

ARTIFICIAL REARING OF LOW BIRTH-WEIGHT PIGS

by

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ABSTRACT

Four experiments were conducted to evaluate a crude immunoglobulin preparation fractionated from abattoir porcine serum with ammonium sulphate. The preparation was used as an additive to milk replacer for colostrum deprived, low birth-weight pigs reared in a non-isolated environment. Six pigs per treatment were used in experiments 1 and 3. Eight pigs per treatment were used in experiments 2 and 4.

In the first experiment, members of the negative control group did not survive and mortality of the other three groups which received some immunity was high. In the second experiment, the negative control group was eliminated from the trial, so those receiving only colostrum for 12 hours died, but the two groups receiving immunoglobulin treatment showed improved survival (63, 50%). In the third experiment, higher levels of immunoglobulin (15g./kg. body weight, initially followed by 5g./kg/day) did not show a significant effect on survival in comparison with the previous levels of 10g./kg. to 2g./kg.. However, rate of gain in body weight was significantly higher in the high dose level of immunoglobulin.

In the fourth experiment the pigs were maintained on immunoglobulin for 10, 15, and 21 days and it was found that 21 day treatment eliminated deaths. The highest rate of gain was achieved with those on 21 day treatment in experiment 4. However, these rates of gain were considerably below those achieved with pigs on the sow.

The causes of mortality were predominately *E. coli* scours, septicaemia due to *E. coli* and other bacteria, pneumonia, and in the last

two experiments, Salmonellosis. The prevention of death in experiment 4 by the immunoglobulin extract, indicated the success of the preparation against the Salmonella species encountered.

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1. INTRODUCTION

Several studies have been done on the artificial rearing of neonatal pigs using supplemental immunoglobulins added to the diet (Owen, Bell, and Williams, 1961; Scoot, 1972; McCallum, 1977; Lodge and Elliot, 1979). The experiments reported herein were attempts to raise low birthweight pigs, including so-called runts, in a farm environment using previously studied levels and periods of serum-derived immunoglobulin supplementation.

Below a birthweight of 900 to 1000 grams, pig mortality during the first week of life dramatically increases (Pomeroy, 1960; Sharpe, 1966; Lefce, 1971; Fahmy and Bernard, 1971; Bereskin et al., 1973; English and Smith, 1975). Previous workers have found that, given an environment removed from competition and trauma, low birthweight pigs can be artificially reared with low mortality and more satisfactory growth rates (Lecce, 1971; England, 1974). However, these earlier studies utilized a semi-isolated environment and sterile food. The current project involved attempts to raise these low birthweight pigs in a non-isolated environment using added immunoglobulins to eliminate the need for an isolated environment.

2. LITERATURE REVIEW

2.1 The Mammalian Immune System

2.1.1 The Development of Immunocytes¹

The major effector cells in immunity are the lymphocytes, of which there are two classes. There are T lymphocytes where "T" refers to their derivation in the thymus gland and there are B lymphocytes where the "B" refers to the major differentiation region in the bird called the Bursa of Fabricius (Cooper and Lawton, 1974). The bursa has no analogous region yet demonstrated in the mammal although similar development of B cells here seems to occur in the fetal liver or bone marrow. (Cooper & Lawton 1974). These lymphocytes are derived from a common stem cell for hemopoiesis from which other circulating cells are derived. Thus, lymphocytes, monocytes, polymorphonuclear leucocytes, macrophages, megakaryocytes (from which platelets are derived), mast cells, eosinophils and erythrocytes are all derived from the same stem cell. All, except erythrocytes, are involved in the immune system. The primordial stem cell first develops in the blood islands of the embryonic yolk sac and then migrates to the liver of the fetus. Later in gestation and during adult life these stem cells are found in the bone marrow, having migrated from the fetal liver, and they continue the very active process of hemopoiesis throughout the life of the individual (Hood et al., 1978).

In the hemopoietic tissues a subtle and as yet undetermined differentiation occurs giving rise to cells which will individually home to a specific tissue microenvironment. Here further differentiation and a commitment occurs which means that the cell is set on a uni-directional path of development. T cell precursors become committed in the microenvironment of the thymus under the control of thymus hormone; B cells are committed in

the fetal liver, bone marrow and, possibly Peyer's patches (Bienstock, 1979); macrophages and polymorphonuclear leucocytes in the bone marrow and spleen; and mast cells in the bone marrow (Hood et al., 1978).

The lymphocytes develop within these specific tissues in an antigen independent differentiation until they are capable of responding to antigens. At this stage both B and T cells carry antigen receptor molecules on their membranes. The B cell receptor molecule is an immunoglobulin. The T cell receptor molecule is as yet unidentified but does not seem to be immunoglobulin (Benaceraf and Unanue, 1979). The B cell immunoglobulin is first IgM but later bears the isotype to be produced by the end cells. (Hood et al., 1978).

If these lymphocytes bind a cognate antigen to these surface receptors they become activated; they enlarge with the formation of polysomes, microtubules and macromolecules in a process called blast transformation (Hood et al., 1978). These activated cells proliferate and continue to differentiate. Thus clones of T cells are formed containing both effector cells and memory T cells. Similarly, clones of effector and memory B cells are formed. The memory cells remain in circulation and provide a continuously reactive clone of cells which respond in a secondary immune response upon later antigen stimulation. The major effector cells are directly involved in antibody production as with T helper and T suppressor and plasma cells (B end cells) or in cell mediated immunity as with T killer and T - delayed hypersensitivity cells. (Hood et al., 1978).

The function of the lymphoid system is to provide maximal contact of any antigen with its repertoire of antigen specific lymphocytes. Foreign material is phagocytized by macrophages which present antigen from

the foreign material to the T cells. These T cells (T helpers) are then activated and stimulate maximal antibody production by the plasma cells. Any antigen which enters tissue is gathered into the lymphatic system by the lymph fluid and carried to a nearby filtering lymph node or the spleen to stimulate antibody production. Antigens which enter the upper respiratory tract or gastro-intestinal tract encounter local lymph nodes as well as specialized organs: tonsils, adenoids, Peyer's patches, and the appendix. Blood borne antigens are dealt with mainly by the spleen. (Hood et al., 1978).

2.1.2 The Intestinal Secretory Immune System

A major area of contact with antigenic material in the environment is at the mucosal lining of the gastro-intestinal system. Consequent to the development of gut microflora, the lamina of the gut becomes infiltrated with immunocytes producing predominately IgM and IgA which are transported across the gut epithelium into the lumen (Porter et al., 1976). Initially in the development of a young animal IgM producing cells predominate but later there is a greater proliferation of IgA cells (Allen and Porter, 1973).

The intestinal tract is especially adapted for production of antibody to the lumenally occurring antigens of bacteria, viruses, and other food borne materials. The mucosa overlying the Peyer's patches appears to be adapted to the uptake of large molecules and even intact microorganisms (McClelland, 1979). In this way precursor lymphocytes are sensitized in the Peyer's patches and are released to circulate and home into the intestinal lamina propria where they develop into IgA secreting plasma cells (Pierce and Cowans, 1975; Hasland et al., 1976).

The development of a systemic antibody production due to the gastro-intestinal immunization route may also occur from IgG or IgM forming

cells which are seeded by Peyer's patches in extra-intestinal lymphoid tissues. Also there is the possibility of antigen reaching lymphocytes in the spleen or peripheral lymph nodes from the gut (Craig and Cebra, 1971). Serum IgA also originates from gastrointestinal secretion (Tomasi, 1976).

Mammary secretions of antibody are found to be sensitized to intestinal antigens (Chidlow and Porter, 1979). This is the gut-to-mammary axis referred to by Parmely et al., (1976). IgA secreting lymphocytes, possibly originating in Peyer's patches and travelling through the mesenteric lymph nodes into circulation are trapped by active mammary tissue under the influence of mammatropic hormones (Lamm et al., 1978).

2.1.3 Antibody

Antibody is produced by plasma cells which are end B cells (Nossal, 1976). The antigen is held by the immunoglobulin receptor in the cell membrane and is processed by internalization or endocytosis. The binding of antigen by B cells is essential for the development of production of antibody (Benaceraf and Unanue, 1979). The antibody is of five isotypes: M, G, A, D, or E. M, G, or A are responsible for humoral and secretory defense while IgD is presently only known as a membrane bound receptor on many lymphocytes, and IgE is concerned with the allergic response (Benaceraf and Unanue, 1979).

2.1.3.1 The Structure of Antibodies (figure 2.1)

Antibodies belong to a class of proteins called immunoglobulins. The basic unit of structure is a complex of four polypeptides, two identical "light" (low molecular weight) chains and two identical "heavy" (high molecular weight) chains linked together by disulphide

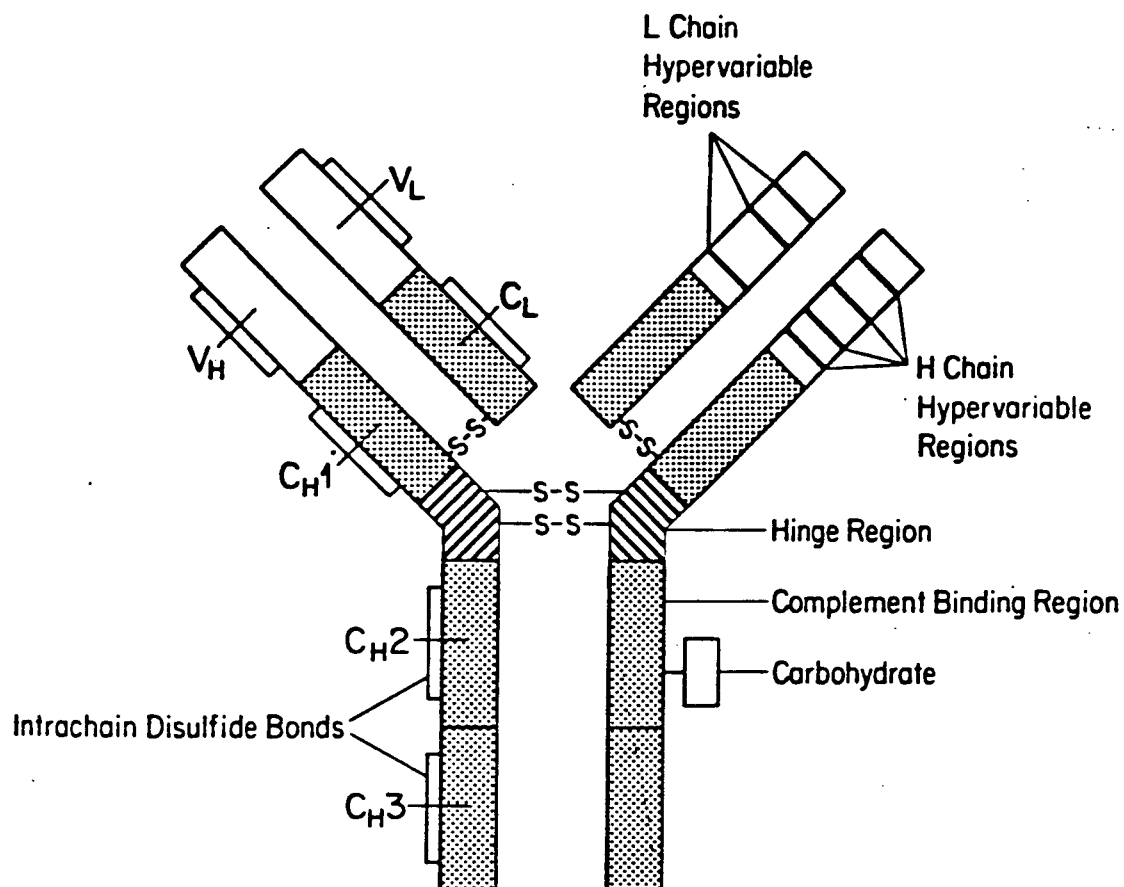


Figure 2.1. The IgG Antibody Molecule: a Schematic Presentation of Structure (Benaceraf and Unanue, 1979)

bonds (Hood et.al., 1978). The five classes of antibodies are called isotypes and their different structures are determined by their heavy chains. Thus, IgG has gamma heavy chains, IgM has mu, IgA has alpha, IgD has delta, and IgE has epsilon heavy chains. All isotypes may have one of two types of light chain, either kappa or lambda. The heavy chains have three constant regions and one variable region (at the amino-terminal end) while the light chains have one constant with one variable region. The variable regions are further composed of framework regions, and hypervariable regions which are the least homogenous regions of the immunoglobulin (Beneceraf and Unanue, 1979). The variable regions of the heavy and light chains form a cleft which is the specific binding site of the antibody for an antigen (Gearhart et al., 1981). The binding site is precisely complementary to the structure of the antigen and binds to the antigen by weak electrostatic attraction, hydrogen bonding, and Van der Waal forces (Bach, 1978).

The synthesis of these immunoglobulins is controlled by gene clusters (Hood et al., 1978). For each heavy or light chain a diverse cluster of V (variable) region genes of unknown number is linked with a cluster of C (constant) region genes on one chromosome. Thus, heavy chain clusters of V and C regions may be on one chromosome, a kappa C region with a cluster of V regions on another, and a lambda C region with a cluster of V regions on another. A single V region on the chromosome for the heavy chain can be associated with two or more C region genes during the differentiation of antibody producing cells (Hood et.al., 1978) resulting in identical idiotypes (i.e. identical variable regions) among different isotypes. Further work by Tonegawa et al., (1978) found the presence of J (joining)

loci on light chain chromosomes, and both J and D (diversity) loci on heavy chain chromosomes.

Diversity of antibody production to deal with the many possible antigens in the environment, in theory, results from two possible mechanisms (Hood et al., (1978). The first is diversity within the same germ line (i.e. diversity of V region genes). Thus, a combination from the selection of variable light chain genes with variable heavy chain genes produces a multiple of them in antibody clefts. In combination with J and D loci there is a further multiplication of diversity (Gearhart et al., 1981). The second mechanism of antibody diversity could result from somatic mutation or recombination which could occur during an individual's immune development. The consequence of these mechanisms is a very diverse repertoire of antibody specificities which, in the presence of antigen stimulation, leads to a continuously circulating array of antigen specific lymphocytes ready to proliferate under heavier antigen stimulation. The newborn is inexperienced in the environment which is constantly presenting diverse antigens and consequently has an undeveloped repertoire of lymphocytes to respond.

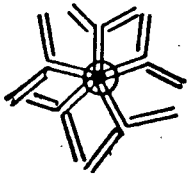




The antibody classes differ in gross formation (Table 2.1) IgG is a monomer of two heavy and two light chains of immunoglobulin.




IgM is a pentamer which is held together by a joining (J) chain with disulphide bonds (Tomasi, 1976). IgM may also be associated in disulphide or non-covalent bonds with another protein, the secretory component (Brandtzaeg & Baklien, 1977) at least in human IgM.

IgA may exist as a monomer, or as a dimer with a J chain as in serum. It may also exist as secretory IgA (S-IgA) which is a dimer with J chain and secretory component (SC). The binding of SC in IgM is weaker (more non-covalent bonding) than in S-IgA (Brandtzaeg & Baklien, 1977). IgD

Table 2.1

Immunoglobulin Structure

Immunoglobulin	Light Chain Type	Heavy Chain Type	Other Components	Structure
IgM	κ λ	μ	J, SC	
IgG	κ λ	γ		
IgA	κ λ	α	J, SC	
IgD	κ λ	δ		
IgE	κ λ	ϵ		

 = SC (Secretory Component)
 = J chain
 = Immunoglobulin Unit

and IgE both exist as monomers.

Both J chain and Secretory Component are polypeptides (Tomasi, 1976). According to Brandtzaeg & Baklien (1977) J chain is synthesized in the plasma cell with immunoglobulin where it polymerizes the IgA or IgM. SC is produced by serous type epithelial cells and acts as a specific receptor for the immunoglobulin. Covalent and non-covalent interaction between SC and immunoglobulin are completed during the passage of the complex through the epithelial cells and, conjugated IgA (or IgM) with SC, plus free SC is transported into the lumen by exocytosis (Benaceraf and Unanue, 1979). Since S-IgA is the predominate immunoglobulin in all body fluids outside the vascular system (with the exception of colostrum which contains mostly IgG) (Porter, 1976), this model probably applies to all exosecretions of secretory IgA. SC conjugated IgA is resistant to proteolysis by gastro-intestinal enzymes (Tomasi and Calvanico, 1968). SC is also thought to facilitate binding of the immunoglobulin to the mucosa coat of the intestinal epithelium (Porter et al., 1972).

