SYMPOSIUM ON
'NUTRITION OF THE YOUNG FARM ANIMAL'

The immunoglobulin system of the suckling pig

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The new-born pig is devoid of immunoglobulins, relying on colostrum as the sole source for serum antibody and depending on milk for its intestinal antibody during most of the post-natal period. It will be shown that colostrum and milk are well adapted to perform their very different immune functions.

Pig immunoglobulins

The pig immunoglobulin system has now been well characterized and has been the subject of two recent reviews (Bourne, 1971*a*; Porter & Allen, 1972). Three immunoglobulin classes are recognized which on the basis of their physicochemical properties and antigenic relationships may be regarded as analogous to human IgG, IgM and IgA. Some of the properties of pig immunoglobulins are shown in Table 1 (Bourne, 1971*a*).

Immunoglobulin IgG is quantitatively the most important in pig serum and colostrum; two main sub-classes exist—IgG_1 and IgG_2. Both sub-classes have a sedimentation coefficient (s) of 7. Immunoglobulin IgM occurs as a macroglobulin and, like the corresponding immunoglobulin in other species, possesses an s value of 19 s.

The pig IgA system has been shown to be similar to that present in other species and it has been suggested that the main biological role of IgA is associated with local defence of mucosal surfaces (Tomasi, 1967).

Table 1. *Some properties of porcine immunoglobulins*

	Sedimentation coefficient	Nitrogen	Carbohydrate (μmol/mg)							Extinction coefficient (E)
			Fuc	Man	Gal	Glc	Glc NAc	Gal NAc	SA	
Serum IgG_1	7 s	14·6	9	65	17	13	56	6	6	13·7
Serum IgG_2	7 s	14·5	6	53	17	11	51	—	4	—
Serum IgM	19 s	13·4	25	178	76	21	146	—	75	12·5
Serum IgA	7 s, 9·5 s	—	—	—	—	—	—	—	—	—
Milk IgA	9·5 s, 11·6 s	14·0	27	102	75	33	110	17	43	13·9

Fuc, Fucose; Man, Mannose; Gal, Galactose; Glc, Glucose; GlcNAc, *N*-acetyl glucosamine; GalNAc, *N*-acetyl galactosamine; SA, Sialic acid; E, $E_{1cm}^{1\%}$ at 282 nm in 0·1 M-NaOH.

Immunoglobulin IgA of porcine external secretions is composed principally of two molecular types—a 9·5 s and a 11·6 s molecule, the smaller molecule making up to 60–70% of colostral and milk IgA, although variation among sows does exist. The 11·6 s molecule is the classical secretory IgA molecule that predominates in external secretions of other species. It is a dimer containing an additional peptide called secretory component. The 9·5 s molecule is a dimer but without secretory component (Bourne, unpublished observation). Vaerman (1970) has also demonstrated two molecular types of porcine milk IgA of 8·9 and 11·2 s and similar findings have been reported by Porter & Allen (1972).

A similar situation is seen in pig salivary and urinary IgA (Bourne, Curtis & Loh, 1973).

Two structural types of IgA molecule are recognized in the pig, IgA_1, which is equivalent to human IgA_1 and IgA_2 (AM–), and pig IgA_2, which is equivalent to human IgA_2 (AM+). As in man the pig IgA_2 protein predominates in secretions (Bourne, 1971b).

The predominance of a non-secretory IgA dimer in pig secretions is an unusual and interesting feature which could have important biological implications.

The functions of the secretory component have not been established, although it would seem to protect the molecule against the action of proteolytic enzymes (Tomasi & Calvanico, 1968) and may assist in binding the IgA antibody to epithelial cells of mucosal surfaces or to the mucus. Some immunoglobulin antibody activities may be influenced by quaternary structure (Newcomb & DeVald, 1969) and secretory component may contribute to the effectiveness of antibody activity by influencing molecular orientation.

