A STUDY

OF

SOME OF THE FACTORS AFFECTING THE TRANSFER OF IMMUNOGLOBULINS FROM COWS TO CALVES

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SUMMARY

The yields of colostral immunoglobulins were estimated from the four quarters of 18 cows soon after birth (Experiment A). The concentrations of IgG, IgG2, IgA and IgM were found to be similar between the four quarters with means ranging from 82-88 mg/ml for IgG, 4.8-4.9 mg/ml for IgG2, 9.6-11.1 RSA/ml for IgA (RSA = relative to concentration of serum IgA) and 7.1-7.4 mg/ml for IgM. The calculated total yields of the four immunoglobulins were (means $\pm$ S.E.) 411$\pm$44 g, 23.4$\pm$3.0 g, 42.6$\pm$4.9 (RSA) and 35.0$\pm$4.8g for IgG, IgG2, IgA and IgM respectively. These amounts were considered adequate for the requirements of passive immunity in newborn calves. Most calves which were allowed to suckle their dams for the first two days were able to absorb high levels of these immunoglobulins in their sera. The means $\pm$ S.E. of the 24-hour serum levels of the immunoglobulin in these calves were 37.1$\pm$5.3 mg/ml for IgG, 1.3$\pm$0.1 mg/ml for IgG2, 3.2$\pm$0.4 RSA/ml for IgA and 2.1$\pm$0.2 mg/ml for IgM (Experiment C).

The apparent absorption efficiencies of the four immunoglobulins by newborn calves fed with colostrum within 6 hours of birth were similar at (means $\pm$ S.E.) 33.3$\pm$2.7% for IgG, 26.8$\pm$3.5% for IgG2, 32.8$\pm$3.1% for IgA and 36.0$\pm$3.8% for IgM. However sheep IgG2 was absorbed at lower efficiency (18.5-1.6%) by ten of these calves ($P < 0.05$) (Experiment B). In contrast the apparent absorption efficiency of sheep IgG2 was significantly greater (26.0$\pm$1.4%) in 20

calves which were allowed to remain with their dams for two days ($P < 0.01$) (Experiment C). This indicates that the absorption efficiency of immunoglobulins by calves which were allowed to nurse their dams was superior to the calves which were removed from their dams and fed from a nipple feeder.

The results obtained in the present studies were discussed in relation to relevant data reported in the literature.

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INTRODUCTION

The immunological importance of passive transfer of colostral immunoglobulins to the young has been well recognised in cattle and other farm animals including pigs, horses, goats and sheep (Brambell, 1958; Gay, 1965; Butler, 1969; and Simpson-Morgan and Smeaton, 1972). All these animals lack prepartum maternofoetal transfer of immunoglobulins probably due to the epitheliochorial (pigs and horses) or syndesmochorial placentation (cows, goats and sheep) (Sterzl and Silverstein, 1967), which are impermeable to macromolecules such as immunoglobulins, antitoxin (Mason et al, 1930) and even to smaller molecules such as growth hormone (Alexander et al, 1973). In the calves, this lack of prenatal transfer, coupled with the apparent inability of the newborn calves to actively produce antibodies in sufficient quantities during the immediate prenatal and postnatal period, means that the newborn are ill-equipped to resist pathogens. Their early ability to obtain and absorb adequate maternal colostral antibodies is therefore of paramount importance to ensure survival against most neonatal diseases.

In order to fully understand the need for antibody transfer in newborn calves, some knowledge of the physiology of the bovine immune system is required. For this reason part of the present thesis will include a brief review of some of these aspects, particularly the characteristics of the immunoglobulins and the inherent factors which lead to the necessity for passive acquisition of immunity, and factors affecting this transfer in newborn calves.

CHAPTER I

1.1 Bovine Immune System

The basic structure of the immune system in adult cattle is probably similar to that in other mammals. The latter has been extensively reviewed (Dutton, 1967; Sterzl and Silverstein, 1967; Edelman and Gall, 1969; Owen, 1972). Briefly it involves both the primary lymphoid organs, the bone marrow and the thymus, and all the peripheral lymphoid tissues (secondary lymphoid organs) such as the lymph nodes and the spleen. The bone marrow is responsible for the production of small lymphocytes, which originate from primitive haemopoetic stem cells, and which later migrate to the peripheral lymphoid tissues either directly or by way of the thymus. Those lymphocytes which pass directly from the bone marrow to the peripheral lymphoid tissues are known as bone marrow-derived lymphocytes or B-cells, while those lymphocytes which first enter the thymus before migrating to the lymphoid tissues are called thymus-dependent, thymus-derived lymphocytes or T-cells. B-cells and T-cells attain immunocompetence while in bone marrow and in thymus respectively (Abdou, 1972; Owen, 1972).

The nature of immune responses initiated by the two populations of lymphocytes is also different. T-lymphocytes which are predominantly found in the paracortical area of the lymph nodes and in the centre of the Malpighian body of the spleen, are generally associated with cell mediated reactions which include delayed sensitivity, graft rejection and various autoimmune phenomena. These reactions are, however, not

relevant to the present thesis and will not therefore be discussed further.

The reaction which results in the production of humoral antibodies is generally initiated by B-cells. Soluble or particulate antigens are initially 'processed' by macrophages in the medulla of the lymph nodes or in the red pulp of the spleen and the macrophages later relay these 'antigenic messages' to the B-cells which are found in the corticomedullary junction. This stimulates the B-cells to proliferate into clones of plasma cells which produce antibody (or immunoglobulin) directed specifically against the particular antigen.