2.1.4 Complement

The term complement refers to a complex group of enzymes in normal blood serum (Mayer, 1973). Thus, the complement system consists of 17 plasma proteins (Benaceraf and Unanue, 1979) which comprise a significant portion of the serum globulin fraction (Hood et al., 1978; Mayer, 1973). These proteins fall into two functional groups. One group is the classical components which are symbolized with a capital C and a number, one to nine. The other group is the components of the alternate pathway which are denoted with a capital letter and are B, D, and P (properdin). Many of these proteins are cleaved during complement reactions and cleavage fragments are suffixed with

lower case letters, for example, C3a and C3b, with the suffix b denoting the larger of the two fragments (Benaceraf and Unanue, 1979).

The functions of complement are several-fold. Completion of the complement sequence on an attacked cell leads to cytolysis. The cleavage product of C3, C3B, binds to microorganism cell surfaces producing immune adherence which facilitates phagocytosis (Mayer, 1973; Benaceraf and Unanue, 1979). C3a and C5a cleavage fragments are anaphylotoxins which are noted for causing lethal bronchospasm in guinea pigs and wheal and flare reactions in human skin by degranulating mast cells (Benaceraf and Unanue, 1979). Anaphylotoxins are active in other mammals also, causing acute inflammation (Hood et al., 1978). C5a and free C5b-6-7 complex are chemotactic factors which attract polymorphonuclear leucocytes (PMN), which are phagocytes, to a site of complement activity (Hood et al., 1978).

The sequence of the complement cascade is most simply demonstrated in figure 2.2. Essentially, there is an effector pathway (C5 to C9) which "punches" a hole in the affected cell and can be activated by either the classical sequence involving C1, C2, C4 and calcium or the alternate sequence involving B, D, P and magnesium. The classical sequence requires specific antibody which must be either IgM or IgG. A single molecule of IgM bound to an antigen on a cell membrane will initiate the classical sequence whereas two adjacent IgG molecules are necessary. Since antibody is scattered over the cell surface quite randomly, the probability of two IgG molecules occupying adjacent sites is quite small and therefore the frequency with which IgG activates the classical sequence is low (Mayer, 1973). The alternate pathway is a non-specific mechanism of immune defense (Mayer, 1977) since it doesn't require antibody for initiation. It is important in defense against

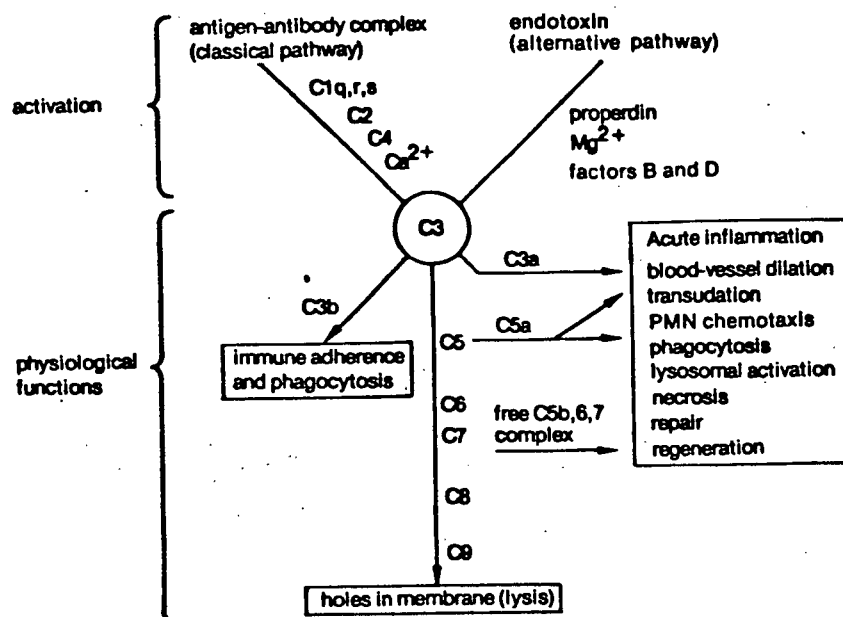


Figure 2.2 Activation Pathways and Physiological Functions of Complement Components (Hood et al., 1978)

gram-negative bacteria that inhabit the gut. Lipopolysaccharide from the cell walls of these organisms (endotoxins) combine directly with the serum factor, properdin (Hood et al., 1978). There is indication that S-IgA will also act with lysozyme to activate complement fixation by the alternate pathway (Hill and Porter, 1974). Both the classical activation sequence and the alternate pathway lead to cleavage of C3 which initiates the effector sequence.

2.1.5 Antibody Reactions in the Gut Associated Lymphoid Tissue (GALT)

Table 2.2 lists most of the known activities of antibody both systemically and externally as "secretory antibody". Within the gut associated lymphoid tissue (GALT) there are essentially two lines of antibody defense as interpreted by Tomasi (1976). The first line of defense involves reactions within the intestinal lumen and the second line of defense involves reactions in the tissues of the GALT allied to systemic immunity. The first line of defense is mainly carried out by S-IgA (as copra antibodies). Miler et al. (1975), using ligated porcine intestinal segments and live *E. coli* cultures as challenge injections, found that local protective effects of immunoglobulins IgG, IgM, and IgA were different, the minimum effective concentrations being 0.5, 0.05, and 0.005 mg/ml respectively for IgG, M, and A. Miler et al. (1975) further stated that IgA does not possess enterotoxin neutralizing activity and that the mechanisms of action might be due to inhibition of absorption of bacteria or by blocking of binding of enterotoxin to receptors on epithelial cells. The efficacy of IgA was further demonstrated by Steele et al. (1974). They found that of the immunoglobulins, S-IgA was most effective in reducing mortality in rabbits infected with live cholera organisms.

Table 2.2 Mechanisms of Antibody Anti-bacterial Activity

Class of Antibody	Activity
IgG	Toxin neutralization ¹ Agglutination ¹ Opsonization ¹ Bacteriolysis by complement fixation ¹ Inhibition of bacterial adherence ²
IgM	Toxin neutralization ¹ Agglutination ¹ Opsonization ³ Bacteriolysis by complement fixation ¹
IgA	Anti-toxin activity ⁴ Agglutination ⁴ Inhibition of bacterial motility ⁴ Bacteriostasis ⁵ Inhibition of bacterial adherence ⁴ Complement fixing by the alternate pathway ⁴ Binding to mucin of mucosa ⁴ Inhibition of bacterial enzymes ⁴ Inhibition of foreign antigen uptake ^{4,1}

1. Benaceraf and Unanue, 1979
2. Tomasi, 1976
3. Brandenburg and Wilson, 1974
4. McClelland, 1979
5. Porter, et al., 1976

The second line of defense, or systemic immunity, depends largely on IgG and IgM. The activities of IgG and IgM are quite different. Due to its pentameric form, IgM has more active antibody binding sites than IgG (Bach, 1978). Thus, the activity of IgM is considered to be greater in antibody reactions. Rowley and Turner (1966) found the 8 IgM molecules/bacterium were necessary as opsonins whereas 2200 IgG molecules/bacterium were necessary. Brandenbourg and Wilson (1974) stated that IgM was 500 to 1000 times more effective as an opsonin than IgG. They also stated that IgM was much more effective in complement fixation (See Section 2.1.4). Steele et al., (1974) developed data supporting the greater activity of IgM using rabbit Ig against *V. cholerae*. Porter et al., (1977) say that the dominant immunoglobulins found in the lamina propria are IgM and IgA. Since IgM may form only a small part of the secreted Ig, it may form a strong second line of defense in tissues behind the epithelium (Allen and Porter, 1973) where it can carry out complement fixation, opsonization agglutination, and toxin neutralization against pathogenic invaders.

2.2 Prenatal Development of the Porcine Immune System (see Table 2.3)

Working with the fetal lamb and fetal mouse it was found that development of competence in the mammalian fetus occurs in a controlled, step-wise fashion. "A hierarchy of antigens exists to which the fetus develops competence at different stages of gestation" (Silverstein, 1977). The immunologic competence maturation is independent of lymphoid development. The fetus before having organized lymphoid tissue is able to respond to some antigens presumably utilizing lymphoid cells in the fetal liver. Once it is competent to a certain antigen, the fetus is able to produce comparable antibody titres to an adult, and forms them in the usual sequence of immunoglobulin classes (IgM followed by IgG) (Silverstein, 1977). However, in contradiction, there may be some restrictions to fetal response. The level of antigen needed for stimulation may be higher (Sterzl, 1963), or there may be fewer stem cells, or there may be clonal restriction in the initial response (Klinman and Press, 1975).

Table 2.3 Prenatal Development of the Porcine Immune System

<u>Gestational Time of Occurrence</u>	<u>Event</u>
Day 28	Lymphoid cells are detected in the region of the thymus ¹
Day 32	The lymph nodes begin forming without germinal centres (unless the animal is infected) ²
Day 38	Lymphoid cells are detected in the liver and blood ¹
Day 40	Components of the complement system are present ³
Day 51	Number of lymphocytes gradually begin to increase in the spleen ¹
Before 65 days	Less than 1% of the lymphocytes have surface immunoglobulin ⁴
Day 77	Histologically, the thymus is identical to development at birth ¹ In the lamina propria of the terminal ileum lymphocytes occur in follicular aggregations ¹
After 70 - 80 days	Approximately 10% of blood lymphocytes have surface immunoglobulin indicating development of immunocompetence ⁴
After 80 days	A more generalized follicular lymphoid structure is developing with larger populations of lymphocytes ¹ . Immune responses can be shown against several antigens: formalized Salmonella ⁵ , sheep red blood cells ⁵ and phage ⁶ . Transplantation immunity to allogeneic cells was induced after 80 days ⁷ ; if injected at 60 days, allogeneic cells induce tolerance to skin grafts. ⁷
At birth	Peyer's patches are present ¹

1. Kruml et al., 1970
2. Pestana et al., 1965
3. Day et al., 1969
4. Binns, 1973
5. Binns and Symons, 1973
6. Tlaskalova et al., 1970
7. Binns, 1967

2.3 Postnatal Development of the Porcine Immune System

In germ-free neonatal piglets, no immunoglobulin is found with the exception of a small "half molecule" of IgG type which has been shown to consist of one heavy and one light chain and is not antigen responsive (Prokesova et al., 1970). This fragment has been theorized as a released, membrane-associated immune receptor molecule from lymphocytes (Porter, 1979). Due to an epitheliochorial placenta, the pig fetus is prevented from receiving antibody from the dam's circulation. The reason for this is unclear, because, although in the early embryonic stages as many as six histologically distinct layers separate the two bloodstreams, in later pregnancy the embryonic capillaries develop more in sub-epithelial positions and invade the embryonic trophoblast so that maternal and fetal bloods are in close proximity (Marrable, 1971). Whatever the situation of the circulatory supply, no maternal antibodies (or immeasurable quantities) pass (Kim et al., 1967). The placenta is also thought to block antigen stimulation (Prokesova et al., 1970).

Immunization of a newborn piglet with a large dose of antigen results in a relatively rapid onset of antibody formation in the blood. Antibodies are produced within 36 hours as IgM, and after 6 days both IgM and IgG which were antigen responsive are found (Prokesova et al., 1970). However, if certain antigens (e.g. Hog Cholera Virus) are used, injection in neonates induces tolerance² to that antigen. (Weide et al., 1962). This neonatal induction of tolerance to certain antigens implies an immaturity of development and is commonly demonstrated in mice (Beneceraf and Unanue, 1979).

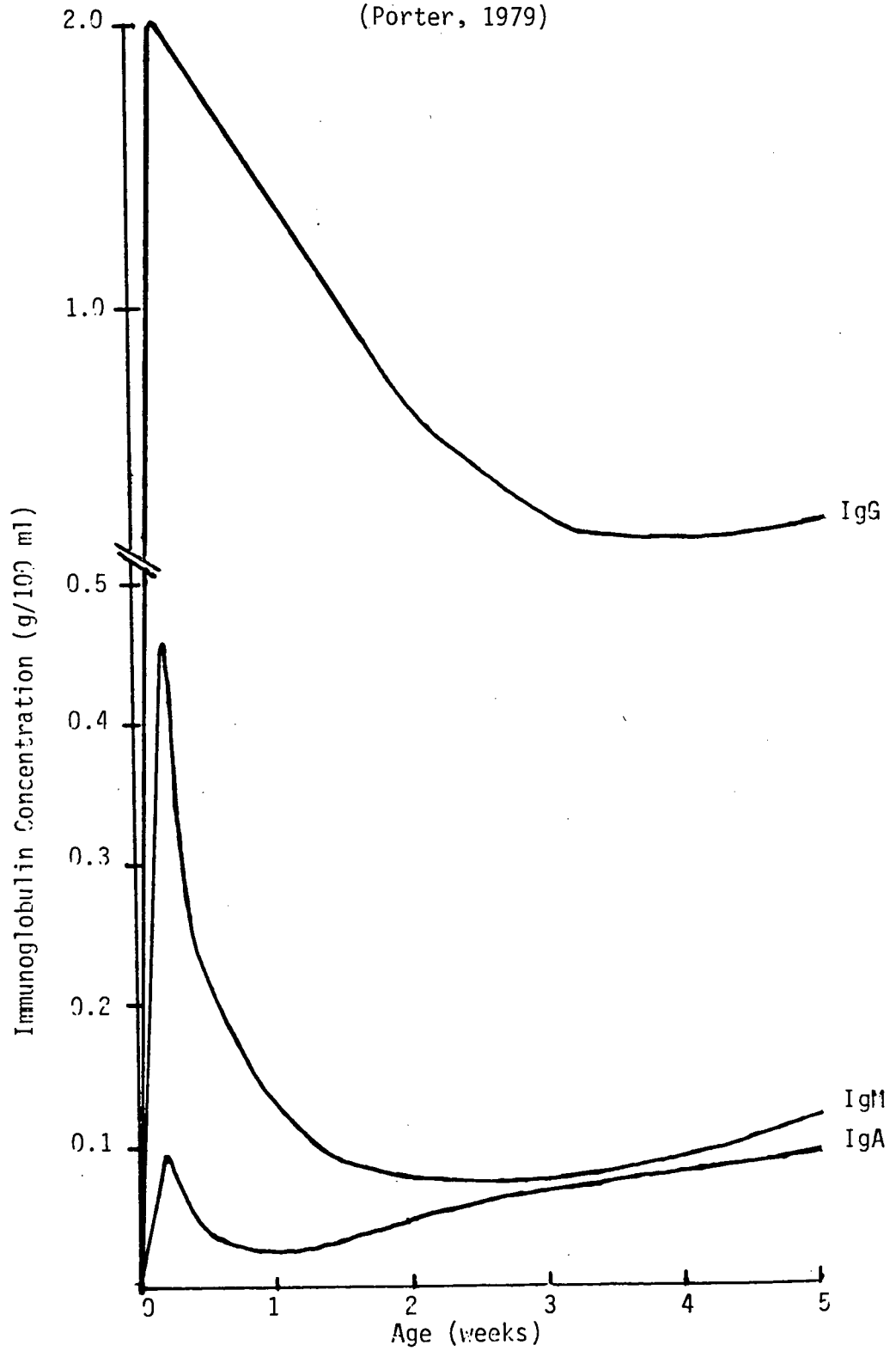
After birth, lymphocytes are antigenically stimulated at the

Peyer's patches and/or the mesenteric lymph nodes to precommitment for IgA production; they then enter the circulation and home mainly to the lamina propria of the small intestine. Thus, a response of the intestinal secretory immune system requires antigenic stimulation. In the duodenum, Brown and Bourne (1976) found considerable numbers of cells staining for each of the three classes of immunoglobulin after the first week. The presence of IgM cells was greater than IgA cells for the first 3 weeks; IgA exceeded IgM cells after 3 weeks during which time IgG cells were present in considerable but smaller numbers. After about 1 month, the plasma cell population of the duodenum was like that of an adult.

Humoral immunity also begins with the presence of synthesized IgM first during the end of the first week; IgA is found in the serum after 7 - 12 days; IgG is found in the serum after 14 days (Bourne and Curtis, 1973).

The immune mechanism of an adult can be contrasted with that of a neonate. The adult has been primed, during its antigen experience, to a great diversity of antigens from bacterial flora, and this response may be cross-reactive to an even larger array of invaders. Thus, a response which appears primary in adults may really be a secondary and more rapidly occurring response, whereas in the young or germ-free animal a primary response is truly primary (Solomon, 1971). It is puzzling why a piglet which is immunocompetent at birth should be so susceptible to infection particularly when deprived of maternal antibody. But this may be answered that because of antigenic virginity, there may be a reasonable delay in response. Essentially a race occurs between the multiplication of antigen stimulated cells and pathogens (Solomon, 1971).