The pig 9·5 s IgA_2 molecule spontaneously dissociates under denaturing conditions into heavy- and light-chain dimer sub-units and does not need to be first reduced. The 11·6 s IgA secretory molecule is resistant to this treatment. The relatively unstable 9·5 s non-secretory IgA molecule will readily combine with free secretory component to produce an 11·6 s secretory IgA molecule that will resist dissociation (Bourne, unpublished results), therefore a further function of secretory component could be to assist in the maintainance of the structural integrity of the pig IgA_2 molecule which does predominate in external secretions. This hypothesis is supported by the observation of Jerry, Kunkel & Adams (1972) that the AM+ genetic variant of human IgA_2 is also stabilized by secretory component.

The observation that the pig 9·5 s non-secretory IgA molecule will freely combine with free secretory component and the relative paucity of free secretory component in porcine colostrum and milk suggests that the low proportion of an 11·6 s secretory IgA molecule in pig external secretions may be the result of a relative deficiency of secretory component—this may be of importance in view of the above-mentioned properties of secretory component.

Serum IgA consists of approximately equal proportions of a 7 s monomer and the 9·5 s dimer. This differs markedly from the situation in man but is similar to that described in the dog (Vaerman, 1970) and may reflect contribution of the intestinal tract to the circulating IgA immunoglobulins in the pig.

Immunoglobulin quantitation

Immunoglobulin IgG constitutes more than 80% of the total immunoglobulin content of colostrum, which exceeds by 2·5–3-fold the IgG content of serum. A 5-fold decrease in colostral IgG concentration is found in the first 24 h of lactation and there is a 30-fold decrease in the first week; IgG accounts for 20–30% of milk immunoglobulin, but in some sows this level may be higher. The IgA concentration in colostrum is four times higher than that in serum. A 3-fold decrease in concentration is found in the first 24 h of lactation and milk IgA levels then remain constant at 3 mg/ml when IgA accounts for 50–60% of milk immunoglobulin. IgM is the smallest component of both colostrum (4%) and milk (18%) immunoglobulin (Table 2, Curtis & Bourne, 1971).

Immunoglobulin IgA is also the main component of pig intestinal juice (Table 3, Bourne, Pickup & Honour, 1971). Colostrum, therefore, is suited to its immune role to provide for circulating immunoglobulins in the serum of the new-born pig and milk is suited to its role to provide intestinal antibody during the post-natal and pre-weaning periods. The protective value of orally-presented antibody has been clearly shown in gnotobiotic pigs (Kohler & Bohl, 1966; Rejnek, Travnicek, Kostka, Stertzl & Lanc, 1968; Wilson & Svendsen, 1971).

Immunoglobulin levels in young pigs

Immunoglobulins IgG, IgM and IgA are all absorbed non-specifically from colostrum by the new-born piglet. Peak serum levels are obtained at 24 h of age but there is great variation in levels between litters and within litters (Fig. 1, Curtis & Bourne, 1971).

Table 2. *Immunoglobulin levels in serum, colostrum, and milk of pigs*

	Mean immunoglobulin concentration (mg/ml)								Total serum and whey protein (mg/ml)
	IgG		IgG_2		IgA		IgM		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Serum from pork pigs	18·31	0·67	12·41	0·48	1·44	0·12	3·15	0·19	70
Sow Serum	24·33	0·94	14·08	0·49	2·37	0·20	2·92	0·18	70
Colostrum (o h)	61·80	2·44	40·29	1·66	9·66	0·59	3·19	0·21	196
Milk (24 h)	11·83	4·82	8·04	3·21	3·76	0·99	1·79	0·31	41
Milk (2 d)	8·16	3·17	5·02	1·80	2·72	0·67	1·81	0·41	35
Milk (3–7 d)	1·91	0·64	1·31	0·32	3·41	1·01	1·17	0·23	33
Milk (8–35 d)	1·37	0·62	0·99	0·45	3·04	0·74	0·89	0·25	33

Table 3. *Immunoglobulin levels (mg/ml) in serum, colostrum, milk and intestinal juice of pigs*

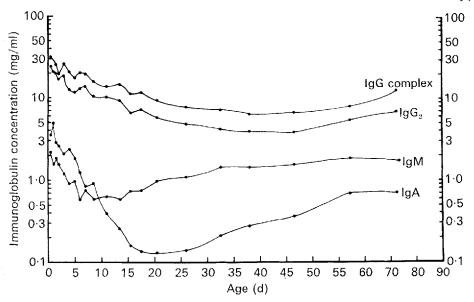|	IgG	IgA	IgM	IgA:IgG
Serum	24·3	2·4	2·9	0·1
Colostrum (o h)	61·8	9·7	3·2	0·16
Milk	1·4	3·0	0·9	2·1
Intestinal juice (relative levels)	0·2	2·6	Trace	13·0

Fig. 1.   Immunoglobulin levels in serum of young pigs.