This first response to antigen, also know as primary response, is usually limited in intensity and duration. Often it is also characterised by high production of IgM which precedes the production of IgG.

After this primary response, a second and subsequent exposure to the same antigen is manifested by a much more extensive proliferation of the plasma cells. This secondary or anamnestic response is believed to be dependent on immunological memory which is carried on a special population of cells (memory cells). During the secondary response, a much more extensive and lasting production of immunoglobulins, particularly IgG, occurs. Small quantities of IgM are still however, produced, but only for a limited period.

1.2 The General Structure of Immunoglobulins

A comprehensive review of the literature on the molecular structure of immunoglobulins is beyond the scope of the present thesis.

References may be found in Putnam (1969), Edelman and Gall (1969), Leslie and Cohen (1973), and Porter (1973). Suffice to describe the basic structure of an immunoglobulins which consists of a pair of heavy chains (H chains) and a pair of light chains (L chains) joined together by disulphide bond(s) to form a tetrachain molecule, with two specific antigen combining sites (Figure 1). A great variability is required for the structure of these combining sites in order to meet the multitudes of antigens that exist. This is achieved by the hypervariable region (V_H and V_L) involving some 110 to 120 amino acids on the NH_2 terminal end of the H and L chains.

An antibody molecule is classified into different classes on the basis of antigenic and structural differences on its heavy chains. These differences not only contribute to differences in class-specific physicochemical properties but also determine their biological properties. Nevertheless, antibody molecules which are classified within a single class are still heterogenous mixtures of chemically different molecules despite sharing similar overall structure.

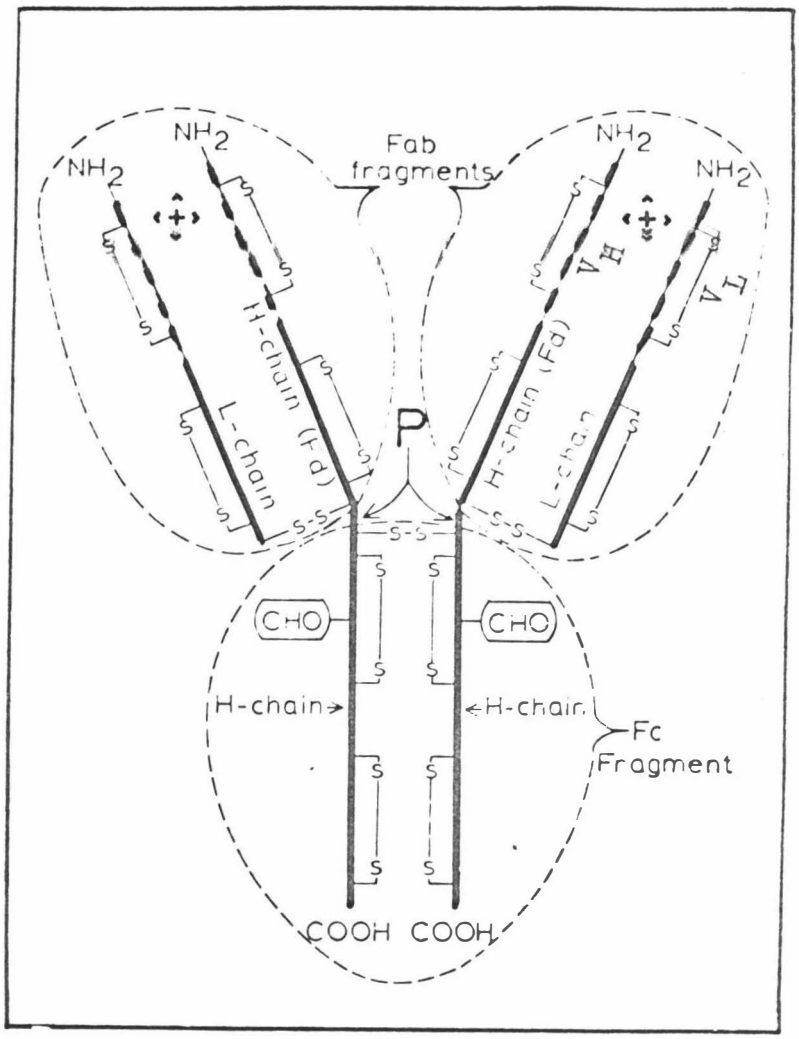


Figure 1. A schematic representation of a four chain structure of an immunoglobulin molecule showing two heavy (H) chains, two light (L) chains and intra and interchain disulphide bridges. The solid line and the broken line indicate the 'constant' and 'variable' portions of the H and L chains respectively. The combining sites of the molecule for antigens (marked ‹+›) are believed to be the function of amino acid sequence in the variable region V_H and V_L. P is the approximate site of enzymatic cleavage by papain which split the molecule into two Fab fragments and an Fc fragment. The nomenclature of the H chain would correspond to the class of the immunoglobulin which is either γ, α, μ, or ε.