Figure 2.3 Serum Immunoglobulin Profiles of the Neonatal Pig
(Porter, 1979)



The general development of the immune system after birth is a continuous maturation due to stimulation by natural antigens. This may be observed in the rapid enlargement of lymphoid organs such as the spleen and the slower development of lymph nodes with age (Solomon, 1971). These structures do not develop nearly as fast in germ-free animals. In the alimentary tract of germ-free pigs there is virtually no development of lymphoid tissues by 31 days (Kenworthy, 1970).

Contrasting results have been found with regard to the effect of colostrum antibody in the newborn pig. Segre and Myers (1964) in their trials found that piglets seemed to respond better to antigen-antibody complexes where the antibody is supplied by colostrum. Adult pigs respond just as well to antigen alone which, according to these workers, is presumably because there is sufficient natural antibody present in adults. However, Sterzl, et al., (1965) found that colostrum tended to inhibit the active production of antibody. Later results tend not to resolve this contradiction except that low levels of antibody will sometimes enhance an immunological response whereas high levels of antibody will cause a poor response (Solomon, 1970). The later case may be understood either to be a case of removal of antigenic stimulation by the antibody (Dixon et al., 1967) or a case of negative feedback. Low levels of antibody may facilitate phagocytosis by macrophages and enhance antigen presentation to T-helper cells (Kim et al., 1966).

Sera from pre-colostral, neonatal, germ-free piglets is bactericidal for rough strains of *E. coli*, and this activity can be correlated with the presence of complement (Sterzl et al., 1964). This is a mechanism which can function non-specifically against gram negative

bacteria without the presence of antibody and could be effective against any gut flora which become invasive.

2.4 The "Runt" Phenomenon

As birthweight decreases, mortality increases (Carroll et al., 1962; Sharpe, 1966; Fahmy and Bernard, 1971). Smaller pigs often require manual assistance to suckle and additional warmth to survive. Failure to suckle can lead to hypoglycaemia and death (Newland et al., 1952); lack of warmth can lead to hypothermia and death.

Exceptionally small pigs at birth are termed "runts" and are distinguished from normal pigs by both their light weight and distinctive physical characteristics. Cooper et al., (1970) defined runts as being the lightest 5% of the total population at birth. Perry and Rowell (1969) took a runt to have a prenatal weight of two-thirds of the average for its uterine horn. Physically, runts are small, thin, and have a disproportionately larger and more domed head than normal progeny.

The physical characteristics of a runt are the result of intra-uterine growth retardation which is not the result of fetal anomaly or maternal illness (Cooper et al., 1978). The runting phenomenon may be a result of intrinsic fetal growth factors or be secondary to placental function (Cooper et al., 1978) but the cause is not clear. When five or fewer feti are in each horn of the uterus, the position of the embryo has no effect on size. But, as the number increases, those at either end tend to have an increasing advantage over those in the middle of the horn, and those at the ovarian end have a greater advantage than those at the cervical end (Perry and Rowell, 1969). Marrable (1971) suggested that even with small litter sizes, the positional effect will be operative later in

pregnancy. Waldorf et al., (1957) suggested that the uterine arteries provided higher blood pressure at terminal sites but, although this may be relevant in other multiparous mammals, the porcine vascular system is such that supply to the cervix and uterine body is augmented by a branch from the urogenital artery (Marrable, 1971).

Several studies have been done about prenatal appearance of runting. Pomeroy (1960) found significant runting appearance at 74 days of gestation but not at 51 days. Perry and Rowell (1969) found feti of less than two-thirds the weight for the average of the uterine horn at 31 to 49 days of gestation and identified these as runts appearing. Cooper et al., (1978) found runts at gestational ages of 44, 53, 56 and 75 days of gestation. Obviously these data do not pin-point a conclusive stage of gestation at which fetal growth retardation occurs. However, since 99 per cent of fetal growth (by weight) occurs after day 40 of gestation (Marrable, 1971), the occurrence of runting this early suggests that intra-uterine retardation may be determined before it becomes recognizable and may occur throughout fetal development in some cases.

Widdowson (1971) compared the development of organs in the runt with that of a fetus taken at an equal size and with that of a litter-mate of normal size. Two outstanding differences were noted: firstly, muscle development, as measured by quadriceps size was significantly less in the runt than in either the fetus of equal weight or the normal litter-mate; secondly, the livers of the fetus and runt were comparable in size yet very much smaller than the liver of the normal littermate with the added characteristic of a very low carbohydrate content in the runt. Heart, spleen and stomach of the runt were intermediate in weight between those

of the fetus and normal littermate. The brain of the runt was nearer to normal weight than any other part of the body. Generally, Widdowson (1971) found that the decrease in organ size of the runt was due to both hypoplasia (decreased cell size) and hypotrophy (decreased cell number). Widdowson (1971) went on to compare the growths of a runt and a normal littermate for three years and found that the runt was 60 kg. lighter at the end of this term. Since there were no restrictions in diet or as a result of environmental effects after weaning, it may indicate that intra-uterine growth retardation may provide a permanent set-back.

2.5 Mechanisms of Action of Bacteria in the Intestine

Within the intestine there are three major ways that bacteria may associate with the intestinal mucosa to produce disease. Firstly, they can attach to the mucosa without penetration and induce disease by multiplying and producing exotoxin³. This occurs in *E. coli* enteritis of the small intestine and is caused by enterotoxin producing *E. coli*. Secondly, bacteria can attach to and penetrate the mucosa but not the subepithelial tissues and induce disease by damaging epithelial cells and also produce exotoxin. This can occur in *E. coli* enteritis of the large intestine and is caused by invasive strains of *E. coli*. Thirdly, bacteria can attach to, and penetrate the mucosa to reach the subepithelial tissues, multiply in the submucosa, and spread systematically also growing intracellularly in phagocytes. This mode of action occurs with *Salmonella* enteritis caused by *S. typhi*. (McClelland, 1979).

A major phenomenon involved in bacterial invasion of epithelial surfaces is bacterial adherence. Since peristalsis and flushing with secretions tends to remove bacteria and maintain a continuously lower level of growth and prevent invaders from proliferating, attachment to the gut epithelium by adherence is a major mechanism affecting the ability of a pathogen to proliferate. Best known in this connection is *E. coli* with its varying adhesion fimbriae. The most studied is the K88 antigen which has non-flagellar filamentous surface pilli carried on the genome of a plasmid. (Smith and Linggood, 1971). Other adherence factors of *E. coli* in the neonatal porcine intestine are the antigens K99, strain 987 type, and type 1 common pilli of which each has a different receptor site on the intestinal epithelium (Dupond and Pickering, 1980). All of these surface

structures are antigenic and elicit antibody responses where the antibody acts as an anti-adherence factor possibly by masking the adherence site of the bacterium. In reference to the K88 antigen, the porcine receptor site for it is controlled by a dominant gene and therefore animals which are double recessive are immune to K88 strains of bacteria unless other factors are present determining virulence. (Rutter, 1975; Rutter et al., 1976).

The mechanism of enterotoxins is not fully understood. However two enterotoxins have been shown to be produced by strains of *E. coli* pathogenic for swine: one is antigenic and heat labile (LT) and the other is non-antigenic and heat stable (ST) (Dupont and Pickering 1980). Both toxins are transmitted by a plasmid and both may be produced by the same strain of *E. coli*. It has been suggested that the non-antigenic ST was important in the diarrhea of swine (Gyles, 1971).

2.6 Diarrhea in Piglets

In a normal, healthy intestinal mucosa, salts and water are absorbed by an energy requiring mechanism. The driving force of water absorption is an active transport of electrolytes, particularly sodium and chlorine. Acute enteritis, which causes diarrhea in newborn animals, produced watery stools from fecal fluid primarily originating in the small intestine (Tennant and Hornbuckle, 1980). The rapid dehydration causes hemoconcentration and along with the movement of ions, leads to metabolic acidosis caused by renal failure to excrete hydrogen ions and by increased production of organic acids resulting from decreased tissue oxygenation. Hyperkalemia (high potassium) also results as does hypoglycemia due to decreased gluconeogenesis and increased anaerobic glycolysis (Tennant and Hornbuckle, 1980).

In the specific case of the enterotoxic mechanism of *E. coli*, the toxin (LT only) acts by binding to receptors on the mucosal cell membranes to activate adenyl cyclase which converts ATP to cyclic AMP. The cAMP acts as a second messenger to influence permeability of the membrane. The net result is that sodium absorption is blocked and chlorine is secreted. The chlorine ion pulls water and cations with it into the gut lumen to maintain osmotic equilibrium. This is the cause of fluid loss (Dupont and Pickering, 1980). *E. coli* heat stable toxin (ST) does not activate adenyl cyclase however, as has been discovered recently, in mice and rabbits, its action may be mediated through the build-up of guanyl cyclase which produces cGMP (Hughes et al., 1978).

In the case of *Salmonella* infection, similar dehydration and hydrogen ion and electrolyte disturbances are the result. *Salmonella*

is an invasive organism and penetrates the mucosa and proliferates in the gut associated lymphoid tissue (Dupont and Pickering, 1980). Its effect can occur throughout the intestine although it usually invades the lower gut mucosa more readily. Using the rat, Powell et al., (1971) showed diarrhea increased especially the ileal secretion of water, sodium and chlorine and potassium. They found an increased hydrostatic pressure in the lamina propria, perhaps caused by venous or lymphatic obstruction, with resultant increased membrane permeability.

2.7 Non-Specific Gastro-Intestinal Immune Mechanisms

There is a series of mechanisms which operate in defense against pathogenic bacteria and in some cases, viruses and parasites.

2.7.1 The Gastric Trap

A gastric trap is provided by the HCl which is anti-bacterial and is highly effective against almost all bacteria. However, if the gastric mucosa is not functional, as, possibly, in the neonate, or if the challenge dose is large enough, or if bacteria are not reached by the acidity of the stomach, bacteria may pass this trap to proliferate later in the gastro-intestinal tract (Giannella et al., 1971).

In neonates, the work on gastric acid secretion seems quite incomplete and contradictory. Cranwell et al., (1968) found that piglets in a clean environment had acid secretion at one week of age, while those raised in a conventional environment didn't secrete until 30 days of age. Later, Cranwell and Titchen (1974) discovered that secretion could begin from 2 days of age using a surgically separated pouch from the stomach and that the secretion was significant. Hill (1970) stated that acid secretion was very minimal during the first 2 weeks. Jones (1972) referred to work which stated that HCl secretion does not begin until 20 days of age of milk fed or 14 days if given cereals. The likely explanation of discrepancies is that the piglet has a competent gastric mucosa at least within a few days of birth, but environmental or nutritional factors control its development (Cranwell and Titchen, 1974).

2.7.2 Intestinal Motility

The rate of passage of chyme will determine to a large extent whether local propagation will occur. There is evidence that the time of

contact between the epithelial lining of the gut and an invasive pathogen such as a *Salmonella* strain of bacteria determines the development of disease (Sprinz, 1969). It has been shown by the use of a ganglion blocking drug which slowed the rate of peristalsis in rats that there was a substantial increase in the number of coliforms in the small intestine. (Dixon and Paulley, 1963). Diarrhea is associated with decreased motility (Christiansen et al., 1972) and gut stasis (Rutter, 1975; White et al., 1972; Kohler, 1972). Related to intestinal motility is the level of secretion of large amounts of sterile fluid by the duodenum which washes out bacteria (Nielson et al., 1968).

2.7.3 Intestinal Microflora

In the developing piglet raised conventionally, while gastric activity is low, bacteria are ingested from the environment and successfully adapted strains proliferate. Probably, the establishment of a healthy and natural microflora occurs here and evolves as the diet changes from milk to solid food. In a healthy young pig the ecology of the gut maintains a homeostasis which is further ensconced in a more mature animal. In the nursing piglet lactobacilli proliferate producing lactic acid which in its acidity inhibits other bacteria. Later, the resident bacteria create antibacterial catabolites including lactic acid and short chain volatile fatty acids to lower the pH. In addition, the resident flora dominate the demand for space and nutrients to prevent other forms from gaining access to grow (Freter and Abrams, 1972; Freter, 1974). Working with continuous flow cultures, Ozawa and Freter (1964) found that resident strains used nearly all energy sources and any invader strain couldn't proliferate unless a source not used by the residents was supplied.

Another factor of the environment of the lumen is that it has a low redox potential which allows only certain strains of bacteria to survive (McClelland, 1979). A further factor influencing bacterial ecology in the gut is that some bacteria can produce specific antimicrobials of limited spectrum which appear to influence the ability of a particular strain to survive (Branche et al., 1963). In treating with antibiotics against an infection (e.g. Salmonella) the magnitude of the challenge dose may be reduced and the infection may be prolonged by reducing the total intestinal flora and allowing the infective strain to proliferate (Aserkoff and Bennett, 1969). The stability of the microflora suggests that immunity is operative against the effects of invasive or mucosal associated bacteria and that low grade pathogens (i.e. the intestinal flora) either are restricted to the gut lumen where immunological interactions are prevented or display a low degree of antigenicity. (Shedlofsky and Freter, 1974).

2.7.4 Non-Immunological Factors in the Secretions of the G.I. Tract

Several non-specific inhibitors of bacterial growth are present in the duodenal secretions and in milk of the nursing individual. Bile salts are metabolized by the resident bacteria and may form anti-metabolites to certain bacteria (McClelland, 1979). Lactoferrin and lysozyme are both present in intestinal secretions and milk and, with secretory IgA, are bactericidal (Hill and Porter, 1974; Knopf et al., 1971). Lactoferrin, which is commonly present in milk chelates iron which restricts bacterial growth (Orson and Reiter, 1981; Bullen et al., 1972). Complement may be involved in reactions in or near the mucosa. The alternate or properdin pathway of complement activation is accepted

as important in the defense against gram negative bacteria of the gut (Hood et al., 1978). This is supported by the discovery that some complement components are synthesized by the intestinal mucosa (Lai A Fat et al., 1976).

2.8 Humoral and Cellular Immunity in Porcine Mammary Secretions

Colostrum is the fluid secreted by the mammary gland during the first 24 hours (Karlsson, 1966) and milk is secreted during the remainder of lactation. IgG is the primary immunoglobulin component of serum and colostrum; IgA is the primary component of milk; and IgM is a minor component of both. In the first 24 hours IgG decreases five-fold and within the first week it decreases thirty-fold. Thus it drops from forming 80% of total colostrum immunoglobulin to 25% of total milk immunoglobulin (Curtis and Bourne, 1971). IgA only decreases three-fold during the first week and hence emerges as the major immunoglobulin in sow milk, accounting for 50 to 60% of milk immunoglobulin (Curtis and Bourne, 1971).

Colostrum functions mainly in protection against systemic infection because the immunoglobulins it contains are absorbed through the gut mucosa into the circulation. This occurs in a period of time during which the piglet intestine absorbs macromolecules intact before gut closure after 24 to 36 hours (Payne and Marsh, 1962). Milk provides copra-antibodies which function within the intestinal lumen during the pre-weaning period (Wilson, 1974; Brandtzaeg, 1973).

Bourne and Curtis (1973) using ^{125}I labelled immunoglobulin determined the proportions of serum derived and locally produced immunoglobulin in mammary secretions. They found that all colostrum IgG came from the serum and that 60% of colostrum IgA was synthesized by the mammary gland (a small portion of total colostrum immunoglobulins), and, consequently, considered that colostrum was a serum transudate (Bourne, 1973). They found that milk, on the other hand, was a true mammary secretion since

more than 90% of the IgA and IgM and 70% of IgG was produced locally.

In conjunction with immunoglobulins, porcine milk contains a trypsin inhibitor which occurs firstly at high levels and falls to a very low level by the fifth day (Laskowski et al., 1957). This probably functions to prevent degradation of colostral immunoglobulins before they can be absorbed in the intestine (Scoot, 1972).

Although no information exists for porcine colostrum, human colostrum shows a relatively high level of T lymphocytes and macrophages (Parmely and Beer, 1977). Parmely and Beer suggested that it was possible, but not proven, that these cells could survive in the gastro-intestinal tract. They further suggested that the mammary tissue may "package" specific cellular components in the colostrum to contribute to immuno-competency in the neonate and that this cell mediated immunity (CMI) from the mammary gland depended upon inductive events at distant mucosal surfaces (bronchial associated and gut associated lymphoid tissues).