At 24 h the mean values for IgG concentrations in litters range from 18·7 to 39·0 mg/ml; levels then decrease gradually to a minimum value of 6·3 mg/ml at 36–40 d. Immunoglobulin IgA levels vary from 2·1 to 8·6 mg/ml and decrease rapidly after 24 h, to their lowest values at 17–22 d of age. IgM levels varied from 1·1 to 2·0 mg/ml at 24 h and then decreased rapidly to their lowest level (0·6 mg/ml) at 8–14 d of age.

The important factors are the great variation in immunoglobulin levels between litters and within litters, and the rapid decline particularly in levels of IgM and IgA. At weaning (5–6 weeks) IgM and IgA are low, the serum concentration of IgG reaches its lowest level and the protection afforded by intestinal antibody from milk is removed. It must be appreciated, however, that while systemic immunoglobulin levels may indicate the general immune status of the animal, they give no indication of the specificity of immunity that may be present, or of the development of the intestinal tract immune system which must be important in relation to gut infections.

The half-lives of absorbed IgM, IgA and IgG are 4·5, 3·5 and 14·2 d respectively in piglet serum (Curtis & Bourne, 1971). An interesting finding is the difference in serum half-life between absorbed 9·5 s non-secretory IgA and 11·6 s secretory IgA molecules. The proportion of these two IgA molecules is the same in colostral whey and piglet serum IgA 24 h after suckling. However, 72 h after suckling the 11·6 s molecule has virtually disappeared from piglet serum (Bourne, 1971b). This has been confirmed by estimating levels of secretory IgA in the serum of the developing piglet and the serum half-life of the 11·6 s secretory IgA molecule, which was 24 h (Bourne, unpublished results). The fate of the 11·6 s molecule is unknown, but it might be selectively removed from the serum to function at mucosal surfaces. These observations contrast with those of Porter (1969a), who found that secretory

IgA is not absorbed by the piglet; on the other hand a similar situation was reported in the calf (Porter, 1972), and a half-life of 24 h for 11 s secretory IgA compared to a 5–6 d half-life for serum IgA monomer has been found in man (Butler, Rossen & Waldman, 1967).

Immunoglobulin formation in the young pig

It is established that the foetal piglet is capable of responding immunogenically to an antigenic stimulation in the last half of gestation (Binns, 1967; Bourne, Curtis, Johnson & Collings, 1973) but normal piglets at birth show little development of the immune system and only traces of immunoglobulins (Porter, 1969*b*; Curtis & Bourne, 1971; Prokesova & Rejnek, 1971) as the result of the efficient protection given by the placenta to passage of antigens and antibodies.

The time of onset of immunoglobulin formation in normal piglets has not been studied in detail. From half-life studies of naturally-absorbed colostral immuno-globulins and ^{125}I-labelled immunoglobulins (Curtis & Bourne, 1973) it was con-cluded that IgA production in the piglet did not contribute significantly to serum IgA levels in the first 7–12 d of life nor IgG production during the first 2–3 weeks of life. However, IgM was produced in the first week of life and this contributed significantly to circulatory IgM levels. This contrasts with the report of Porter & Hill (1970) that IgM could not be detected in the serum of two colostrum-deprived pigs until after the 20th d of life, although they reported that serum IgM levels in a litter of suckled pigs decreased to a minimum of 5 d and then increased at 7 d of age.

Immunoglobulin-containing cells appear in the lamina propria of the intestinal tract after about the 10th d (Allen & Porter, 1973) and by the 4th week of life a fairly well-developed IgA system is present (P. J. Brown, personal communication). However, in this period the circulatory levels of immunoglobulins are very low and the piglet probably relies on milk antibody for the first month of its life.