1.3 Classes of Bovine Immunoglobulins

Four classes and two subclasses of bovine immunoglobulins have been recognised with H chain identified as γ (γ_1, γ_2), α, μ and ε also known as IgG (IgG1 and IgG2), IgA, IgM and IgE (Butler, 1969; Hammer et al, 1971; Duncan et al, 1972). In contrast to the heavy chain, the light chains within individual molecules of all the classes are either 2 κ or 2 λ but never together. In addition, all polymeric immunoglobulins including dimeric IgA, secretory IgA (SIgA) and pentameric IgM but not monomeric IgG and IgA, may possess another chain called J chain. The presence of J chain has been described in humans (Halpern and Koshland, 1970; Mestecky et al, 1971; and Kobayashi et al, 1973), rabbits (Halpern and Koshland, 1970) and pigs (Porter, 1973), but has yet to be found in cattle (Beale and Buttress, 1972). The function of this chain is to join together subunit immunoglobulins as well as to maintain the tertiary structure of the subsequent polymer (Mestecky et al, 1974).

Another polypeptide chain called secretory piece (SP) is also known to be in unique association with exocrine IgA or secretory IgA (SIgA). However the exact function of this chain remains speculative and will be discussed in page 21.

1.4 Characteristics of Bovine Immunoglobulins

The structure and the physicochemical and immunological functions of bovine IgG, IgA and IgM are basically similar to their respective classes in human and other species reviewed extensively by Cohen and Milstein (1967), Tomasi and Bienenstock (1968) and Metzger

(1970). Specific reviews and other similar recent publications on bovine immunoglobulins have been written by Butler (1969; 1971b) and Mukkur and Froese (1971).

1.4.1 IgG (7S)

Two subclasses of bovine IgG have been established on the basis of minor antigenic and structural differences on the γ chain. They are distinguishable electrophoretically as a fast and slow IgG (Murphy et al, 1964; 1965) or IgG1 and IgG2 respectively (Milstein and Feinstein, 1968). IgG1 has a molecular weight of between 163,000 to 165,000 Daltons and sedimentation coefficient (S_{20w}) of 6.6 to 7 S (Table 1), while the more basic IgG2 has a molecular weight of 150,000 and S_{20w} of 6.3 S. In addition it was reported that a pentameric form of IgG (19S) which was characteristically distinct from 19S IgM, existed in colostrum as well as in intestinal secretion of very young calves (Hammer et al, 1968 and Porter et al, 1972).

IgG has a relatively low carbohydrate content of 2-3% compared to 6-10% for IgA and 10-12% for IgM (Table 1). Their components of D-mannose, D-galactose, L-fucose and D,N, acetyl neuraminic acid, but not their proportions, are however similar (Butler, 1969; 1971b).

The protective functions of IgG appear mainly related to systemic defence. This includes neutralisation of viruses, toxins and foreign enzymes, opsonisation of agglutination of bacteria (Murphy et al, 1966; Rice and Carriere, 1969; and Wilkie, 1974). In addition differences in the Fc fragment distinguish some properties of the two subclasses. Only IgG1 has been shown to be capable of fixing complement and is therefore more efficient than IgG2 in bacteriolysis (Beh, 1973;

TABLE 1 - Physico chemical and biological characteristics of bovine immunoglobulins.

	IgG1	IgG2	IgA	IgM
Heavy chain (H chain)	$\gamma 1$	$\gamma 2$	α	μ
Light chain (L chain)	κ or λ	κ or λ	κ or λ	κ or λ
J Chain			+ ?	+ ? (a)
Terminology	IgG1 (γG1)	IgG2 (γG2)	IgA (γA)	(IgM (γM)
Molecular weight (MW)	163,000 (b) 165,000	150,000 (b)	200,000-400,000 (i) 385,000 (e)	1,030,000 (d)
MW_H chain	55,000 - 58,000 (c)	54,000 - 58,000 (c)	60,000-63,000 (c)	61,000 - 76,000 (d)
MW_L chain	20,000	20,000	20,000 ?	22,000 - 28,000 (d)
Electrophoretic mobility	Fast γ and β_2	γ	β_2	β_2
Sedimentation coefficient (S_{20w})	6.6 - 7.0 (c)	6.0 - 7.0 (c)	6 - 13 (g, i)	19.5, 19.7 (d)
Carbohydrate %	2-3 (c)	2-3 (c)	6-10 (c)	10-12 (c)
Half life (days)	9.6 (c)	18, 17.7 (h)	2.1 - 2.8 (h)	4 (f, h)
Antibody activity	+	+	+	+
Complement fixation	+	?	?	+
Lacteal transmission	Selective	Passive ?	?	?
Placental transmission	-	-	-	-

1974). IgG1 is also reportedly capable of skin fixation in heterologous species (c.f. Butler, 1969).

No evidence is reported, however, on the possible role of IgG1 in the defence of intestinal epithelium of the calves, despite its being the major immunoglobulin in the colostrum (Porter et al, 1972).

1.4.2 IgA

The occurrence of IgA in cattle analogous to human IgA has only recently been confirmed. This immunoglobulin forms the bulk of the immunoglobulins in most external secretions including the saliva, gastrointestinal secretion, spermatic fluid, fluid of secretion of respiratory tract (Butler, 1969; Mach et al, 1969; Porter and Noakes, 1970; Mach and Pahud, 1971a; 1971b). IgA is not, however, predominant in bovine colostrum as in human (Tomasi and Bienenstock, 1968).

