10 while

IgG and IgM cells were present in smaller groups of up to 5 cells. At 4 weeks post-farrowing, groups of IgG and IgM cells had increased to about 10, while IgA cells were present in groups of 15 or more.

IgA and IgM cells appeared to be fairly evenly distributed throughout a given section, occurring in both interlobular and intralobular connective tissue, although there were some areas which had few stained cells. IgG cell groups were not so evenly distributed and were most obvious in the interlobular rather than intralobular connective tissue; they were also more difficult to identify than IgA or IgM cell groups because of interstitial staining, which probably reflects intravascular fluid IgG.

Staining of the luminal contents of the glands was seen for IgA, IgG and IgM, although the extent and intensity varied considerably in different parts of a section. In many cases, staining of secretion was concentrated close to the lining epithelium, and occasionally staining associated with epithelial cells could be definitely identified. This was seen in the cuboidal cells lining ducts, rather than the alveoli themselves (Fig. 5); while it was most obvious with IgA antiserum, it was seen also with IgM, but never with IgG antiserum.

Post-weaning (Fig. 6). One week after weaning the numbers of stained cells of all immunoglobulin classes were decreased; in particular they did not occur in the large groups previously seen. Staining of the luminal contents included both a finely granular material, and other structures which were rounded with a peripheral rim of staining, having an appearance similar to that of the plasma cells remaining in the rest of the tissue.

DISCUSSION

Earlier studies of sow mammary glands have been carried out using either histological (Cross, Goodwin & Silver, 1958) or immunofluorescent techniques (Porter *et al.* 1970*a*). The latter authors detected staining of a few IgA-containing cells in the septa of the pre-partum pig as well as staining of colostrum, and cells present in thin sheets around the globules of colostrum. IgG was reported to be generally present throughout the glandular and interlobular connective tissue, and a similar distribution of IgG staining was reported in lactating animals. No IgA was detected in the interlobular septa of the lactating animals, although the luminal contents of the ducts were reported to be strongly stained. These investigations, however, did not demonstrate the changes in the immunocyte populations of the three classes over the period of lactation.

In the present study the presence of IgA cells, but fewer IgM and IgG cells, in the pre-farrowing animal would support the finding of Bourne & Curtis (1973) that nearly all colostral IgG, and a high proportion of colostral IgM, are derived from serum; whereas about 60 % of colostral IgA which represents only about 20 % of the total immunoglobulin content of colostrum, is formed locally. The changes in cell populations during lactation reported here also emphasize the importance of IgA as the major immunoglobulin in milk, and support the concept of local antibody production. IgA cells were at all times more numerous than either IgG or IgM cells; it was, however, less easy to distinguish great differences between IgG and IgM cell populations. Although IgM is present in milk at lower levels than IgG (18 % and 20–30 % respectively of the total immunoglobulin content) 90 % of the milk IgM

is produced locally, while only about 70 % of milk IgG is locally produced (Bourne & Curtis, 1973).

It is particularly interesting that immunoglobulin-containing cell populations were found in the mammary glands of pre-farrowing gilts and this raises the question of the source of these antibody-forming cells. It is unlikely that they would have developed in response to local antigenic challenge via the teat canal. A more likely explanation is that they developed in response to antigenic challenge elsewhere, either as a result of antigen drainage to the mammary gland, or from localization in the mammary gland of stimulated cells arising in other sites. It has been shown in sows that specific antibody is produced in milk following parenteral injection by either the intramuscular or intramammary route (Bourne, 1973). In addition it has recently been shown that oral immunization results in an antibody response in the mammary gland (Bohl *et al.* 1973). Whatever the mechanism, it is obviously important that a mammary gland plasma cell population is present to allow for the rapid changes which occur shortly after parturition, in the transition from colostrum to milk secretion. Furthermore, a possible link between the intestinal tract and mammary gland immune systems would be particularly advantageous to the suckling piglet since it is dependent upon milk as a source of intestinal antibody in its early life.

IgA in milk and colostrum occurs principally in two molecular forms, namely a 9 S and an 11 S molecule (Porter, 1973; Bourne, 1974). The 11 S molecule seems to represent a 9 S dimer with an additional glycopeptide which has been termed 'secretory component', and for which a number of possible functions has been suggested. Recently it has been identified both free and bound to IgA in epithelial cells (Brandtzaeg, 1974; Poger & Lamm, 1974) and has been further implicated in transport of IgA and IgM across epithelial surfaces. Intracellular staining for IgA and IgM, but not for IgG, has been identified in pig intestinal tissues (Brown, Bradley & Bourne, in preparation), suggesting a similar transcellular secretory pathway at all levels of the intestinal tract. It is not known whether the same cells are involved in transport of both IgA and IgM, nor is it known what role may be played by 'secretory component' in this process. Bradley (1974) has demonstrated IgA and IgM staining in serous gland epithelial cells in the nasal and tracheobronchial mucosa of the pig, and in the present study some evidence was obtained for epithelial cell staining with IgA and IgM in the mammary gland, which might suggest a similar transcellular secretory route in these organs. No evidence could be found for a secretory pathway for IgG in spite of the relatively higher proportion of IgG in milk than in other secretions, such as intestinal juice (Bourne, Pickup & Honour, 1971; Porter, Noakes & Allen, 1970*b*).