Local production of immunoglobulins in mammary tissue

There is evidence to suggest that IgA is synthesized at mucosal surfaces and much has been obtained from studies on the mammary gland (Chodirker & Tomasi, 1963; Adinolfi, Glynn, Lindsay & Milne, 1966; Tomasi & Bienenstock, 1968; Genco & Taubman, 1969; Lawton, Asofsky & Mage, 1970; Eddie, Schulkind & Robbins, 1971). The contribution of locally-produced immunoglobulins to pig mammary secretions has been studied by Bourne & Curtis (1973). This was done by measuring the transfer of ^{125}I-labelled immunoglobulins from serum into colostrum and milk and determining the proportion derived from serum. (Fig. 2). All colostral IgG was found to be derived from serum and this proportion declined rapidly with the onset of suckling. Although 60% of colostral IgA is produced locally, this is only a small fraction of the total immunoglobulin as IgA is only a small component of colostral immunoglobulin (Porter, 1969*b*; Curtis & Bourne, 1971). Sow colostrum is, therefore, a transudate from serum and not a true secretion; this further indicates its suitability in performing its immune function.
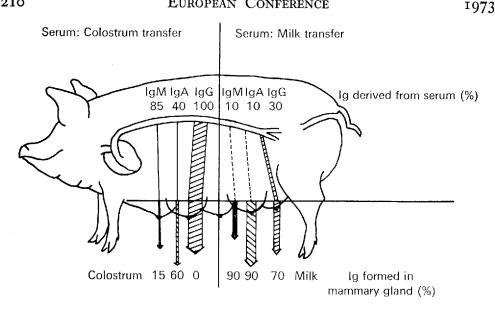
Fig. 2.   Transfer of immuglobulins from serum into colostrum and milk in the sow.

The source of milk immunoglobulins is very different. More than 90% of IgA and IgM and 70% of IgG in milk is produced locally in mammary tissue and milk can be regarded as a true secretion. Another important factor is that the local immune system of the lactating mammary gland contains all classes of immuno-globulins. In all sows studied, IgG and IgM made up 40% of the total of locally-formed immunoglobulin and in some sows this value was higher.

Influence of site of antigenic stimulation on local antibody production

If immune protection of the young suckling pig is to be achieved through colos-trum and milk it is important to know not only the site of formation of the relevant immunoglobulins but also the influence of the site of vaccination on their specific development.

In addition to the evidence of local production of IgA by the mammary gland, mentioned previously, it has been shown in rabbits (Genco & Taubman, 1969; Eddie et al. 1971) and in sheep (Lascelles & McDowell, 1970) that intramammary vaccination of antigen is necessary to produce IgA antibodies in colostrum but the total immunoglobulin response has not been specifically quantified.

In the pig the influence of the route of vaccination on the systemic and local immune response has been studied using an immuno-absorbent technique (Bourne, Newby & Chidlow, unpublished results) which is based on a primary antigen-antibody interaction and can be used to determine the specific antibody activity in each immunoglobulin class. Three methods of vaccination have been investigated: (1) intramuscular vaccination with Freunds Complete Adjuvant (FCA); (2) intra-mammary vaccination without FCA; (3) intramammary vaccination with FCA.

The immune response to two antigens (horse-spleen ferritin and pig γ-globulin)
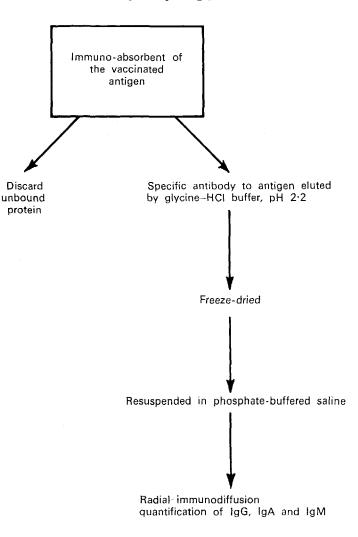
Fig. 3. Schematic outline of the immuno-absorbent technique for isolating and quantifying class-specific antibody.

was measured. Nine sows were injected, by either the intramuscular or intramammary route, 21, 14 and 7 d before parturition. Intramuscular injection was done at different sites for each injection but the intramammary injection was done using one gland. The experimental procedure is outlined in Fig. 3 using an immuno-absorbent prepared by the method of Newby, Bourne, Chidlow & Steel (1973).