The reason for the comparatively larger amount of IgA in external excretions is largely because of the local synthesis of IgA by plasma cells found in the region underlying lamina propria of the seromucosa of the glands concerned (Wilson et al, 1972). In sheep this concept of local immune production associated with IgA is well known. Local production of IgA particularly SIgA were noted in immunised glands of ewes (Lascelles and McDowell, 1970; 1974; Watson and Lascelles, 1973) and in locally immunised intestine (Husband and Lascelles, 1974).

IgA exists in a range of molecular sizes ranging in molecular weight from 200,000 to 400,000. Much of this exists as a dimer of S_{20w} of 10-11S with or without secretory piece (Porter, 1971).

Both dimeric IgA and monomeric IgA are known to occur in serum (Butler, 1971a; Mach and Pahud, 1971a,b; and Butler et al, 1972), whereas in external secretion, SIgA is usually predominant (Porter, 1971; 1972).

The protective function of IgA in different animals including cattle appears to be mainly concerned with local defence, particularly at the mucosal surface of epithelial membrane (Tomasi and Binenstock, 1968; Porter, 1969; 1973; Porter et al, 1970; and South, 1971). Comoglio and Guglielmore (1973) showed that SIgA was capable of anchoring to the intestinal luminal surface through the secretory piece thus forming a barrier against invading organisms. In young calves, local production of SIgA has been shown in the intestinal mucosa (Porter et al, 1972) as well as in saliva (Mach and Pahud, 1971), and is regarded to be the principal antibody providing protection against enteric diseases on the mucosal surface of the gut (Tomasi and Bienenstock, 1968; South, 1971; Penhale et al, 1971; Logan and Penhale, 1972; and Porter, 1972). Moreover, IgA is the most resistant of all the immunoglobulins to proteolysis by intestinal enzymes (Brown et al, 1970; Steward, 1971). Porter (1972) showed that circulating IgA in calf's serum was lost rapidly through the intestine, suggesting that absorbed colostral IgA was able to re-enter intestinal lumen to provide external protection.

The mechanism of protection by IgA in external defence is regarded to be mainly through its strong agglutinating (Beh, 1974) and bactericidal ability (Burdon, 1973). These properties, effectively immobilise most of the invading organisms and assist phagocytosis.

In addition IgA is known to be capable of causing lysis of E . coli in the presence of lysozyme (Adinolfi et al, 1966; Hill and Porter, 1974).

1.4.3 IgM

IgM is the largest of the four immunoglobulins, with a molecular weight of about one million and S_{20w} of 19S. IgM is also the major and first immunoglobulin to appear during primary immune response (Metzger, 1970). Structurally, IgM is a polymer of five 7S IgM subunits joined together, probably by a single J chain (Beale and Buttress, 1972), thus giving a potential of ten antigen combining sites. For unknown reasons, however, only five of these are active (Metzger, 1970). Nevertheless IgM is very effective in systemic defence particularly in complement fixation, agglutination and opsonisation (Robbin et al, 1965; Pike, 1967; and Metzger, 1970). Besides, IgM unlike IgA does not require lysozyme for complement fixation (Metzger, 1970). Work in mice showed that IgM anti pneumococcal antibody was 100,000 fold more effective than IgG (Hill and Robbin, 1966). In rabbits IgM was reportedly 500 to 1,000 gold better as an opsonin than IgG (Robbin et al, 1965). Similarly other reports also indicated that IgM was highly effective in haemolysis and agglutination of bacteria (Onoue et al, 1965; Hammer et al, 1968), and in neutralisation of viruses (Bauer et al, 1963).

The function of IgM in cattle is probably limited to systemic defence due to its relatively large size. This was reflected in the failure of purified IgM fraction in the experiment of Logan et al, (1971) to provide protection against enteric infection while evidently capable of slowing the onset of serological septicaemia.

1.5 Quantity of Bovine Immunoglobulins

Quantitative studies of bovine immunoglobulins in different body fluids have been limited to IgG, IgA and IgM. These are summarised in Table 2. The fourth immunoglobulin class, which is associated with homocytotropic activity, similar to human IgE, has recently been identified in cows following antigenic stimulations with rabbit serum albumin (Hammer et al, 1971). So far this immunoglobulin has only been partially characterised.

As shown in Table 2, IgG is quantitatively the most abundant immunoglobulin in most body fluids. The proportions of the two subclasses varies. Thus, whereas IgG1 and IgG2 occur in approximately equal amounts in serum, the subclass IgG1 is dominant in colostrum and in secretions from the reproductive tract. This is due to the selective transmission of IgG1 which will be discussed later.

IgA and IgM together from about 20% of all immunoglobulins in serum and most secretions. In addition free secretory piece (FSP) is also found in milk (Porter and Noakes, 1970; Mach and Pahud, 1971), gastrointestinal secretion, colostrum and in saliva (Mach et al, 1969; Porter and Noaks, 1970).

TABLE 2 - The concentrations of the different immunoglobulins in various body fluids of cattle.