SUMMARY

Peroxidase-labelled antisera to pig immunoglobulins A, G and M were used to study immunoglobulin-containing cell populations in pig mammary gland at different stages of gestation and lactation. Immunoglobulin-containing cells of all three classes were present several weeks before farrowing; IgA-containing cells were most numerous at all stages of lactation.

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REFERENCES

AVRAMEAS, S. (1969). Coupling of enzymes to proteins with glutaraldehyde. Use of the conjugates for the detection of antigens and antibodies. *Immunochemistry* **6**, 43–52.

BOHL, E. H., SAIF, L. J., GUPTA, R. K. P. & FREDERICK, G. T. (1973). Secretory antibodies in milk of swine against transmissible gastroenteritis virus. *Advances in Experimental Medicine and Biology* **45**, 337–342.

BOURNE, F. J. (1969). IgA from porcine serum. *Biochimica et biophysica acta* **181**, 485–487.

BOURNE, F. J. (1973). The immunoglobulin system of the suckling pig. *Proceedings of the Nutrition Society* **32**, 205–215.

BOURNE, F. J. (1974). Structural features of pig IgA. *Immunological Communications* **3**, 157–173.

BOURNE, F. J. & CURTIS, J. (1973). The transfer of immunoglobulins IgG, IgA and IgM from serum to colostrum and milk in the sow. *Immunology* **24**, 157–162.

BOURNE, F. J., PICKUP, J. & HONOUR, J. W. (1971). Intestinal immunoglobulins in the pig. *Biochimica et biophysica acta* **229**, 18–25.

BRADLEY, P. A. (1974). The development of the respiratory tract immune system in the pig and the calf. A morphological study. M.Sc. Thesis, University of Bristol.

BRANDTZAEG, P. (1974). Mucosal and glandular distribution of immunoglobulin components: differential localisation of free and bound SC in secretory epithelial cells. *Journal of Immunology* **112**, 1553–1559.

BROWN, P. J., BOURNE, F. J. & STEEL, M. J. (1974). Immunoperoxidase and immunofluorescence techniques in pig tissues. *Histochemistry* **40**, 343–348.

CROSS, B. A., GOODWIN, R. F. W. & SILVER, I. A. (1958). A histological and functional study of the mammary gland in normal and agalactic sows. *Journal of Endocrinology* **17**, 63–74.

CURTIS, J. & BOURNE, F. J. (1971). Immunoglobulin quantitation in sow serum. colostrum and milk and the serum of young pigs. *Biochimica et biophysica acta* **236**, 319–332.

KOHLER, E. M. & BOHL, E. H. (1966). Studies on *Escherichia coli* in gnotobiotic pigs. III. Evaluation of orally administered specific antiserum. *Canadian Journal of Comparative Medicine and Veterinary Science* **30**, 233–237.

POGER, M. E. & LAMM, M. E. (1974). Localisation of free and bound secretory component in human intestinal epithelial cells. A model for the assembly of secretory IgA. *Journal of Experimental Medicine* **139**, 629–642.

PORTER, P. (1969). Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the parturient sow and their absorption by the neonatal piglet. *Biochimica et biophysica acta* **181**, 381–392.

PORTER, P. (1973). Studies of porcine secretory IgA and its component chains in relation to intestinal absorption of colostral immunoglobulins by the neonatal pig. *Immunology* **24**, 163–176.

PORTER, P., NOAKES, D. E. & ALLEN, D. W. (1970*a*). Secretory IgA and antibodies to *Escherichia coli* in porcine colostrum and milk and their significance in the alimentary tract of the young pig. *Immunology* **18**, 245–257.

PORTER, P., NOAKES, D. E. & ALLEN, D. W. (1970*b*). Intestinal secretion of immunoglobulins and antibodies to *Escherichia coli* in the pig. *Immunology* **18**, 909–920.

SAINTE-MARIE, G. (1962). A paraffin embedding technique for studies employing immunofluorescence. *Journal of Histochemistry and Cytochemistry* **10**, 250–256.

STRAUS, W. (1971). Inhibition of peroxidase by methanol and by methanol-nitroferricyanide for use in immunoperoxidase procedures. *Journal of Histochemistry and Cytochemistry* **19**, 682–688.

WILSON, M. R. & SVENDSEN, J. (1971). Immunity to *Escherichia coli* in pigs. The role of milk in protective immunity to *E. coli* enteritis. *Canadian Journal of Comparative Medicine* **35**, 239–243.