2.9 Prewaning Mortality

In a survey of the literature from 1937 to 1976 McCallum (1977) found that levels of preweaning mortality have consistently ranged between 18 and 30 per cent of pigs born (including stillbirths). Two studies (Fahmy and Bernard, 1971; English and Smith, 1974) recorded that 50 per cent of the total preweaning mortality occurred before 3 days of age. Pomeroy (1960) noted that 70.2 per cent of total preweaning mortality occurred before 3 days of age, and Jones (1972) recorded 63.1 per cent. Thus, any attempt to reduce mortality must affect the earliest days of post natal existence.

2.9.1 Causes of Prewaning Mortality

The causes were fairly consistent among the authors cited and are summarized in Table 2.3. These are trauma, starvation, general weakness, disease, congenital abnormalities, and other causes. Trauma involves crushing or trampling of pigs by the sow. Some of the data may reflect the effect of other primary causes predisposing trauma such as malnutrition, general weakness, or chilling. Starvation, a major primary cause of death, could result from two different contributing factors. The first is agalactia of the sow which, in the studies of English and Smith (1974) contributed to 27 per cent of the starvation. The other factor, contributing 73 per cent, is severe competition within litters due either to supernumary pigs in relation to available teats or to disparity in birthweights which results in unequal competitive advantage for heavier pigs (English and Smith, 1974). The common result of starvation is hypoglycaemia (Sharpe, 1966; Edwards, 1972) which may be aggravated by chilling (Curtis, 1974). General weakness, which contributes to high

Table 2.4

A Summary of Major Causes Contributing to Death in Baby Pigs

(per cent contribution)

Reference	Trauma	Starvation	Relative Weakness	Disease	Congenital Abnormality	Other
Sharpe (1966)	21.2	17.1	9.6	9.6	2.1	40.4
Fahmy and Bernard (1971)	19.2	--	26.9	17.5	14.2	22.2
English and Smith (1974)	18.2	42.8	14.9	--	12.3	11.8
Rodeffer <u>et al.</u> , (1975)	30.9	17.6	14.7	18.2	--	13.1

losses, is most clearly associated with parturition aberrations of which the most common result is prenatal anoxia or hypoxia (English and Smith, 1974). "Parturition aberrations" include premature umbilical cord rupture and a prolonged farrowing interval between births (Rodeffer et al., 1975). Disease, as a cause of death, includes different diseases depending on the study. Sharpe (1966) listed the major causes as gastroenteritis and septicaemia. Fahmy and Bernard (1971) listed scours and pneumonia as major disease factors. Rodeffer et al., (1975) listed transmissible gastroenteritis (T.G.E.), other diarrheas, and pneumonia. Congenital abnormalities varied among the studies. English and Smith (1974) listed the major traits including atresi ani, cardiac abnormality, congenital splay-leg, cleft palate, and hypoplasia kidney.

2.9.2 Effect of Low Birthweight on Survival

"The effect of birth weight on survival is essentially linear within a wide range of values" (Bereskin et al., 1973). Thus, low birth-weight is a major contributing factor to reducing survival (Sharpe, 1966; Fahmy and Bernard, 1971; Bereskin et al., 1973). Table 2.4 lists mortality values found for low birthweight pigs by several researchers. These high mortality rates may vary with strain of pig or environment which, in turn, may include temperature and milking ability of the sow (Pomeroy, 1960). These high mortality rates reflect a greater susceptibility to the major causes of death. There was a higher incidence of trauma in low birthweight pigs (Sharpe, 1966); a markedly higher incidence of starvation (Sharpe, 1966; English and Smith, 1974); and low birthweight had some effect on the incidence of septicaemia (Sharpe, 1966). Also, low birth-weight pigs are much more susceptible to chilling. Newland et al., (1952)

Table 2.5

Effect of Birth Weight on Mortality

Reference	Weight Range	Per Cent Mortality
Pomeroy (1960)	<900g	83.0 (before 3 days)
Sharpe (1966)	<800g	82.1
Fahmy and Bernard (1971)	<910g	60.0
English and Smith (1974)	<907g	74.6

found that the drop in body temperature after chilling was inversely related to body weight.

Pomeroy (1960) cited three factors contributing to higher mortality in low birth weight pigs:

1. they may be less vigorous and active, and less competitive at the teats during nursing.
2. they have a larger surface area in relation to body weight. Therefore there is a relatively greater heat loss per unit of body weight.
3. they may be physiologically immature.

Foot-Notes

1. Immunocytes include all cells of the immune system.
2. The failure of the immune system, as a result of previous contact with antigen, to respond to the same antigen, although capable of responding to others. Tolerance is best established by neonatal injection of an antigen (Benaceraf and Unanue, 1979).
3. An exotoxin is a soluble poisonous substance passing into the medium during growth of a microorganism. An endotoxin is a poisonous substance present in bacteria but separable from the cell body only on its disintegration. An enterotoxin is a toxin of bacteria produced within and affecting the intestine. (Websters 7th New Collegiate dictionary, 1965)

3. Experimental

3.1 Introduction

Colostrum-deprived pigs have been successfully reared in a non-isolated environment since 1961 (Owen et al., 1961). After work by Owen and Bell (1964), Scoot (1972), and McCallum (1977), McCallum (1977) concluded that it was possible "to raise colostrum-deprived neonatal pigs in an ordinary swine barn environment and achieve survival rates comparable to those presently tolerated under natural conditions" using abattoir serum-derived immunoglobulins in milk replacers.

Both Scoot (1972) and McCallum (1977) concluded that oral administration of 10 grams per kilogram of body weight of immunoglobulins on the first day followed by 2 grams per kilogram on succeeding days could confer adequate passive immunity on colostrum-deprived pigs. Scoot (1972) found that administration of immunoglobulins over 21 days increased survival compared to 10 day treatment (from 75 per cent to 88 per cent) and McCallum (1977) observed a sharp increase in mortality following removal of immunoglobulin after 10 days compared to those continued on treatment for 20 days.

The purpose of experiments reported herein was to evaluate the effect of orally administered immunoglobulins on colostrum-deprived pigs of low birthweight. Experiments of Lodge and Elliot (1979), using the artificial rearing technique of McCallum et al., (1977), found that 21 day weight, 56 day weight and age at 90kg. were highly correlated with birthweight, but that low birthweight was less inhibiting under artificial rearing than under natural rearing. Lodge and Elliot (1979) also found that artificial rearing reduced mortality of low birthweight pigs.

The trials described herein were carried out in a barn environment on a commercial farm ¹ in an environmentally controlled room adjacent to the farrowing rooms. A series of 4 experiments was carried out to determine effects of low birth weight on survival and growth.

3.2 Experiment I

3.2.1 Objective

The objective of this experiment was to assess the efficacy of abattoir derived porcine immunoglobulin extract from serum as a milk replacer additive for rearing low birthweight pigs.

3.2.2 Materials and Methods

3.2.2.1 Experimental Animals

Twenty-four low birth weight pigs weighing less than 1000g of Yorkshire X Landrace breeding were allotted within a ten day period. Farrowings were attended and experimental pigs were removed from the sow at birth or 12 hours later. Pigs were weighed at birth, ear tagged, and individually penned in a nursery room. Pigs were randomly assigned to treatments. Cages were adjoining wire mesh 46cm x 25cm x 20cm in two tiers. Prior to the trial the room and cages were thoroughly cleaned and disinfected. Cages were washed periodically during the trial. Temperature in the nursery room was maintained between 30°C and 33°C. All pigs received iron dextran injections and had their needle teeth and tails clipped at two days of age. A second iron dextran injection was given at 10 days of age.

3.2.2.2 Preparation of Porcine Immunoglobulins

Immunoglobulins used in all trials were prepared by Canada Packers Ltd., Toronto, Ontario. The immunoglobulin fraction derived at 40 per cent saturation with $(\text{NH}_4)_2\text{SO}_4$ was washed, dialyzed, mixed with condensed whole milk and spray dried (Owen, personal communication).

3.2.2.3 Dietary Treatments

In all treatments, pigs received a non-medicated commercial milk replacer². Feed intake was adjusted to 7.5 per cent of their body

weight in air dry matter which was diluted 1 : 4 with water. For the first 3 days diets were formulated with a 10 per cent dextrose solution. Pigs were weighed every other day and feed allowances were adjusted accordingly. Immunoglobulin was mixed with the milk replacer and fed at levels set out in the following schedule:

1. No colostrum, milk replacer only.
2. Colostrum for 12 hours, followed by milk replacer only.
3. Colostrum for 12 hours, plus immunoglobulin at 2g/kg body weight/day for 9.5 days.
4. Immunoglobulin extract on first day (10g/kg body weight), plus immunoglobulin (2g/kg body weight/day) for 9 days.

Scouring animals were treated, as necessary, on an individual pig basis with Furoxone³. The trial was of 21 days duration.

3.2.2.4 Feeding Regimen

Since the pigs would not feed from shallow bowls immediately after birth, they were force fed with syringes until they would nipple feed. In later trials nipple feeding was successful from birth but during this trial some difficulty was encountered. At 10 days of age all pigs were switched to shallow bowls. Feeding was every 2 hours for the first 4 days, every 4 hours on days 5 to 8 and every 6 hours from days 9 to 21. Fresh water was made available from day 5 and creep feed was offered fresh daily after 10 days of age.

3.2.3 Measurements and Observations

Pigs were weighed every 2 days. Mortality was recorded and post mortems were carried out on all dead pigs by the Provincial Veterinary Laboratory, Abbotsford, B.C. Infectious agents and causative factors were

identified whenever possible.

Mortality was analyzed statistically by giving a survivor a zero value and a dead pig a 1 value. The analysis used was analysis of variance (ANOVA) with the design model being:

$$Y_{ij} = \mu + T_i + \epsilon_{ij} \quad \text{where } T_i = \text{Treatment Effects}$$

$$\mu = \text{Population Mean}$$

$$\epsilon_{ij} = \text{Experimental Error}$$

Rate of gain was also analyzed with this model.

The mean birth-weights of survivors and dead pigs were compared with a "t" test (Choi, 1978).

3.2.4 Results and Discussion

Mortality was high (Table 3.1.b) with all pigs in the negative control group dying before 10 days of age. Those which received colostrum plus supplemental immunoglobulin extract for 10 days showed the highest survival. The differences between treatments were not significant ($P > 0.05$). The mean birth-weight of the survivors was significantly higher than that of the dead pigs ($P < 0.01$). This difference suggests that higher birth-weight pigs had an advantage in this trial.

The cause of death was predominantly aspiration pneumonia complicated by *E. coli* septicaemia. The pneumonia was invariably caused by the presence of foreign material in the bronchi and lungs introduced while force feeding with syringes. A major characteristic of dead pigs, regardless of treatment, was a flaccid, dilated intestine or distended stomach. The cause of this state could have been over-feeding and could have involved *E. coli* enteritis. All pigs were diarrhetic before death. Most pigs which died showed a marked lack of depot fat indicating no energetic reserves for survival, and

Table 3.1a Experiment 1: Experimental Animals

<u>Treatment</u>	<u>Number in Treatment Group</u>	<u>Mean Birth-Weight ± S.D.</u>
No colostrum; no immuno-globulin	6	765 ± 157 g
Colostrum; no immuno-globulin	6	854 ± 121 g
Colostrum + immunoglobulin (2g/kg/day)	6	843 ± 190 g
Immunoglobulin (10g/kg/day to 2g/kg/day)	6	752 ± 190 g

Table 3.1b Experiment 1: Results

<u>Treatment</u>	<u>% Survival to Day 10</u>	<u>% Survival to Day 21</u>	<u>Rate of Gain (g/day)</u>
No colostrum; no immuno-globulin	0	0	
Colostrum; no immuno-globulin	33	33	46.5 ± 24.2
Colostrum + immunoglobulin (2g/kg/day)	50	50	42.1 ± 23.5
Immunoglobulin (10g/kg/day to 2g/kg/day)	16	16	7.5 (1 pig)-

dehydration, no doubt a result of the diarrhea.

A higher level of disease carrying microbes in the environment might have been present due to the reservoir of infection carried by the negative control animals which were all ill from the beginning of the trial. Sanitation and high moisture levels were difficult to control by washing the cages. The resulting moist, humid, and non-sterile conditions may have been ideal for the growth of microbes.

It was assumed that a 12 hour nursing period was adequate for attaining sufficient immunoglobulin levels in the plasma. This is supported by work of Scoot (1972) and Carlson and Lecce (1973).

3.3 Experiment II

3.3.1 Objective

The objective of this experiment was to further assess the efficacy of abbatoir derived porcine serum immunoglobulin extract as a milk replacer additive for rearing low birth weight pigs. To reduce the pool of infection, negative controls receiving no immunoglobulins (artificial or colostrum derived) were not included in this trial.

3.3.2 Materials and Methods

3.3.2.1 Experimental Animals

Twenty-four low birth weight pigs weighing less than 1000g of Yorkshire X Landrace breeding were allotted within a ten day period. Farrowings were attended and experimental animals were removed at birth or 12 hours later. Pigs were weighed at birth, ear tagged, and individually penned in a nursery room. Pigs were randomly assigned to treatments. Cages were adjoining wire mesh 46cm x 25cm x 20cm in two tiers. Prior to the trial the room and cages were thoroughly cleaned and disinfected. Cages were left unwashed throughout the trial period. Temperature in the nursery room was maintained between 30°C and 33°C. All pigs received iron dextran injections and had their needle teeth and tails clipped at two days of age. A second iron dextran injection was given at 10 days of age.

3.3.2.2 Dietary Treatments

Pigs received a non-medicated commercial milk replacer ². Feed intake was reduced from the level in experiment I to 6 per cent of body weight in air dry matter which was diluted 1 : 4 with water. For the first 3 days diets were formulated with a 10 per cent dextrose solution. Pigs were weighed every other day and feed allowances were adjusted accord-

ingly. Immunoglobulin extract was mixed with the milk replacer and fed at levels set out in the following schedule of treatments:

1. Colostrum for 12 hours, followed by milk replacer only.
2. Colostrum for 12 hours, followed by immunoglobulin at 2g/kg body weight/day for 9.5 days.
3. Immunoglobulin (10g/kg body weight) on the first day followed by immunoglobulin at 2g/kg body weight/day for 9.5 days.

Scouring animals were treated, as necessary, on an individual pig basis with Anistat⁴, a broad spectrum antibiotic. The trial was of 21 days duration.

3.3.2.3 Feeding Regimen

Pigs were nipple fed for one week then changed to bowl feeding for the remainder of the trial. Feeding was every 2 hours for the first 4 days, every 4 hours on days 5 to 8, and every 6 hours from days 9 to 21. Fresh water was available to them from day 5 and creep feed was offered fresh daily after 10 days of age.

3.3.3 Measurements and Observations

Pigs were weighed every 2 days. Mortality was recorded and post mortems were conducted on all dead pigs by the Provincial Veterinary Laboratory, Abbotsford, B.C.. Infectious agents and causative factors were identified whenever possible.

Mortality and rate of gain were analysed as in Experiment 1, using analysis of variance. The mean birth weights of survivors and dead pigs were compared with a "t" test (Choi, 1978).

3.3.4 Results and Discussion

Mortality (Table 3.2.b) decreased from the level achieved in Experiment 1. This may be attributed to improved technique, elimination of the negative control group (as a pool of infection), and improved environmental conditions. The group receiving only colostrum for 12 hours showed a mortality rate at 21 days significantly higher than the other two groups ($P = 0.05$). There was no significant difference in mortality between those receiving colostrum with immunoglobulin and those receiving immunoglobulin extract instead of colostrum.

Since it was observed in Experiment 1 that feeding at 7.5 per cent of body weight led to a distended stomach and possible gastric stasis producing scours, the level of feeding was reduced to 6 per cent of body weight in air dry matter, and this problem was consequently reduced. White et al., (1969) found in their trials, that scour was always preceded by gastric stasis. They found that a direct relationship existed between pH and growth of bacteria in the stomach. A lack of lactic acid producing organisms in the stomach of the achlorhydric pig, with resultant high pH in the stomach, might lead to rapid growth conditions for coliforms which could then pass to the intestine and cause a scour syndrome. Endotoxin release in the stomach by coliform bacteria could also lead to reduced gastric acid secretion exacerbating the conditions.

There was no significant difference between birth weights of surviving versus dead pigs.