Body Fluids		IgG (mg/ml)	IgG1 (mg/ml)	IgG2 (mg/ml)	IgA (mg/ml)	IgM (mg/ml)	Ref.
SERUM	*	20.3±4.6	10.7±5.7	ND	0.1±.04	ND	a
	*	26.4±7.3	12.9±6.1	ND	.06±.04	ND	a
	*	26.4±12.6	ND	ND	ND	2.6±.08	b
	**	12.9±.66	ND	ND	ND	2.8±.01	c
			9.2-12.0	6.2-9.6	.07-1.2	1.12-3.1	d
					SIgA .065		d
		16.9-22.3	8.67-12.6	7.67-12.2	.12-.28	3.6-5.1	e
	**		13.4±.75	10.1±.4			f
MILK			.31-.40	.03-.08	ND	ND	d
	*	2.0	1.38±1.9	ND	.23±.4	ND	a
COLOSTRUM	*	34.5+21.1	31.6+19.3	ND	1.82+1.9	ND	a
	*	43±14	ND	ND	ND	3.2±1.7	b
			28.1-70.7	1.4-5.5	1.07-11.2	5.7-12.9	g
			71.2-124	1.1-3.0	2.5-4.8	5.8-13.4	h
	**		79.2±6.2	5.18±.75	6.85±1.11	10.7±1.3	f
			52-87	1.6-2.1	3.2-6.2	3.7-6.1	d
	**	78±2.2					
SALIVA	*	2.0	.05±.07	ND	.3±.2	ND	a
			.01-.08	.01	.28-.70	.01	d
TEARS	*	2.0	.54±.39	ND	3.88±2.4	ND	a
			.08-.18	.03-.08	.66-1.01	.05-.12	e
GASTRO-INTESTINAL SECRETION			.08-.70	.04 .11	.09-.60	ND	d
LACRIMAL			.20-.50	.08-.13	1.55-3.10	.006	d
SPERMATIC FLUID			.09-.12	.08-.13	.08-.16	Trace	d
BILE			.07-.14	.06-.12	.07-.10	.05	d
VAGINAL FLUID			.16-.30	.10-.20	.11-.32	ND	e

TABLE 2 - continued

References

a = Butler et al (1972)
b = Klaus et al (1969)
c = Penhale and Christie (1969)
d = Mach and Pahud (1971)
e = Duncan et al (1972)
f = Brandon et al (1971)
g = Husband et al (1972)
h = Porter (1972)
i = Fey and Hunyady (1962) (cited from Kruse 1970a)

ND = not determined
* = Mean $\pm$ standard deviation
** = Mean $\pm$ standard error

1.6 Immune Status of Neonatal Calves

The lympho-reticular tissues and related immunological components in neonatal calves are mostly relatively inactive because of the effective intrauterine protection given to the foetus against most antigenic stimulations. Subsequently the calf is born with little or no antibody (Butler, 1969; Klaus et al, 1969; and Husband et al, 1972) and only begins active endogenous production of the immunoglobulins after birth. This becomes apparent during the first to second weeks of post natal life, particularly with IgM and IgG (Porter et al, 1972; Husband et al, 1972). The production of IgA was shown by Husband et al, (1972) to be slightly delayed, although Porter et al (1972) showed that both IgA and SP were produced by the young calves at the same time as IgG and IgM. This means that the onset of endogenous production of all immunoglobulins in neoborn calves is similar, and commences soon after birth. Nevertheless the time of this onset of active antibody production

may be delayed by the presence of passively acquired immunoglobulins (Graves, 1963; McEwan et al, 1970b; Logan et al, 1972). This inhibitory effect may be due to removal of invading organisms by the acquired immunoglobulins before the former can stimulate the antibody synthesising mechanism.

In spite of the low immune activity in newly born calves, experimental data is available which indicates that the maturation of most immunological capabilities occurs much earlier during gestation. Schultz (1971) and Schultz et al (1973) using immunofluorescence studies showed that the spleen of foetal calves subjected to antigenic stimulation while in utero, began active production of IgM and IgG at gestational ages of 60 and 145 days respectively. Others (Tranin and Metrom, 1973; Sawyer et al, 1973) have also reported similar observation of high production of IgG and IgM by foetuses which were infected either naturally or artificially. Conner et al (1973) further demonstrated that such animals if adequately stimulated will even elicit secondary responses while in utero or soon after parturition.

Another significant characteristic of the process of prenatal immunological maturation is that it occurs in a series of discrete stepwise events involving the attainment of competence from one antigen to the other. This maturation sequence will go on until all the potential antigens are covered. An illustration of the sequence is given in Table 3.

TABLE 3 - Foetal ages of calves at which immunological response to infections or antigens was first observed.

Infectious agents or antigens	Gestational age (days)	References
Parainfluenza-3 virus	145	Swift and Kennedy (1972)
Leptospira saxkoebing	132-168	Fennestad and Petersen (1962)
Bovine viral diarrhoea	168 180 200	Casaro et al (1971) Gibson and Zemjanis (1973) Braun et al (1973)
Anaplasma marginale	100	Trueblood et al (1971)
Vibrio fetus	212	Osburn and Hoskin (1971)
Ferritin	120)	
Ovalbumin	150)	Gibson and Zemjanis (1973)
Brucella abortus	120-150)	

Yet, despite this inherent capacity of neonatal calves to employ their immunological resources against potential pathogens, they are very much dependent on colostral immunoglobulins for their early defence. This is indicated by the inability of colostrum-deprived calves to cope with the multitude of pathogenic organisms to which they are exposed following birth. Moreover, the antibodies which may have been synthesised prior to birth are usually highly specific and only to the immunising antigen and will be ineffective for protection against differing diseases.

1.7 Protective Roles of Colostral Immunoglobulin in Calves

Of the many diseases in neonatal calves, colibacillosis caused by pathogenic strains of E. coli is well known. The role of E. coli in this respect has been reviewed by Lovel (1955) and Gay (1965).