Immunoglobulins in serum, colostral whey and milk whey were quantified. Immunoglobulins with specific antibody activity were isolated, by absorption on to the solid-phase immuno-absorbent and after elution they were quantified and the total amount of antibody activity in each class and the percentage involvement of that class was obtained.

The results from three sows for the three methods of vaccination with horse-spleen ferritin antigen are shown in Tables 4–6. Horse-spleen ferritin and pig γ-globulin antigens gave similar results but ferritin produced a higher quantitative response than pig γ-globulins.

Intramuscular vaccination (Table 4) produced only a poor systemic response and, although all three classes of immunoglobulin were involved, most antibody activity was in the IgG fraction. In colostrum the immune response was also poor and mainly associated with IgG, but in milk, where IgA is the predominant immunoglobulin, antibody activity was found in all classes, but at a very low level. Seven d after parturition vaccination of ferritin in FCA was made into one mammary gland of sow no. 8. A marked increase in IgA and IgM activity was found in milk from the vaccinated and non-vaccinated glands, an increase that was also reflected in the systemic response. Serum IgG activity increased 2-fold but milk activity of IgG only marginally increased.

Intramammary vaccination of ferritin antigen without FCA (Table 5) produced a systemic response at parturition that was better than that obtained by intramuscular vaccination except that no serum IgA activity was found, although this did develop later. A higher percentage of colostral IgA was committed to a specific antibody than

Table 4. *Changes in immunoglobulin levels in sow no. 8 in response to intramuscular vaccination with horse-spleen ferritin antigen in Freunds complete adjuvant*

(Values in parentheses represent percentage involvement of each class of immunoglobulin in the immune response)

	Serum (mg/ml)	Colostrum (mg/ml)	
Farrowing			
IgG	17·4	51·4	
IgA	2·2	8·7	
IgM	2·7	3·1	
Specific antibody			
IgG	0·3　(1·7)	0·8　(1·5)	
IgA	0·04 (1·8)	0·03 (0·3)	
IgM	0·07 (2·5)	0·02 (0·6)	
7 d post-partum		Milk (mg/ml)	
IgG	16·8	1·9	
IgA	2·1	2·4	
IgM	3·0	0·9	
Specific antibody			
IgG	0·4　(2·4)	0·02 (1·0)	
IgA	0·04 (1·9)	0·02 (0·8)	
IgM	0·1　(3·3)	0·01 (1·1)	
		Injected gland	Non-injected gland
21 d post-partum			
IgG	17·2	1·8	1·7
IgA	2·3	2·7	2·4
IgM	3·0	1·0	0·9
Specific antibody			
IgG	0·8　(4·6)	0·03 (1·7)	0·025 (1·4)
IgA	0·06 (2·6)	0·21 (7·8)	0·17 (7·1)
IgM	0·2　(6·6)	0·07 (7·0)	0·05 (5·6)

Table 5. *Changes in immunoglobulin levels in sow no. 2 in response to intramammary vaccination with horse-spleen ferritin antigen without Freunds complete adjuvant*

(Values in parentheses represent the percentage involvement of each class of immunoglobulin in the immune response)

		Colostrum (mg/ml)	
	Serum (mg/ml)	Injected gland	Non-injected gland
Farrowing			
IgG	14·0	32·9	34·0
IgA	2·17	5·5	5·4
IgM	3·6	2·0	2·1
Specific antibody			
IgG	0·4 (2·9)	0·5 (1·5)	0·4 (1·2)
IgA	0 (0)	0·2 (3·6)	0·18 (3·3)
IgM	0·12 (3·3)	0·08 (4)	0·06 (2·8)
7 d post-partum		Milk (mg/ml)	
IgG	16·1	2·15	2·2
IgA	2·2	2·6	2·4
IgM	3·4	0·9	0·8
Specific antibody			
IgG	0·5 (3·1)	0·03 (1·4)	0·03 (1·3)
IgA	0·02 (0·9)	0·08 (3·1)	0·06 (2·5)
IgM	0·1 (2·9)	0·05 (5·5)	0·05 (6·3)
21 d post-partum			
IgG	16·3	1·8	1·87
IgA	2·1	2·74	2·3
IgM	3·3	0·8	0·7
Specific antibody			
IgG	0·6 (3·6)	0·03 (1·7)	0·02 (1·1)
IgA	0·04 (1·9)	0·10 (3·6)	0·08 (3·4)
IgM	0·2 (6·0)	0·05 (6·3)	0·03 (4·3)

either IgG or IgM but the main activity was in the IgG component. This was expected as IgG constitutes 80% of colostral antibody activity.