Table 3.2a Experiment 2: Experimental Animals

<u>Treatment</u>	<u>Number in Treatment Group</u>	<u>Mean Birth-weight ± S.D.</u>
Colostrum; no immuno- globulin	8	677 ± 111 g
Colostrum + immunoglobulin (2g/kg/day)	8	707 ± 170 g
Immunoglobulin (10g/kg/day to 2g/kg/day)	8	736 ± 143 g

Table 3.2b Experiment 2: Results

<u>Treatment</u>	<u>% Survival to Day 10</u>	<u>% Survival to Day 21</u>	<u>Rate of Gain (g/day)</u>
Colostrum; no immuno- globulin	37	0 ^a	
Colostrum + immunoglobulin (2g/kg/day)	75	63 ^b	35.2 ± 15.4 g
Immunoglobulin (10g/kg/day to 2g/kg/day)	63	50 ^b	39.8 ± 16.9 g

(differing superscripts denote statistically different values at
P= .05)

Causes of mortality were general septicaemia for 6 pigs probably due to coliform infection, definite coliform septicaemia for 3 pigs, and coliform enteritis with septicaemia for 7 pigs. All dead pigs showed characteristics of emaciation and dehydration which may be a result of the diarrhea which occurred in all animals which died.

Rate of gain did not differ significantly between the two groups of surviving pigs (Table 3.2 b) and overall remained at a very low level. Consumption of creep feed and water supplied ad libitum could not be weighed accurately because of wastage.

3.4 Experiment III

3.4.1 Objective

The objective of this experiment was to compare a lower level of immunoglobulin extract administration (10g/kg body weight for the first day followed by 2g/kg body weight/day for 9 days) with a higher immunoglobulin level (15g/kg body weight for the first day followed by 5g/kg body weight/day). Treatment groups of pigs nursing colostrum for 12 hours instead of high initial doses of immunoglobulin were also included for comparison.

3.4.2 Materials and Methods

3.4.2.1 Experimental Animals

Two consecutive replicates were carried out. To each replicate 24 low birthweight pigs of less than 1000g of Yorkshire X Landrace breeding were allotted within a 10 day period. Farrowings were attended and experimental pigs were removed at birth or 12 hours later. Pigs were weighed at birth, ear tagged, and individually penned in a nursery room. Pigs were randomly assigned to treatments. Cages were adjoining wire mesh 46cm x 25cm x 20cm in two tiers. Prior to the trial the room and cages were thoroughly cleaned and disinfected. Cages were left unwashed throughout the trial period. Temperature in the nursery was maintained between 30°C and 33°C. All pigs received iron dextran injections and had their needle teeth and tails clipped at two days of age. A second iron dextran injection was given at 10 days of age.

3.4.2.2 Dietary Treatments

Pigs received a non-medicated commercial milk replacer². Feed intake was set at 6% of body weight of air dry matter which was diluted

1 : 4 with water. For the first 3 days diets were formulated with a 10 per cent dextrose solution. Pigs were weighed every other day and fed accordingly. Immunoglobulin extract was mixed with the milk replacer and fed at levels set out in the following schedule of treatments:

1. 15g/kg bodyweight immunoglobulin on the first day followed by 5g/kg body weight/day for 9 days.
2. 10g/kg body weight immunoglobulin on the first day followed by 2g/kg body weight/day for 9 days.
3. 12 hours of colostrum nursing followed by 9.5 days of immunoglobulin at 5g/kg body weight/day.
4. 12 hours of colostrum nursing followed by 9.5 days of immunoglobulin at 2g/kg/day.

In replicate 1 the antibiotic Anistat⁴ was used. In replicate 2 the antibacterial Furoxone³ was used.

3.4.2.3 Feeding Regimen

Pigs were nipple fed for one week then changed to bowl feeding for the remainder of the trial. Feeding was every 2 hours for the first 4 days, every 4 hours on days 5 to 8, and every 6 hours from days 9 to 21. Fresh water was available from day 5 and creep feed was offered fresh daily after 10 days of age.

3.4.3 Measurments and Observations

Measurments and statistical analyses were the same as in the two previous experiments except that another dependant variable was included, frequency of scouring. The design was a randomized block design since two replicates were carried out.

3.4.4 Results and Discussion

Mortality (Table 3.3.b) was similar to that in Experiment 2 where it was approximately 50 per cent in the treated groups. There was no significant difference between treatments for mortality. Thus, the results indicated no significant difference between those receiving colostrum and those receiving immunoglobulin extract, or between those receiving a higher level of immunoglobulin and those receiving a lower level. The assumption that 12 hours of nursing was adequate was based on the findings of Scoot (1972) and Carlson and Lecce (1973). This assumption relies on the pig receiving adequate colostrum during nursing. However, low birth weight pigs are frequently disadvantaged in this respect, not being as competitive or not being able to reach a teat. Thus, in some cases within this trial, individuals may not receive an adequate level of systemic passive immunity which may lead to susceptibility to infection affecting the mortality rate. As was the case, those receiving artificial immunoglobulin extract instead of colostrum, showed a high survival before day 10, but the difference was not significant. The difference between mean birth weights of survivors and dead pigs was not significant. It was observed that

Table 3.3a Experiment 3: Experimental Animals

<u>Replicate</u>	<u>Treatment</u>	<u>Number in Treat- ment Group</u>	<u>Mean Birth-Weight ± S.D.</u>
1	Immunoglobulin (15g/kg /day to 5g/kg/day)	6	761 ± 166 g
	Immunoglobulin (10g/kg /day to 2g/kg/day)	6	729 ± 227 g
	Colostrum + immuno- globulin (5g/kg/day)	6	835 ± 99 g
	Colostrum + immuno- globulin (2g/kg/day)	6	746 ± 192 g
2	Immunoglobulin (15 to 5 g/kg/day)	6	814 ± 83 g
	Immunoglobulin (10 to 2 g/kg/day)	6	671 ± 96 g
	Colostrum + immuno- globulin (5g/kg/day)	6	704 ± 218 g
	Colostrum + immuno- globulin (2g/kg/day)	6	834 ± 59 g

Table 3.3b Experiment 3: Experimental Results

Replicate	Treatments	% Survival to Day 10	% Survival to Day 21	Rate of Gain (g/day)	Scouring Frequency days scouring/survivor)	Mean Scouring Frequency
1	15 g/kg for Day 1 then 5 g/kg/day for 9 days		50	46.3 ± 4.0 ^a	7	
	10 g/kg for Day 1 then 2 g/kg/day for 9 days		50	39.7 ± 1.2 ^b	5.3	
	Colostrum for 12 hrs.; 5g/kg/day for 9.5 days		33	51.5 ± 0.7 ^a	5	
	Colostrum for 12 hrs.; 2g/kg/day for 9.5 days		66	55.3 ± 8.8 ^a	3.3	5.0 ± 2.6 ^c
2	15 g/kg for Day 1 then 5 g/kg/day for 9 days		50	55.7 ± 3.5 ^a	9	
	10 g/kg for Day 1 then 2 g/kg/day for 9 days		83	55.7 ± 3.5 ^a	8.8	
	Colostrum for 12 hrs.; 5 g/kg/day for 9.5 days		17	59.0(1 only) ^a	15	
	Colostrum for 12 hrs.; 2 g/kg/day for 9.5 days		50	60.0 ± 8.7 ^a	7	8.9 ± 3.5 ^d
Combined	15 g/kg for Day 1 then 5 g/kg/day for 9 days	84	50	51.0 ± 6.1 ^a		
	10 g/kg for Day 1 then 2 g/kg/day for 9 days	84	66	41.0 ± 6.5 ^b		
	Colostrum for 12 hrs.; 5 g/kg/day for 9.5 days	75	25	54.0 ± 4.4 ^a		
	Colostrum for 12 hrs.; 2 g/kg/day for 9.5 days	75	58	57.3 ± 8.4 ^a		

under 500g birth weight there was no survival, however the number of observations was few.

Cause of death during the first replicate, in 11 cases was Salmonellosis causing diarrhea and septicaemia. The actual serotype was identified as Group B *Salmonella* in 4 cases while in the remaining cases serotype was unidentified. In one case death was due to *E. coli* enteritis. During the second replicate, *Salmonella* were heavily implicated, but, due to the interference of antibiotics given during treatment, could not be isolated in all cases. *Klebsiella* was also isolated from two pigs.

Rate of gain (Table 3.3.b) was increased over that found in Experiment II. Those receiving immunoglobulin at the lower level showed a significantly lower rate of gain in both replicates ($P = 0.05$) (Figures 3.1.a and 3.1.b). Since the pigs receiving 2g/kg body weight/day of immunoglobulin after receiving colostrum showed a significantly higher ($P = 0.05$) growth rate than those which received artificial immunoglobulin (10g/kg body weight on the first day) instead of colostrum, the effect may be due to an insufficient initiating dose of 10g/kg body weight for passive systemic immunity. However, even if this treatment affected growth rate, it did not show any significant affect on mortality rate. Both Scoot (1972) and McCallum (1977) found that the higher dosage of immunoglobulins (15 and 5g/kg body weight/day) increased rate of gain slightly. Scoot (1972) indicates that blood globulin levels were not as high with artificially supplemented as with sow nursed pigs.

Frequency of scouring of the survivors showed no significant differences between the scheduled treatments, however, the frequency of scouring was significantly different between the two replicates (Table

Figure 3.1.a Growth in Experiment 3, Replicate 1

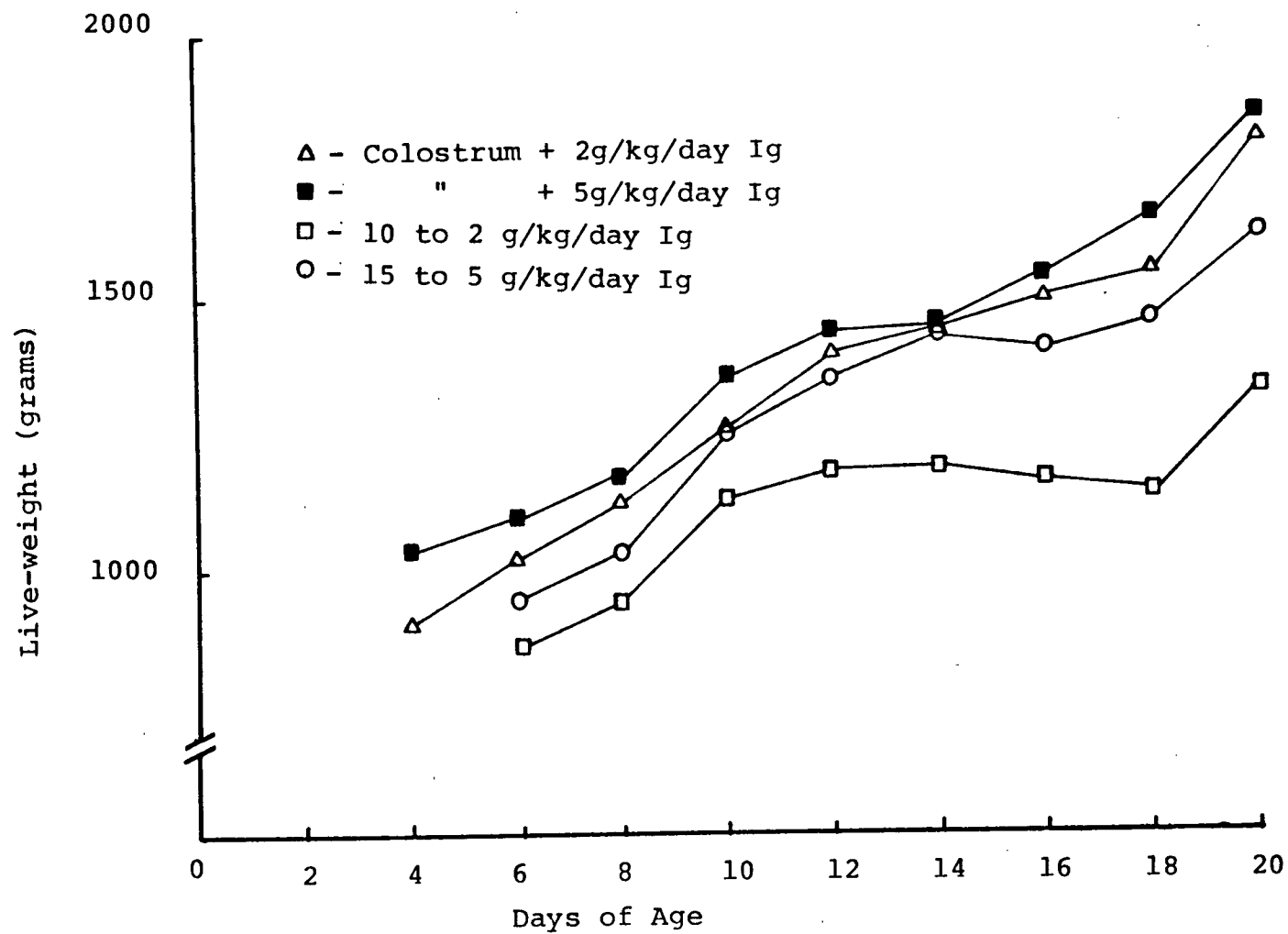
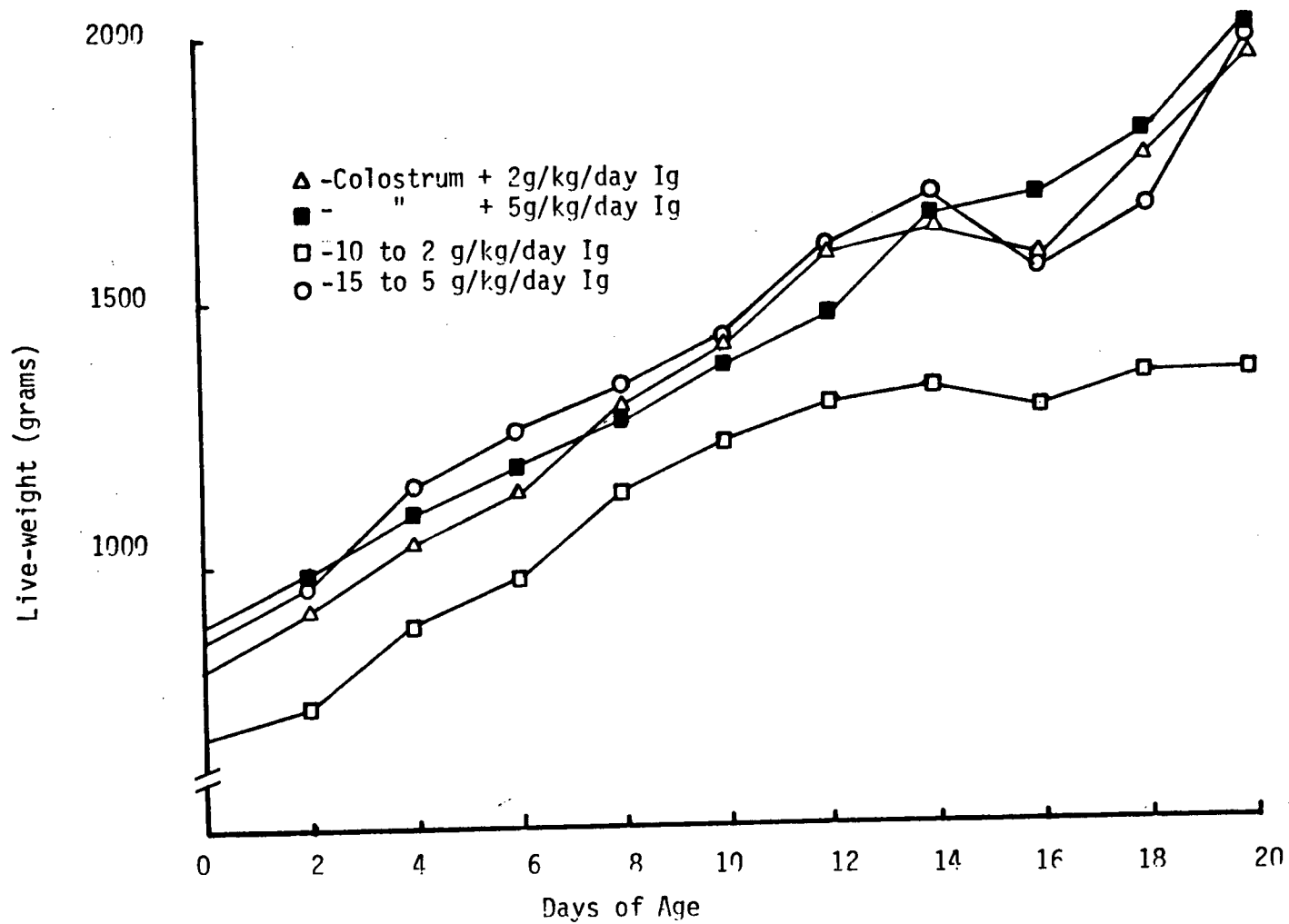
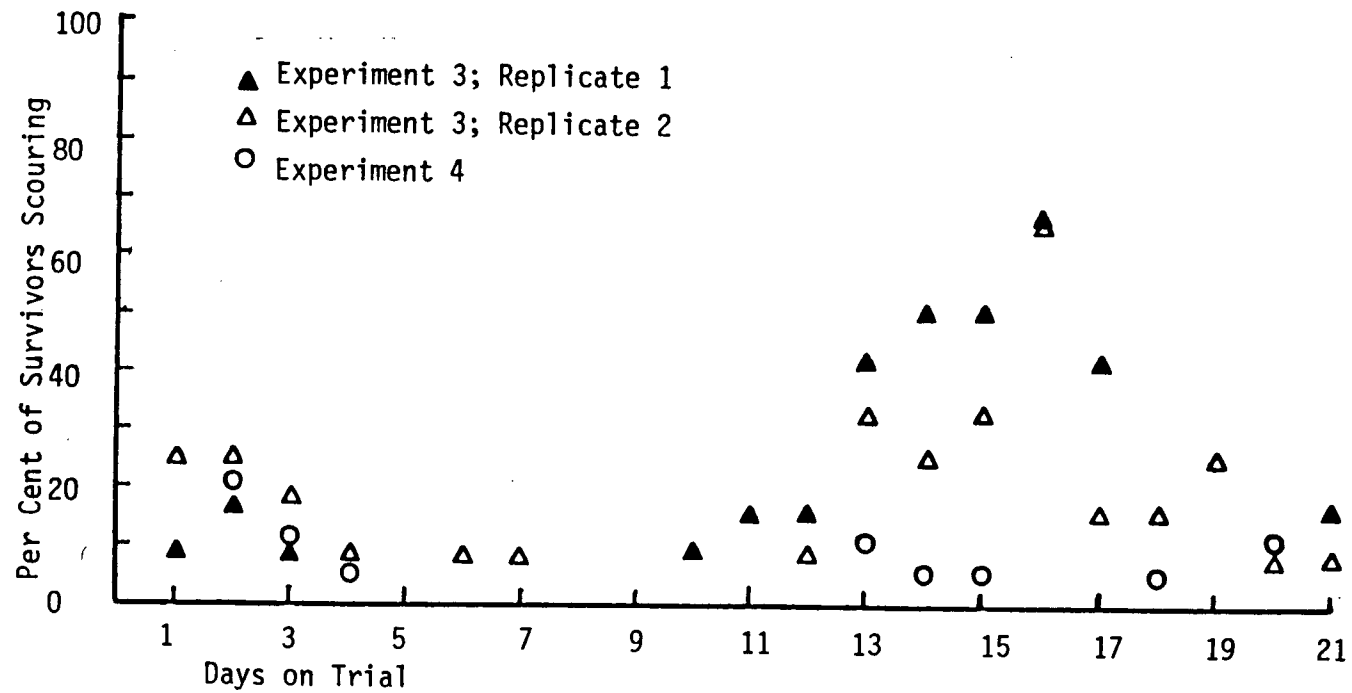