Earlier, observations have been reported on the role of colostrum against this disease (Smith and Little, 1922; Aschaffenburg et al, 1949), and since then many other reports have shown the relationship of low serum immunoglobulin levels and the incidence of fatal diseases in young calves arising from similar pathogens (Pierce, 1955; Smith, 1962; Smith et al, (1967; Klaus et al, 1969; McEwan et al, 1970; Penhale et al, 1970; Fey, 1971). Similarly surveys of dead calves indicate a high proportion with low immunoglobulin concentrations in their sera (Smith, 1962; Gay et al, 1965; McEwan et al, 1970; Boyd, 1972). Moreover experimental reproduction of colisepticaemia was more easily shown in calves deprived of colostrum than those fed with colostrum soon after birth (Smith, 1962).

Protective functions of ingested colostrum were also noted in calves infected while in utero in spite of some ability of the foetus to produce specific antibody. Reports of calves suffering from congenital diseases, for example, showed that the rate of survival was better if they were given their mother's colostrum (Dunne et al, 1974; Lambert and Fernelius, 1974).

There is little doubt therefore that low serum levels of immunoglobulins is one of the most important predisposing factors to bacterial invasion in calves. It appears that the ability of pathogenic microorganisms to establish and subsequently cause septicaemia, or enteric disease in the calves is inhibited or delayed by the presence of absorbed colostral immunoglobulins (Gay et al, 1965; Penhale et al, 1971; Logan and Penhale, 1971).

1.8 Immunoglobulins in the Colostrum

Over 70% of the immunoglobulins in the colustrum of cows belong to the subclass IgG1. IgA and IgM make the bulk of the remainder. In contrast the subclass IgG2 is almost completely excluded from the colostrum (Table 2). This is unlike the situation in the colostrum of human and other species receiving immunoglobulins while *in utero* which is predominantly IgA (Brambell, 1966; Tomasi and Bienenstock, 1968).

The origin of most of these immunoglobulins in colostrum was earlier regarded as locally synthesised. This was based on observations by Campbell *et al* (1950) on transient plasmacytosis in the colostrum-forming udder. However, we now know that this is only partially true and will be discussed later.

1.8.1 The origin of colostral IgG

The cells of bovine udder are known to be capable of concentrating preformed IgG from circulation to lacteal secretion, particularly during the formation of colostrum (Blackmore and Garner, 1956; Larson and Gillespie, 1957; Garner and Crawley, 1958; Dixon *et al*, 1961). Later it has been shown that the immunoglobulin which is transferred is almcst exclusively IgG1 (Murphy *et al*, 1964; 1965; Pierce and Fienstein, 1965; Sullivan *et al*, 1969). This indicates that a selective transport mechanism is operating in the mammary glands in favour of IgG1. More recently, Brandon *et al* (1971) showed that colostral IgG1 was indeed derived preferentially from serum. This evidence was based on the temporal changes in the concentration of both

IgG1 and IgG2 in the serum and colostrum before and after parturition, clearly indicating selective removal of IgG1 in preference to IgG2.

1.8.2 The origin of colostral IgA and IgM

The concentrations of both IgA and IgM in the colostrum are greater than that in the serum (Table 2). Much of the IgA and IgM is locally synthesised in the mammary glands (Lascelles, 1970). Thus sheep mammary glands either immunised or infected naturally produced immunoglobulins which are largely IgA (Lascelles et al, 1966; Outteridge and Lascelles, 1967; McDowell and Lascelles, 1969; 1970; Lee and Lascelles, 1970). IgM and small amounts of IgG are also produced (Lascelles and McDowell, 1970). It is regarded that most IgA producing systems in ruminants will become active following antigenic stimulation (Lascelles and McDowell, 1974). Unstimulated primaparous glands are therefore mostly lacking in IgA production activity, while the glands of the older ewes, particularly in ewes which were antigenically stimulated, were heavily populated with lymphocytes concerned with IgA production (Lee and Lascelles, 1969). More recently Watson and Lascelles (1973), were able to obtain quantitative data showing that local synthesis was the main source of colostral IgA in both immunised and non-immunised glands of ewes.

Evidence on the local production of the two immunoglobulins in cow's mammary glands is still sparse. Studies by Mach and Pahud (1971) using tissue culture did reveal that IgA producing cells were present in the colostrum-forming udder. This indicates that the phenomenon as described for sheep is also common to other ruminants.

The proportion of colostral IgA and IgM synthesised locally

has also not been fully investigated in sheep and cows. In the pig it is known that all colostral IgG and a large proportion of IgM and about 40% of IgA are derived from serum (Bourne and Curtis, 1973). In the milk local production of IgA and IgM is believed to be at 90% while IgG is at 70%.

1.8.3 The mechanism of transport of colostral immunoglobulins

The manner of how selective transfer of IgG1 from circulation across the epithelial cells of the mammary gland is achieved has been the subject of a number of investigations and speculations. *In vitro* studies by Hammer and coworkers (c.f. Brandon *et al*, 1971) have shown that this selectivity occurred in the glandular epithelial cells. Lascelles (1970) suggested that this selective mechanism involved the need for Fc type specific receptors similar to that postulated earlier by Brambell (1966), but which are specific to IgG1. The presence of these receptors, believed to be located on the basal and intercellular membrane of the glandular epithelial cells (Brandon *et al*, 1971) would allow formation of transport vehicles which would contain considerably more IgG1 than IgG2.