In milk the antibody response was far better than that obtained by intramuscular injection and was principally in the IgA and IgM immunoglobulin classes.

Intramammary vaccination of ferritin with FCA (Table 6) was found to give a response that was 2- to 4-fold greater than either of the other two methods of vaccination for the systemic response and up to 10-fold greater for the mammary-gland response. The activity in colostrum and serum was mainly associated with IgG although the largest percentage involvement in the immune response was found in IgA and IgM; in milk IgA showed the greatest percentage involvement and also the highest antibody activity.

A feature of this study is that intramuscular vaccination in the sow gives a very poor immune response, both systemic and local, although all classes of immuno-globulin are stimulated. The poor response obtained by this method in the pig would correlate with the results of Anderson (1973) who found that little plasma-cell activity develops around intramuscular sites of antigen injection in the pig. Intra-mammary vaccination is far superior in stimulating a local as well as a systemic

Table 6. *Changes in immunoglobulin levels in sow no. 4 in response to intramammary vaccination with horse-spleen ferritin antigen in Freunds complete adjuvant*

(Values in parentheses represent the percentage involvement of each class of immunoglobulin in the immune response)

	Serum (mg/ml)	Colostrum (mg/ml)	
		Injected gland	Non-injected gland
Farrowing			
IgG	19·2	48·3	43·4
IgA	2·1	8·4	8·9
IgM	2·9	3·0	2·7
Specific antibody			
IgG	0·9 (4·7)	2·3 (4·8)	2·2 (5·1)
IgA	0·08 (3·8)	0·6 (7·1)	0·6 (6·7)
IgM	0·29 (10)	0·2 (6·7)	0·15 (5·6)
7 d post-partum		Milk (mg/ml)	
IgG	23·2	1·8	1·6
IgA	2·4	3·4	3·2
IgM	2·3	1·0	1·1
Specific antibody			
IgG	1·3 (5·6)	0·14 (7·8)	0·1 (6·3)
IgA	0·15 (6·2)	0·4 (11·8)	0·25 (7·8)
IgM	0·17 (7·4)	0·07 (7·0)	0·05 (4·5)
21 d post-partum			
IgG	21·9	1·1	1·3
IgA	2·4	3·5	3·2
IgM	2·3	0·9	0·9
Specific antibody			
IgG	2·0 (9·1)	0·13 (11·8)	0·09 (6·9)
IgA	0·16 (6·7)	0·4 (11·4)	0·27 (8·4)
IgM	0·1 (4·3)	0·04 (4·4)	0·02 (2·2)

immune response; all three classes of immunoglobulin are stimulated and the involvement of the IgA class is apparently greater; the largest amount of antibody activity can be correlated with the main immunoglobulin class present in the secretion. Of particular interest and importance is the fact that vaccination of one mammary gland results in the development of antibody activity in other glands. The lower percentage involvement of serum immunoglobulins indicated that this was not the result of passive transfer from serum in the secretions. Alternatively there is a general dissemination of antigen or immunoglobulin-precursor cells from the vaccinated area to other immunoglobulin-producing areas.

This evidence for local production of all three classes of immunoglobulin supports the observations of Bourne & Curtis (1973) that the production of IgA, IgM and IgG is localized and emphasizes that all classes of immunoglobulin are involved in local immune systems.

In most of the suckling period, therefore, the piglet is dependent on the dam for systemic and intestinal antibody. We are now just beginning to appreciate how to manipulate these immune systems to the piglets' advantage, although much work remains to be done on the interrelationship of the various immune systems, the

presentation of antigen and on the development of the piglets' immune system and the mechanism of intestinal immunity.

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