Figure 3.1.b Growth in Experiment 3, Replicate 2



3.3.b). The obvious reason for this difference seems to have been that different anti-scour drugs were used in each replicate suggesting that the antibiotic Anistat⁴ was more effective in controlling scours than the antibacterial Furoxone³. In both replicates, the scouring frequency of survivors increased the highest level after removal of pigs from immunoglobulin supplementation at 10 days of age (Figure 3.2). This suggests that the pigs required further passive protection with immunoglobulins beyond the tenth day.

Figure 3.2. Scouring Frequency (per cent of survivors scouring)



3.5 Experiment IV

3.5.1 Objective

The objective of this experiment was to compare periods of immunoglobulin feeding. The periods selected for comparison were 10, 15 and 21 days on immunoglobulin extract at a level of 10g/kg body weight for the first day followed by 2g/kg body weight/day for 9 days.

3.5.2 Materials and Methods

3.5.2.1 Experimental Animals

Twenty-four low birthweight pigs of less than 1000g of Yorkshire X Landrace breeding were allotted within a ten day period. Farrowings were attended and experimental pigs were removed at birth. Pigs were weighed at birth, ear tagged, and individually penned in a nursery room. Pigs were randomly assigned to treatments. Cages were adjoining wire mesh 46cm x 25cm x 20cm in two tiers. Prior to the trial the room and cages were thoroughly cleaned and disinfected. Cages were left unwashed throughout the trial period. Temperature in the nursery room was maintained between 30°C and 33°C. All pigs received iron dextran injections and had their needle teeth and tails clipped at two days of age. A second iron dextran injection was given at 10 days of age.

3.5.2.2 Dietary Treatments

Pigs received a non-medicated commercial milk replacer². Feed intake was adjusted to 6 per cent of body weight in air dry matter which was diluted 1 : 4 with water. For the first 3 days diets were formulated with a 10 per cent dextrose solution. Pigs were weighed every other day and feed allowances were adjusted accordingly. Immunoglobulin was mixed with the milk replacer and fed at one level: 10g/kg body weight

on the first day followed by 2g/kg body weight/day for succeeding days. Three periods of immunoglobulin feeding were applied: 10 days, 15 days, and 21 days.

Scouring was treated, as necessary, on an individual pig basis, with Furoxone³.

3.5.2.3 Feeding Regimen

Pigs were nipple-fed throughout the 21 day trial period. Creep feed was offered from day 10 but water was offered periodically, only, by bottle, to prevent spillage. Feeding was every 2 hours for the first 4 days, every 4 hours on days 5 to 8 and every 6 hours from days 9 to 21.

3.5.3 Measurements and Observations

Pigs were weighed every 2 days. Mortality was recorded and post mortems were conducted on all dead pigs by the Provincial Veterinary Laboratory, Abbotsford, B.C. Infectious agents and causative factors were identified whenever possible.

Mortality, rate of gain, and frequency of scouring (for survivors) were statistically analysed with analysis of variance using the same model as in Experiment 1. Also the mean birth weights of survivors and dead pigs were compared with a "t" test.

3.5.4 Results and Discussion

Mortality rate (Table 3.4.b) improved compared to previous

Table 3.4a Experiment 4: Experimental Animals

<u>Treatment</u>	<u>Number in Treatment Group</u>	<u>Mean Birth-weight ± S.D.</u>
10 days on immunoglobulin	8	674 ± 148 g.
15 days on immunoglobulin	8	708 ± 111 g.
21 days on immunoglobulin	8	749 ± 116 g.

Table 3.4b Experiment 4: Results

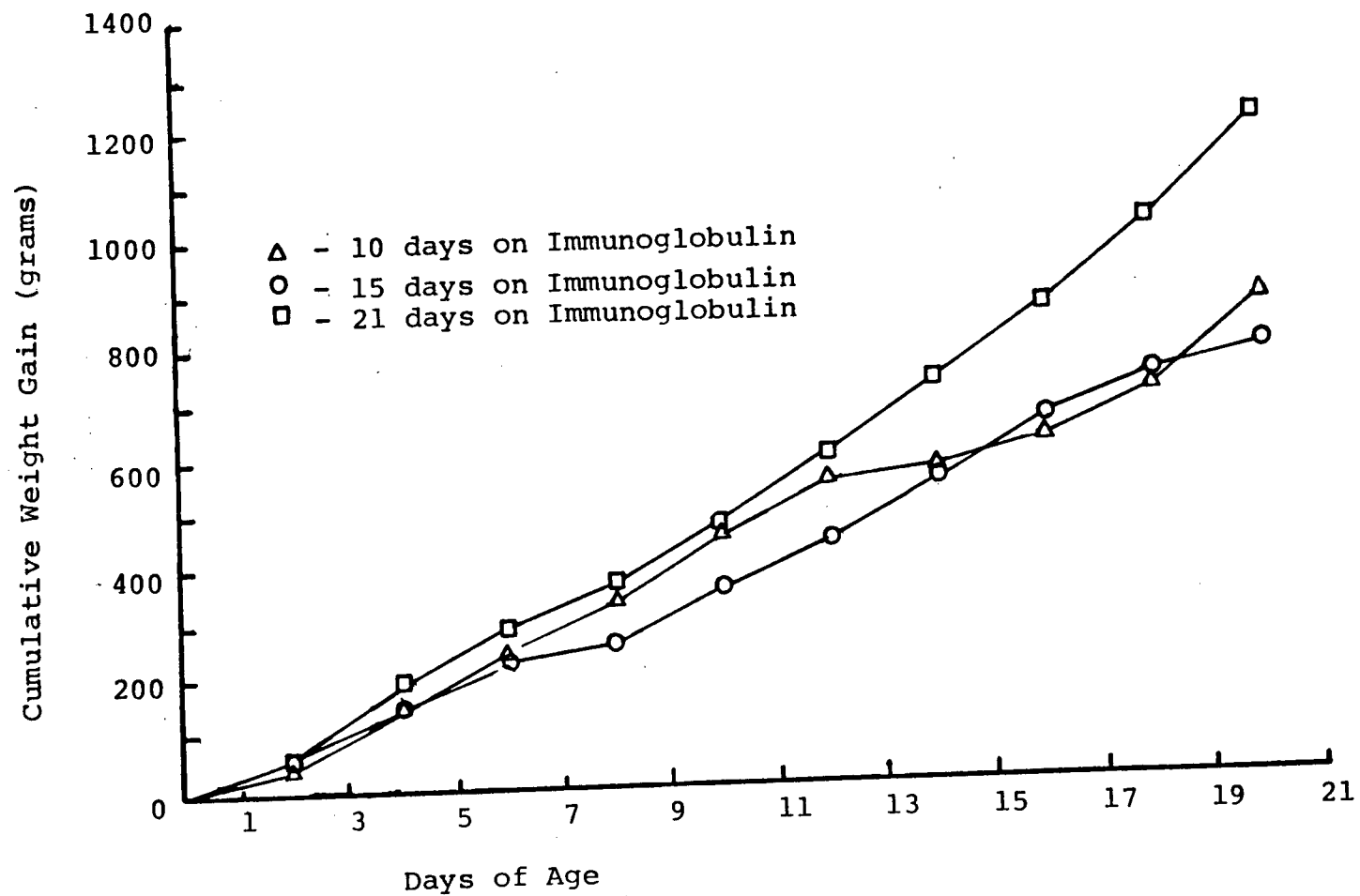
<u>Treatment</u>	<u>% Survival to Day 10</u>	<u>% Survival to Day 21</u>	<u>Rate of Gain (g/day)</u>	<u>Frequency of Scouring (Days scouring/ survivor)</u>
10 days	63	50 ^a	40.8 ± 23.2 ^{cd}	1.0
15 days	100	88 ^{ab}	37.4 ± 14.5 ^c	1.1
21 days	100	100 ^b	60.3 ± 10.6 ^d	0.3

trials. Mortality was significantly higher ($P = 0.05$) on the 10 day treatment compared to the 21 day treatment. However, 3 out of 4 which died in the 10 day treatment group did so while receiving immunoglobulin before 10 days of age. Therefore, the result is spurious. The effect of prolonged feeding of immunoglobulin definitely reduced mortality by maintaining better health and preventing scours in those pigs receiving immunoglobulin after 10 days. Death was due to bacterial infection causing diarrhea in 4 cases and pneumonia in 1 case. Salmonella was isolated from 4 of the 5 dead and presumed present also in the fifth. Thus the infection causing death was diagnosed as Salmonellosis in all cases. There was no significant difference between mean birth weights of survivors and dead pigs.

Rate of gain (Table 3.4.b and Figure 3.3) was highest in the group receiving immunoglobulins for 21 days, but only significantly higher ($P = 0.05$) than the group receiving immunoglobulins for 15 days. The 21 day group had the highest mean rate of gain of all groups in the four experiments carried out.

Frequency of scouring (Table 3.4.b) was very markedly reduced during this trial compared to Experiment III where it was also recorded. The group receiving immunoglobulin for 21 days showed no diarrhea after 4 days of age and the other two groups showed a much reduced frequency between 13 and 20 days of age (Figure 3.2). Since bowl feeding was eliminated and nipple-feeding was continued throughout, chances of spreading contamination in the feed were likely markedly reduced. Water provision was limited to periodic bottle feeding since it couldn't be provided ad libitum without spilling leading to a wet

Figure 3.3. Cumulative Weight Gain. Experiment 4



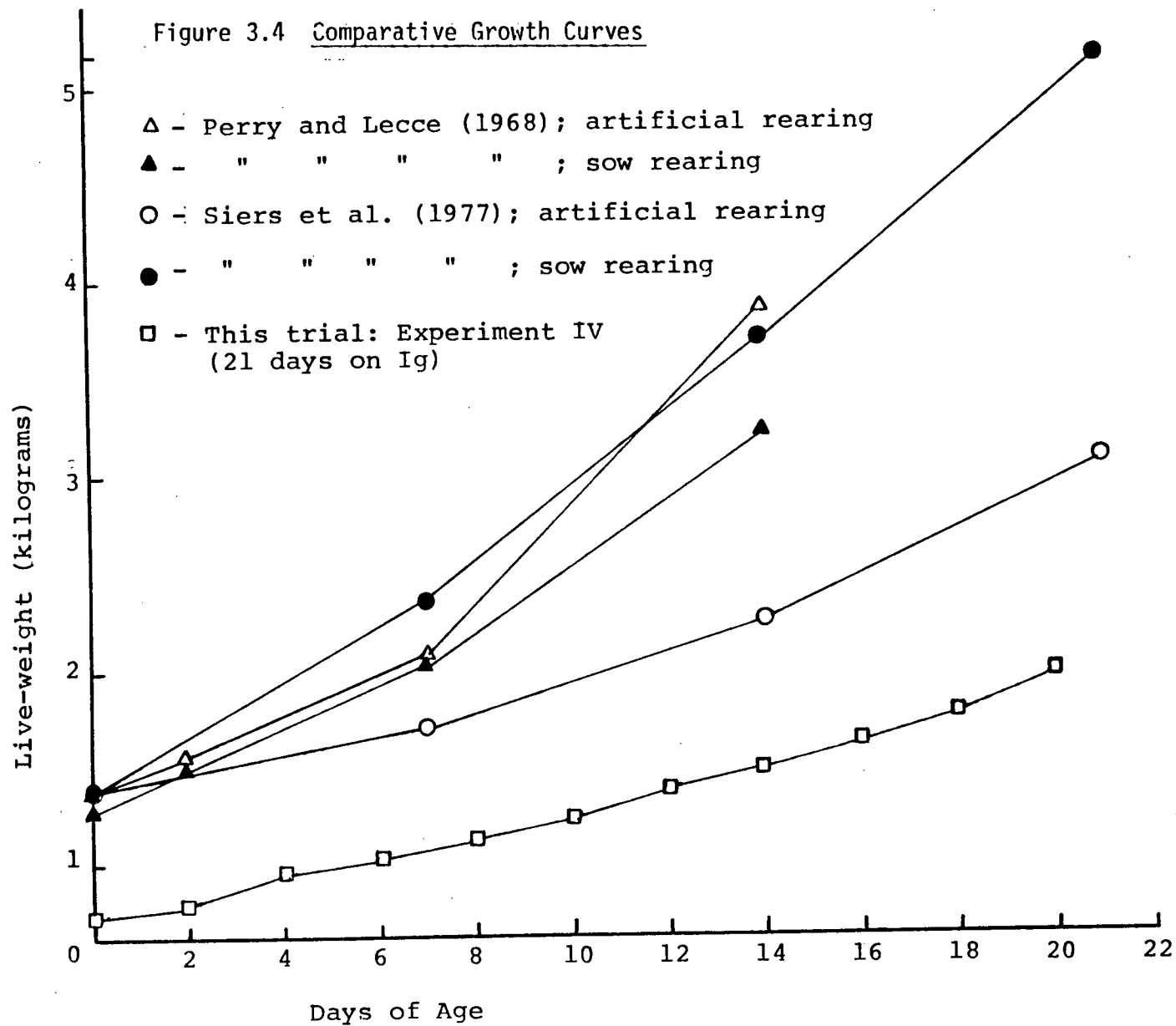
environment which seemed to be detrimental to the pigs from experience in the preceding trials.

3.6 General Discussion

The approach of this study was two-fold. Firstly, artificial rearing was seen as a mean for increasing survival of low birth-weight pigs. Secondly, this artificial rearing technique with immunoglobulin extract was applied in a non-isolated environment. Thus, the results can be discussed from both perspectives.