The mechanism of transport of IgA and IgM into colostrum is even less well understood. Watson and Lascelles (1973) recently showed that the magnitude of preferential transfer of IgA and IgM in the immunised and non-immunised mammary gland of ewes was closely related to the activity of the local immune system. On the basis of this finding they proposed that these two immunoglobulins were transported across the glandular epithelium within vesicles assisted by the concentration gradient created through local synthesis of these immunoglobulins,

which occur in the proximity of the glandular epithelium.

Others have postulated that the SP may play an important role in assisting the secretion of IgA across the epithelium cells by complexing with IgA (Tomasi and Bienenstock, 1968; Tourville et al, 1969). However evidence presented by Allen and Porter (1973) and Allen et al (1973) from immunofluorescent and electron microscope studies in pig intestine showed that the transport of IgA across the epithelial cells which occur by way of vesicles was quite independent of the requirement for SP. This has also been shown in immunohistochemical study in mice by Comoglio and Guglielmone (1973) who also showed that SP was probably concerned more with local defence by anchoring IgA to luminal surface. They maintained that the joining of SP to IgA took place in the lumen rather than at the base of the epithelial cells as postulated by Tourville et al (1969).

The function of SP in bovine mammary glands is not known. Nonetheless, SP is found in the colostrum as well as in milk (Porter and Noakes, 1970; Mach and Pahud, 1971).

1.9 Absorption of Colostral Immunoglobulins by the Newborn Calves

The intestines of newborn calves are generally permeable to intact immunoglobulins during and up to 36 hours postnatal life (Comline and Robert, 1951; Deutch and Smith, 1957; Smith and Erwin, 1959; McCoy et al, 1970) and with declining efficiency with time (Kruse, 1970b). During this period, ingested colostral immunoglobulins are absorbed exclusively via the lymphatic system and reach the circulation through the thoracic duct (Comline and Robert, 1951;

Balfour and Comline, 1962). Peak serum concentrations are usually attained by twelve hours following feeding for IgA and IgM, and about 24 hours for IgG (Husband et al, 1972). Thereafter the serum immunoglobulins decline progressively at a rate depending on the biological half life of the protein (Husband et al, 1972; Logan et al, 1972) as well as the state of health of the calves (McDougall and Mulligan, 1969).

The absorption of the immunoglobulins by the intestine is largely non selective (Deutch and Smith, 1957; Bangam et al, 1958; Pierce, 1961a; 1961b; Pierce and Fienstein, 1965) and independent of requirement of Fc type specific receptor (Fey, 1971). Nevertheless only the large immunoglobulin molecules are retained in the circulation; most of the non-antibody colostral proteins, particularly beta-lactoglobulin, are rapidly filtered by the kidney giving rise to transient protenuria which usually occurs during the period of colostral intake (Deutch and Smith, 1957; Pierce, 1959; 1960; 1961a and b; Pierce and Johnson, 1960; Hardy 1969b).

The region of the calves' intestines where absorption takes place has not been shown conclusively. Hardy (1969a) has, however, suggested that the terminal part of small intestine was probably the only site capable of absorbing colostral immunoglobulins. Thus, absorption of labelled serum immunoglobulins did not occur when infused through the upper part of the small intestine.

1.10 Cessation of Uptake of Intact Immunoglobulins by the Intestine of Newborn Calves

The cessation of uptake and transfer of immunoglobulins by

the intestines of newborn calves was, at one stage, regarded to be associated with the onset of peptic activity which digests the colostral proteins in the young animal (Hill, 1956). This was however disputed by Deutch and Smith (1957), Smith and Erwin (1959). In the former instance, inhibition of gastric proteolytic activity by aluminium hydroxide gel and probanthine did not result in absorption of immunoglobulin after the 36-hour period of postnatal life. Smith and Erwin were also unable to show absorption of colostral immunoglobulins in calves of two days and older when their gastric juice was prevented from entering the intestine by ligature.

In other newborn animals such as lambs and piglets, absorption of macromolecules also ceases by 30 to 48 hours of postnatal life (Lecce et al, 1961; Simpson-Morgan Smeaton, 1972). However in the case of piglets, the time of intestinal 'closure' to macromolecule absorption may be prolonged up to 86 hours by withholding food (Lecce et al, 1961). This, however, is not possible in calves (McCoy et al, 1970). On the contrary it has been stated that premature loss of permeability can occur in calves as young as 6 to 8 hours old (Gay, 1965; Gay et al, 1965). In these instances the authors showed that calves which had been given colostrum or assumed to have suckled, were found to be agammaglobulinaemic. The volume and concentration of immunoglobulins in the colostrum used, however, was not stated.

The effect of feeding prior to ingestion of colostrum on the absorption of immunoglobulins was demonstration by Graves (1963). He showed that feeding of protein or immune serum to newborn calves prior to feeding colostrum inhibited absorption of the immunoglobulins from the latter. This indicates that the presence of food could depress the

mechanism involved in the absorption.