An important observation in these trials was a lower rate of gain than previously found by McCallum (1977) using a very similar diet, and by other workers (Figure 3.4). The rate was highest with the 21 day treatment in Experiment IV where scouring was markedly reduced but was still well below rates found with sow reared pigs of low birth weight within the same herd (60g/day vs 125g/day). Two outstanding reasons for this are evident. Firstly, the pigs on trial may have been restricted in their food intake too much for optimum growth. Considering the diet to be adequate, the main contributing factor could be too infrequent feeding since their intake at each feeding was limited by their capacity. However, without automatic feeding, more frequent feeding would be difficult. Secondly, the lower rate of gain may have been due to their low birth weights.

Low birthweight seems to have a definite effect on rate of gain. However, Lodge and Elliot (1979) indicated that a lower birth-weight was less inhibiting on growth under artificial rearing than under natural rearing. Widdowson (1971), in a trial, showed that runt pigs never reach the potential of large litter mates. One must distinguish between low birthweight pigs which are normal anatomically, but small, and true runts. Not all pigs under 1000g are necessarily runts. The



manifestation of runt characteristics is largely indicated by size, however pigs above 600g don't seem to show any runting appearance. True runts seem underdeveloped, possibly due to prenatal undernourishment. Their physical appearance of very small size and disproportionately large domed heads suggests this. Small, but non-runt pigs may not be as disadvantaged as true runts when provided adequate nutrition and hence may show adequate compensatory growth to make up for low birthweight. Indeed, in all but the first trial, birthweight did not affect survival. However, all those below 500g at birth, died. It is possible that the survival of very small true runts (e.g. below 500g birthweight) requires more extraordinary attention to hygiene and environment.

During sampling of the sow-reared low birthweight pigs, variability on growth rate was found to be high (coefficient of variation = 30.4 per cent). This is understandable if the operative factors determining growth at this pre-weaning age are considered. Such factors include:

1. Nursing success which is determined by aggressiveness, milk production of the sow, and access to a productive teat.
2. Freedom from scours or other disease maladies.
3. Genetic potential of the individual.

Since low birthweight pigs are less competitive during nursing and are more susceptible to chilling (as explained by the relationship between weight, surface area, and heat loss (Monteith and Mount, 1974)), artificial rearing in a controlled environment would eliminate many of the stressful factors and allow a more equitable situation. Runts may be even

further disadvantaged for survival because of a low reserve of carbohydrate in the liver and muscles at birth for energy (Widdowson, 1971). The high survival levels in Experiment IV provide support for the advantage of artificial rearing of low birthweight pigs.

The factors contributing to the efficacy of the immunoglobulin extract can be analysed. This extract, which is a precipitate "salted out" with a 40 per cent $(\text{NH}_4)_2\text{SO}_4$ solution, contains all of the gamma-globulin and much of the beta-globulin fractions of serum (Owen, 1961). Like colostrum, which is considered a serum transudate (Bourne, 1973), it would contain over 80 per cent IgG, less IgA than colostrum (8 vs 13 per cent), and more IgM than colostrum (10 vs 4 per cent) (Bourne, 1973). Also, unlike colostrum, serum derived immunoglobulin contains no secretory IgA (S-IgA). One could presume that the efficacy of the extract was predominately due to IgG. However, the presence of IgM and non-secretory IgA cannot be ignored. Miler *et al.*, (1975) found that the minimum effective concentrations for local protective effect in ligated loop tests with pigs were 0.5, 0.05, and 0.005 mg/ml of IgG, IgM, and IgA respectively. Therefore, even lower levels of IgM and IgA may have a significant protective effect. IgG itself, has been found to be of significant protection against enteric colibacillosis (Brandenburg and Wilson, 1972). How IgG functions within the gut lumen is open to question but it has antitoxin and agglutinating properties (Beneceraf and Unanue, 1979). Although it may not be able to adhere to the gut lining as S-IgA does, agglutination of bacteria would facilitate their peristaltic removal.

Finally, the efficacy of this extract against *Salmonella* bacteria is of considerable interest. To date, this technique has been

used to prevent mortality due to *E. coli* enteritis (Owen et al., 1961; Scoot, 1972; McCallum, 1977; and Kenelley et al., 1979). Since it protects against *Salmonella*, as shown in the present study, many of the herds from which the blood for immunoglobulin extraction was taken must have antigenic quantities of *Salmonella* infesting them. The contribution of these herds must have been great enough to provide a sufficiently high antibody titre against *Salmonella* to be preventive in these experiments.

3.7

Conclusions

1. The artificial rearing technique with artificial immunoglobulins used in these trials will save low birthweight pigs if weaned at birth. However, growth rate is restrained, possibly by restrictive feeding or by their low birth weight.
2. The level of immunoglobulin of 10 g/kg body weight on the first day followed by 2 g/kg/day on succeeding days seems to be effective as a dosing level.
3. Longer periods of immunoglobulin administration may be necessary in a non-isolated environment. Whether this is a result of low birthweight is not known.
4. This immunoglobulin extract used was effective protection against the Salmonella bacteria encountered in these trials. Certain types of Salmonella may be present at endemic levels in many swine herds.
5. An increased understanding of the causes of runting is necessary in order to justify the effort of attempting to save the smallest ones.

Foot-Notes

1. R and H Farms, Aldergrove, B.C.
2. Federated Cooperatives Ltd. Milk Replacer - 30.
(for pigs and lambs).
3. Furoxone is manufactured by the Austin Company, Cleveland, Ohio. It contains the active ingredient furazolidone, a nitrofurantoin, and it is a broad spectrum, synthetic antibacterial compound.
4. Anistat is a broad spectrum antibiotic containing Chloramphenicol, Neomycin Sulfate, Sulfathiazole, and Sulfamethazine. The manufacturer is not known.

BIBLIOGRAPHY

- Allen, W.D. and Porter, P. 1973. The relative distribution of IgM and IgA cells in intestinal mucosa and lymphoid tissues of the young unweaned pig and their significance in ontogenesis of secretory immunity.
- Bach, J.F. 1976. Antigen and Antibody Reactions; pp.248-288 in "Immunology", edited by Bach, J.F., John Wiley and Sons, Toronto.
- Beneceraf, B. and Unanue, E.R. 1979. Textbook of Immunology; Williams and Wilkins, Baltimore and London.
- Bereskin, B.; Shelby, C.E.; and Cox, D.F. 1973. Some factors affecting pig survival. J. of An. Sc. 36:821-827.
- Bienenstock, J. 1979. The Physiology of the Local Immune Response; p.3 in "Immunology of the Gastrointestinal Tract" edited by Asquith, P., Churchill Livingstone, N.Y.
- Binns, R.M. 1967. Bone marrow and lymphoid cell injection of the pig fetus resulting in transplantation tolerance of immunity and immunoglobulin production. Nature (London) 214: 179-181
- Binns, R.M. 1973. Cellular Immunology in the Pig. Proc. R. Soc. Med. 66: 1155-1160.
- Binns, R.M. and Symons, D.B.A. 1974. The Ontogeny of Lymphocyte Immunoglobulin Detriments and their Relationship to Antibody Production in the Fetal Pig. Res. in Vet. Sc. 16: 260-262
- Blecha, F. and Kelley, K.W. 1981. Cold Stress Reduces the Acquisition of Colostral Immunoglobulin in Piglets. J. An. Sc. 52:594-600
- Bourne, F.J. 1973. The Immunoglobulin System of the Suckling Pig. Proc. Nutr. Soc. 32:205-215.
- Bourne, F.J. and Curtis, J. 1973. The Transfer of Immunoglobulins IgG, IgA and IgM from Serum to Colostrum and Milk in the Sow. Immunol. 24:157-162.
- Bowland, J.P. 1966. Swine Milk Composition - A Summary; in "Swine in Bio-Medical Research"; pp. 97 - 108 edited by Bustad, L.K. and McClellan, R.O.; Batelle Memorial Institute, Richland, U.S.A.
- Branche, W.; Young, V; Robinet, H.; and Massey, E. 1963. The Effect of Colicine Production on E. coli in the Normal Human Intestine. Proc. of the Soc. of Exp't'l Biol. and Med. 114:198.
- Brandenburg, A.C. and Wilson, M.R. 1972. Immunity to E. coli in Pigs: IgG Immunoglobulin in Passive Immunity to E. coli Enteritis. Immunology 24:119.

- Brandenburg, A.C. and Wilson, M.R. 1974. Immunity to *E. coli* in pigs: IgE and Blood Clearance. *Res. in Vet. Sc.* 16:171-175.
- Brandtzaeg, P. 1973. Structure, Syntheses and External Transfer of Mucosal Immunoglobulins. *Ann. Immunol (Inst. Pasteur)* 124C: 417-438.
- Brandtzaeg, P. and Baklien, K. 1977. Intestinal Secretion of IgA and IgM: a Hypothetical Model, pp. 77-144 in *Immunology of Ciba Foundation Symposium 46 Elsevier, Excerpta Medica, North Holland, N.Y.*
- Brandtzaeg, P. and Swilahti, E. 1976. Further Evidence for a Role of Secretory Component (S.C.) and of J chain in the Glandular Transport of IgA, pp. 219-226 in "Secretory Immunity and Infection", edited by McGhee, J. R., Mestecky, J., and Balb, J. L.; Plenum Press, N.Y. 1978.
- Brambell, F.W. Rogers. 1970. The Transmission of Passive Immunity from Mother to Young. North-Holland Publishing Co., London.
- Brown, P.J. and Bourne, F.J. 1976. Development of Immunoglobulin-containing Cell Populations in Intestine, Spleen and Mesenteric Lymph Nodes of the Young Pig, as Demonstrated by Peroxidase-conjugated Antiserum. *Ann. J. Vet. Res.* 37:1309-1314.
- Bullen, J.J.; Rogers, H.J.; Leigh, L. 1972. Iron-binding Proteins in Milk and Resistance to *E. coli* Infection in Infants. *Brit. Med. J.* 1:69-73.
- Carroll, W.E.; Krider, J.L.; and Andrews, F.N. 1962. *Swine Production* 3rd Edition p.137. McGraw-Hill Book Co. N.Y.
- Chidlow, J.W. and Porter, P. 1979. Intestinal Defense of the Neonatal Pig. Interrelationship of Gut and Mammary Function Providing Surface Immunity Against Colibacillosis. *Vet. Rec.* 104:496-500.
- Christensen, J.; Weisbrodt, N.; and Hansen, R. 1972. Electrical Slow Wave of the Proximal Colon of the Cat in Diarrhea. *Gastroenterology* 62:1167-1168.
- Coalson, J.A. and Lecce, J.G. 1973. Herd Differences in the Expression of Fatal Diarrhea in Artificially Reared Piglets Weaned After 12 hours vs. 36 hours of Nursing. *J. An. Sc.* 36:1114-1121.
- Coalson, J.A. and Lecce, J.G. 1973. Influence of Nursing Intervals on Changes in Serum Protein (immunoglobulins) in Neonatal Pigs. *J. An. Sc.* 36:381-385.

- Colten, H.R. 1977. "Development of Host Defense: the Complement and Properdin Systems", in Development of Host Defenses; editors Cooper, M.D. and Dayton D.H. , Raven Press, N.Y.
- Cooper, M.D. and Lawton, A.R. 1974. "The Development of the Immune System", in Readings from the Scientific American: "Immunology"; W.H. Freeman and Co., San Francisco, 1976.
- Cooper, J.E.; John, M.; McFayden, I.R.; and Wooten, R. 1978. Early Appearance of Runting in Piglets. Vet. Rec. 102:529.
- Craig, S.W. and Cebra, J.J. 1971. Peyer's Patches - an Enriched Source of Precursors of IgA Producing Immunocytes in the Rabbit. J. of Expt'l. Med. 134:188-200.
- Cranwell, P.D.; Noakes, D.E.; and Hill, K.J. 1968. Observations of the Stomach Content of the Suckling Pig. Proc. Nutr. Soc. 27:26A.
- Cranwell, P.D. and Titchen, D.A. 1974. Gastric Acid Secretion in Newly Born Piglets. Res in Vet. Sc. 16:105-107.
- Curtis, J. and Bourne, F.J. 1971. Immunoglobulin Quantitation on Sow Serum, Colostrum, and Milk in the Serum of Young Pigs. Biochim Biophys Acta 236:319-332.
- Curtis, S.E. 1974. Pig Barn Environment: How It Affects Baby Pig Performance. Canadian Pork, Feb. 1974, p.9.
- Day, N.K.B.; Pickering, R.J.; Gewarz, H.; and Good, R.A. 1969. Ontogenic Development of the Complement System. Imm. 16:319-322.
- Dixon, F.J.; Jacot-Guillarmod, H.; and McConahey, P.J. 1967. The Effect of Passively Administered Antibody on Antibody Synthesis. J. of Expt'l Med. 125:1119-1121.
- Dixon, J.M. and Paulley, J.W. 1963. The Bacteriological and Histological Studies of the Small Intestine of Rats Treated with Mecamylamine. Gut 4:169-172.
- Dupont, H.L. and Pickering, L.K. 1980. Observations on the Pathogen Properties of the K88, HLY, and Ent. Plasmids of E. coli with Particular Reference to Porcine Diarrhea. J. Med. Microb. 4:467-469.
- Edwards, B.L. 1972. Causes of Death in New-born Pigs. Vet. Bull. 42: 249-258.

- Elliot, J.I.; Lodge, G.A.; and Hartsock, T.G. 1978. Practical Artificial Rearing of Neonatal Piglets under Non-isolated Conditions. *Can. J. An. Sc.* 58:799-800.
- England, D.C. 1974. Husbandry Components in Prenatal and Perinatal Development in Swine. *J. of An. Sc.* 38:1045-1049.
- English, P.R. and Smith, W.J. 1975. Some Causes of Death in Neonatal Piglets. *Vet. Annual* 15:95-104.
- Fahmy, M.H. and Bernard, C. 1971. Causes of Mortality in Yorkshire Pigs from Birth to 20 Weeks of Age. *Can J. An. Sc.* 51: 351-359.
- Freter, R. 1974. Interactions Between Mechanisms Controlling the Intestinal Microflora. *Amer. J. of Clin. Nutr.* 27:1409-1411.
- Freter, R. and Abrams, G. 1972. Function of Various Intestinal Bacteria in Converting Germ-free Mice to Normal State. *Infect. Imm.* 6:119-121.
- Gearhart, P.J.; Johnson, N.D.; Douglas R.; and Hood, L. 1981. IgG Antibodies to Phosphorylcholine Exhibit More Diversity than Their IgM Counterparts. *Nature* 291:29-34.
- Gianella, R.A.; Broitman, S.A.; and Zinchek, N. 1971. Salmonella Enteritis: I. Role of Reduced Gastric Secretion in Pathogenesis. *Amer. J. Dig. Dis.* 16:1000-1010.
- Gyles, C.L. 1971. Heat-labile and Heat-stable Forms of the Enterotoxin from *E. coli* Strains Enteropathogenic for Pigs. *Ann. N.Y. Academy Sc.* 176:314-322.
- Hasland, A.J.; Monie, H.J.; and Gowans, J.L. 1977. The Natural History of the Cells Producing IgA in the Gut. p.29 in "Immunology of the Gut". Ciba Foundation 46, Elsevier, Excerpta Medica, North Holland, N.Y.
- Haye, S.N. and Kornegay, E.T. 1979. Immunoglobulin G, A, and M and Antibody Response in Sow-reared and Artificially Reared Pigs. *J. An. Sc.* 48:1116-1122.
- Hill, K.J. 1970. Development and Comparative Aspects of Digestion. in "Dukes Physiology", 8th Edition Swenson, M.J. editor, Cornell Univ. Press, N.Y.
- Hill, I.R. and Porter, P. 1974. Studies of Bactericidal Activity to *E.coli* of Porcine Serum and Colostral Immunoglobulins and the Role of Lysozyme with Secretory IgA. *Imm.* 26: 1239-1250.

- Hood, L.E.; Weissman, I.L.; and Wood, W.B. 1978. Immunology. Benjamin/Cummings Publishing C. Inc., Don Mills, Ont.
- Hughes, J.M. ; Murad, F.; and Chang, B. 1978. Role of Cyclic AMP in the Action of Heat-stable Enterotoxin of *E. coli*. Nature 271:755-756.
- Jones, A.S. 1972. Problems of Nutrition and Management of Early Weaned Piglets. Proceedings of the Brit. Soc. of An. Prod. 19-32.
- Kennelly, J.J.; Ball, R.O.; and Aherne, F.X. 1979. Influence of Porcine Immunoglobulin Administration on Survival and Growth of Pigs Weaned at 2 and 3 Weeks of Age. Can. J. An. Sc. 59:693-698.
- Kenworthy, R. 1970. Effect of *E. coli* on Germ-free and Gnotobiotic Pigs. I. Light and Electron Microscopy of the Small Intestine. J. of Comp. Path. 70:53-63.
- Kim, Y.B.; Bradley, S.G.; and Watson, D.W. 1966. Antibody Synthesis with Germ-free Colostrum-deprived Miniature Piglets. in "Swine in Biomedical Research", edited by Bustad, L.K. and McClellan, R.D., Batelle Memorial Institute, Richland, Wash.
- Kim, Y.B.; Bradley, G.; and Watson, D. 1967. Ontogeny of the Immune Response. IV. Role of Antigen Elimination in the True Primary Immune Response in Germ-free Colostrum-deprived Piglets. J. Immunology 99:320-325.
- Klinman, N.R. and Press, J.L. 1975. The Characterization of the B Cell Repertoire Specific for 2,4-Dinitrophenyl and 2,4,6-Trinitrophenyl Determinants in neonatal BALB/c Mice. J. Expt'l Med. 141:1132-1134.
- Knop, J.; Bren, H.; Wernet, D.; and Rowley, D. 1971. The Relative Antibacterial Efficiency of IgM, IgG, and IgA from Pig Colostrum. Austr. J. of Exp. Biol and Med. Sc. 49:405-408.
- Kohler, E.M. 1972. Pathogenesis of Neonatal Enteric Colibacillosis in Pigs. J.A.V.M.A. 160:574-582.
- Kruml, J.; Kovann, F.; Pospisil, M.; and Trebichavsky, I. 1970. The Development of Lymphatic Tissue During Ontogeny, pp. 35-54 in "Developmental Aspects of Antibody Formation and Structure", Vol. I. edited by Sterzl, J. and Riha, I., Academic Press, N.Y.

- Lai A Fat,; McClelland, D.B.L.; and Van Furth, R. 1976. In Vitro Synthesis of Immunoglobulins, Secretory Component, Complement, and Lysozyme by Human Gastro-intestinal Tissues. I. Normal Tissues. Clin. Exp. Imm. 23:9-19.
- Lamm, M.E.; Weisz-Carrington, P.; Roux, M.E.; McWilliams, M.; and Phillips-Quagliata, J.M. 1978. Development of the IgA System and the Mammary Gland. pp. 35-42 in "Secretory Immunity and Infection", edited by McGhee, J.R.; Mestecky, J.; and Babb, J.L. Plenum Press, N.Y.
- Laskowski, M.; Kassell, B.; and Hagerty, G. 1957. A Crystalline Trypsin Inhibitor from Swine Colostrum. Biochim. Biophysica Acta 24:300-305.
- Lecce, J.G. 1969. Rearing Colostrum-free Pigs in an Automatic Feeding Device. J. An. Sc. 28:27-33.
- Lecce, J.G. 1971. Rearing Neonatal Piglets of Low Birth-weight with an Automatic Feeding Device. J. An. Sc. 33:47-51.
- Lecce, J.G. 1975. Rearing Piglets Artificially in a Farm Environment: a Promise Unfulfilled. J. An. Sc. 41:659-666.
- Lodge, G.A. and Elliot, J.I. 1979. The Influence of Birth-weight on the Subsequent Growth of Hysterectomy-derived Pigs. Can J. An. Sc. 59:215-216.
- Lucas, I.A.M. and Lodge, G.E. 1961. The Nutrition of the Young Pig: A Review. Tech. Communication of the Commonwealth Bureau of Animal Nutrition #22, Rowett Institute, Aberdeen, Scotland.
- Manners, M.J. 1976. The Development of Digestive Function in the Pig, from "A Symposium in Quantitative Aspects of Pig Nutrition". Proc. of Nutr. Soc. 35:49-56.
- Marrable, A.W. 1971. The Embryonic Pig: a Chronological Account. Pitman Medical.
- Mayer, M.M. 1973. The Complement System, pp. 143-157 in "Readings from Scientific American, "Immunology"". W.H. Freeman and Co. San Francisco.
- McCallum, I.M. 1977. Masters Thesis. University of Sask., Saskatoon.

- McCallum, I.M.; Elliot, J.I.; and Owen, B.D. 1977. Survival of Colostrum-deprived Neonatal Piglets Fed Gamma-globulins. *Can. J. An. Sc.* 57:151-158.
- McClelland, D.B.L. 1979. Bacterial and Viral Infections of the Gastro-intestinal Tract. pp. 214-245 in "Immunology of the Gastro-intestinal Tract." Edited by Asquith, P., Churchill Livingstone, N.Y.
- Miler, I.; Cerna, J.; Travnicsek, J.; Rejnek, J.; and Kruml, J. 1975. The Role of Immune Pig Colostrum, Serum and Immunoglobulins IgG, IgM, and IgA in Local Intestinal Immunity Against Enterotoxic Strains of *E. coli* 055 in Germ-free Piglets. *Folia Microb.* 20:433-438.
- Monteith, J.L. and Mount, L.E., editors of "Heat Loss from Animals and Man", Butterworths, London, 1974.
- Newland, H.W.; McMillan, W.N.; and Reineke, E.P. 1952. Temperature Adaptation in the Baby Pig. *J. An. Sc.* 11:118-133.
- Nielsen, N.O.; Moon, H.W.; and Roe, W.E. 1968. Enteric Colibacillosis in Swine. *J.A.V.M.A.* 153:1590-1606.
- Oram, J.D. and Reiter, B. 1968. Inhibition of Bacteria by Lactoferrin and other Iron-chelating Agents. *Biochimica et Biophysica Acta* 170:351-353.
- Owen, B.D. 1961. Effects of Porcine Immune Globulin Administration on the Survival and Serum Protein Composition of Colostrum-deprived Pigs Reared in a Natural Environment. Phd Thesis, Univ. of Sask., Saskatoon.
- Owen, B.D. and Bell, J.M. 1964. Further Studies of Survival and Serum Protein Composition in Colostrum-deprived Pigs Reared in a Non-isolated Environment. *Can. J. An. Sc.* 44:1-7.
- Owen, B.D.; Bell, J.M.; Williams, C.M.; and Oakes, P.G. 1961. Effects of Porcine Immunoglobulin Administration on the Survival and Serum Protein Composition of Colostrum-deprived Piglets Reared in a Non-isolated Environment. *Can. J. An. Sc.* 41:236-252.

- Ozawa, A. and Freter, R. 1964. Ecological Mechanisms Controlling Growth of *E. coli* in Continuous Flow Cultures and the Mouse Intestine. *J. Infect. Dis.* 114:235
- Parmely, M.J. and Beer, A.E. 1977. Colostral Cell-mediated Immunity and the Concept of a Common Secretory Immune System. *J. Dairy Sc.* 60:655-665.
- Parmely, M.J.; Beer, A.E.; and Billingham, R.E. 1976. In Vito Studies on the T-Lymphocyte Population of Human Milk. *J. Expt'l Med.* 144:358-370.
- Payne, L.C. and Marsh, C.L. 1962. Gamma-globulin Absorption in the Baby Pig: The Non-selective Absorption of Heterologous Globulins and Factors Influencing Absorption Time. *J. Nutrition* 76:151-158.
- Perry, G.C. and Lecce, S.G. 1968. The Rearing of Colostrum Deprived Piglets. *An. Prod.* 10:433-444.
- Perry, J.S. and Rowe ll, J.G. 1969. Variations in Fetal Weight and Vascular Supply Along the Uterine Horn of the Pig. *J. of Reprod. and Fert.* 19:527-530.
- Pestana, C.; Shorter, R.G.; and Hallenbeck, G.A. 1965. *J. of Surgical Res.* 5:471.
- Pierce, N.F. and Gowans, J.L. 1975. Cellular Kinetics of the Intestinal Immune Response to Cholera Toxoid in Rats. *J. of Expt'l. Med.* 142:1550-1563.
- Pomeroy, R.W. 1960. Infertility and Neonatal Mortality in the Sow. III. Neonatal Mortality and Fetal Development. *J. Ag. Sc.* 54:31-56.
- Porter, P. 1976. Intestinal Absorption of Colostral IgA Anti-*E. coli* Antibodies by the Neonatal Piglet and Calf. pp. 397-408 in "Maternofoetal Transmission of Immunoglobulins". The Proceedings of a Symposium on Transmission of Immunoglobulins from Mother to Young, edited by Hemmings, W.A.; Cambridge Univ. Press, N.Y.
- Porter, P. 1979. Immunobiology of the Alimentary Tract in Relation to Oral Immuno-prophylaxis Against Enteric Infections in the Piglet and Calf. pp.268-287 in "Immunology of the Gastro-intestinal Tract", edited by Asquith, P., Churchill Livingstone, N.Y.

- Porter, P. and Allen, W.D. 1972. Classes of Immunoglobulin Related to Immunity in the Pig. *J.A.V.M.A.* 160:511-518.
- Porter, P.; Noakes, D.E.; and Allen, W.D. 1972. Intestinal Secretion of Immunoglobulins in the Pre-ruminant Calf. *Imm.* 23:299-312.
- Porter, P.; Parry, S.H.; and Allen, W.D. 1977. Significance of Immune Mechanisms in relation to Enteric Infections of the Gastro-intestinal Tract in Animals". p.55 in "Immunology of the Gut", Ciba Foundation Symposium 46, Elsevier, Excerpta Medica, North Holland N.Y.
- Powell, D.; Plotkin, G.; Maenza, R.; Solberg, L.; Cutlin, D.; and Formal, S. 1971. Experimental Diarrhea. I. Intestinal Water and Electrolyte Transport in Rat Salmonella Enterocolitis. *Gastroenterology* 60:1053-1060.
- Prokesova, L.; Rejnek, J.; Sterzl, J.; Travnicek, J. 1970. Ontogenetic Development of Immunoglobulins in Pigs. pp. 757-766 in "Developmental Aspects of Antibody Formation and Structure". Vol. II, edited by Sterzl, J. and Riha, I., Academic Press, N.Y.
- Rodeffer, H.E.; Leman, A.D.; and Mueller, A.E. 1975. Development of a Record System for Measuring Swine Breeding Herd Efficiency. *J. An. Sc.* 40:13-18.
- Rowley, D. and Turner, K.J. 1966. Number of Molecules of Antibody Required to Promote Phagocytosis by One Bacterium. *Nature*, (London) 210:496-498.
- Rutter, J.M. 1975. E. coli Infection in Piglets: Pathogenesis, Virulence, and Vaccination. *Vet. Rec.* 96:171-175.
- Rutter, J.M.; Jones, G.W.; Brown, G.T.H.; Burrows, M.R.; and Luther, P.D. Antibacterial Activity in Colostrum and Milk Associated with Protection of Piglets Against Enteric Disease Caused by K88-Positive E. coli. *Infect. Imm.* 13:667-676
- Scoot, A. 1973. Feeding Immunoglobulin to Colostrum Deprived Pigs. Masters Thesis, Univ. of Sask., Saskatoon.
- Searle, S.R. 1971. Topics in Variance Component Estimation. *Biometrics* 27:1-76.

- Segre, D. 1966. Swine in Immunological Research. pp.269-272 in "Swine in Biomedical Research", edited by Bustad, L.K. and McClellan, R.D., Battelle Memorial Institute, Richland, Wash.
- Segre, D. and Myers, W.L. 1964. Antibody Production in Baby Pigs. Amer. J. Vet. Res. 25:413-419.
- Seirs, D.G.; Danielson, D.M.; Chai, E.T.; and Keasling, H.H. 1977. Late Gestation Feeding of Dichlorvos: The Response of Artificially and Dam-reared Litters. J.An. Sc. 44:1-7.
- Sharpe, H.B.A. 1966. Preweaning Mortality in a Herd of Large White Pigs. Brit. Vet. J. 122:99-111.
- Shedlofsky, S. and Freter, R. Synergism Between Enlogic and Immunologic Control Mechanism of Intestinal Flora. J. Infect. Dis. 129:296-301.
- Silverstein, A.M. 1977. Ontogeny of the Immune Response: A Perspective; in "Development of Host Defenses", edited by Cooper, M.D. and Dayton, D.H., Raven Press, N.Y.
- Smith, H.W. and Linggood, M.A. 1971. Observations on the Pathogenic Properties of the K88, HLY and Ent. Plasmids of E. coli with Particular Reference to Porcine Diarrhea. J. Med. Microb. 4:467-470.
- Snedecor, G.W. and Cochran, W.G. 1967. Statistical Methods. 6th Edition, Iowa State Univ. Press.
- Solomon, J.B. 1971. Foetal and Neonatal Immunology. North-Holland Publishing Co., London.
- Sprinz, H. 1969. Pathogenesis of Intestinal Infections. Arch Pathol. 87:556-558.
- Steele, E.J.; Chaicumpa, W.; Rowley, D. 1974. J. Inf. Dis. 130:93-95.
- Sterzl, J. 1963. Antibody Formation by Cells Isolated from Donors of Different Ages After Transfer to Young Rabbits and to X-irradiated Adult Rabbits. Folia Microb. 8:69-72.
- Sterzl, J.; Kostka, J.; Pesak, V.; and Jilek, M. 1964. The Relation Between Bactericidal Activity of Complement and the Character of the Bacterial Surfaces. Folia Microb. 9:284-286.

- Sterzl, J.; Vesely, J.; Jilek, M.; and Mandel, L. 1965. The Inductive Phase of Antibody Formation Studied with Isolated Cells. p.463 in "Molecular and Cellular Basis of Antibody Formation", edited by Sterzl, J., Pub. House of Czeck Acad. Sc., Prague.
- Tennant, B.C. and Hornbuckle, W.E. 1980. Gastro-intestinal Function. in "Clinical Biochemistry of Domestic Animals", 3rd Edition, edited by Kaneko, J.J., Academic Press, Toronto, Ont..
- Tlaskalova, H.; Sterzl, J.; Hajek, P.; Pospisil, M.; Riha, I.; Marvanova, H.; Kamarytova, V.; Mandel, L.; Kruml, J.; and Kovaru, F. 1970. The Development of Antibody Formation During Embryonal and Postnatal Periods, in "Developmental Aspects of Antibody Formation and Structure", Vol. II, edited by Sterzl, J. and Riha, I., Academic Press, N.Y.
- Tomasi, T.B. 1976. The Immune System of Secretions. Prentice-Hall Inc., N.J., N.Y.
- Tomasi, T.B. and Calvanico. 1968. Human Secretory Gamma-A. Fed. Proc. 27:617 (abstr).
- Tonegawa, S.; Maxim, A.M.; Tizard, R.; Bernard, O.; and Gilbert, W. 1978. Sequence of a Mouse Germ-line Gene for Variable Region of an Immunoglobulin Light Chain. Proc. Nat'l. Acad. Sc., U.S.A. 75:1485-1499.
- Ullrey, D.E.; Long, C.H.; and Miller, E.R. 1966. Absorption of Intact Protein from the Intestinal Lumen of the Neonatal Pig. pp.249-262 in "Swine in Biomedical Research" edited by Bustad, L.K. and McClellan, R.O., Battelle Memorial Institute, Richland Wash.
- Waldorf, D.P.; Foote, W.C.; Self, H.L.; Chapman, A.B.; and Casida, L.E. 1957. Factors Affecting Fetal Pig Weight Late in Gestation. J. An. Sc. 16:976-985.
- Walker, W.A. 1979. Gastro-intestinal Host Defense: Importance of Gut Closure in Control of Macromolecular Transport, in "Development of Mammalian Absorptive Process", Ciba Foundation Symposium, Elsevier, Excerpta Medica, North Holland, N.Y.
- Weide, K.; Sanger, V.; and Lagace, A. 1962. Innoculation of Baby Pigs with Hog Cholera Vaccine. A.J.V.M.A. 141:464-469.

- White, F.; Wenham, G.; Robertson, V.A.W.; and Rattray, E.A.S. 1972. Further Observations on Stomach Function of Scouring Piglets. *Proc. Nutr. Soc.* 31:67-71.
- White, F.; Wenham, G.; Sharman, G.A.M.; Jones, A.S.; Rattray, E.A.S.; and McDonald, I. 1969. Stomach Function in Relation to a Scour Syndrome in the Piglet. *Br. J. Nutr.* 23:847-858.
- Widdowson, E.M. 1971. Intra-uterine Growth Retardation in the Pig. I. Organ Size and Cellular Development at Birth and After Growth to Maturity. *Biol. of the Neonate* 19:329-351.
- Wilson, M.R. 1974. Immunologic Development of the Neonatal Pig. *J. An. Sc.* 38:1018-1021.