A series of histological and morphological investigations on the phenomenon of closure has recently been reported involving different animals (Clark and Hardy, 1969; 1970; 1971a and b). Similar work was also reported by Simpson-Morgan Smeaton (1972) with lambs. These workers noted that the uptake and transfer of macromolecules by the intestine may involve two processes, namely (i) uptake by vacuolated epithelial cells in the intestine and (ii) release of the protein to the lymph. The latter process ceases much earlier and is affected by factors such as feeding and the solution used to fee the macromolecules. Thus it may be possible that colostral immunoglobulins are taken up by the epithelial cells after the 36 hours of life but not released into the circulation.

The reason for the closure is also not known, although corticosteroid hormones may have some influence. Work by Halliday (1959) showed that a decline in absortive ability was brought about by large doses of deoxycorticocosterone acetate or cortisone acetate. This decline also coincides with an increase in the activity of alkaline phosphatase, thus suggesting that the two processes are related. Little is known however on the role of this enzyme.

1.11 Factors Affecting the Amount of Colostral Immunoglobulins absorbed

Under natural mothering conditions, the amount of colostral immunoglobulins in the sera of 2 to 3 day-old calves varies considerably from almost negligible to very high levels, often exceeding that in the adult serum (Smith, 1962; Gay *et al*, 1965; Smith *et al*, 1967; Selman *et al*, 1970). Low concentration of immunoglobulins in the calf's serum

may be produced by a failure of the cow to secrete sufficient immunoglobulin into the colostrum (Kruse, 1970b) or the calf not suckling within the period of permeability or inability of the calf to absorb the immunoglobulin ingested (Fey and Margadant, c.f. Selman et al, 1970b; McCoy et al, 1970).

Where colostrum is fed from buckets, low serum level of immunoglobulins is probably due to feeding too late or too little or both (Smith et al, 1967; Kruse, 1970b; McBeath et al, 1971; Selman et al, 1971). Thus it is important to feed newborn calves with sufficient colostrum very early in life. Apart from that, it has been shown that calves which receive colostrum from buckets tend to have a lower serum immunoglobulin level than those which suckled (Smith, et al, 1967; Selman et al, 1971b). In these instances, however, precise information regarding the volume and concentration of immunoglobulin in the colostrum used was not known. Later, Selman et al (1971a) were able to show that calves which were allowed to remain with their dams after feeding colostrum, absorbed greater amounts of immunoglobulin than a non-mothered group in spite of similar amounts of colostrum ingested.

The effect of certain protein factors present in colostrum which promote absorption of immunoglobulins has been shown by Balfour and Comline (1962) and later by Hardy (1969). When immunoglobulin was dissolved in colostral whey or filtered and boiled colostrum, absorption was much more rapid than immunoglobulin dissolved in saline. A similar effect was produced by lactate pyruvate and salts of certain fatty acids. However the role of these anions is uncertain

since the concentrations necessary to produce the effect were far greater than their concentrations in colostrum.

1.12 The Efficiency of Absorption of Colostral Immunoglobulins in Newborn Calves

Investigation into the absorption efficiency of colostral immunoglobulins by newborn calves have been relatively few. In one of the earlier experiments, Bangam et al (1958) noted an absorption into circulation of about 8 to 16% after feeding 200 to 500 mg of labelled serum globulin. A similar experiment was conducted by Balfour and Comline (1962), using anaesthetised calves realised an absorption of 12 to 25% when sampled from the thoracic duct. Similar results were also obtained by Hardy (1969) in a later experiment.

McEwan et al (1970a) have shown, however, a higher absorption efficiency for colostral immunoglobulin in calves. They obtained an average of 25% absorption into the plasma when fresh colostrum was given and upon taking into consideration the fat and casein content of the colostrum used and diffusion into other fluid compartments, the value of 65% was calculated. In a similar experiment by Kruse (1970b), an efficiency of only 20% was recorded when colostrum was fed within a few hours of life.

More recently, Brandon and Lascelles (1971a) showed that the relative efficiency of absorption of the three classes of immunoglobulins when samples were taken from the thoracic duct lymph soon after feeding, were similar. This was then followed with other experiments by Husband et al (1972; 1973) which showed that the apparent

efficiency of the absorption of the different immunoglobulins were as follows: IgG1 43.9 ± 4%, IgG2 58.9 ± 10%, IgA 71 ± 14%, IgM 94.7 ± 9% (means ± standard errors). From these data and from the earlier observation by Brandon and Lascelles (1971) it was concluded that all the four immunoglobulins were absorbed equally well and at an efficiency approaching 100 percent. This conclusion was based on the absorption efficiency of IgM which, because of its large size, was retained within the intravascular compartment. IgG1, IgG2 and IgA were assumed to diffuse into other body fluids, hence lowering their apparent absorption efficiencies relative to IgM.

1.13 The Present Work

There are still many gaps in the knowledge of many aspects of bovine immunology which require further research and much is still to be learnt on the importance of various immunoglobulins for the early defence of calves against various diseases. One of many practical features of calf immunology is the need for newborn calves to acquire adequate passive immunity via the dam's colostrum to insure survival. Thus, it has been stated that calves receiving less than 80 g. of immunoglobulins invariably died (Meyer and Steinbach c.f. Kruse, 1970a). A cow therefore, as suggested by Kruse (1970a) should produce a minimum of 100 g. of immunoglobulin in the first day of calving. In reality, however, the calf's ability to acquire adequate immunity is influenced considerably by the total yield of colostrum and the absorption efficiency of ingested colostrum, all of which vary.

In the present work the following aspects of cow's and calf's immunology have been investigated under local conditions:

(i) A survey to estimate the yeild of colostrum at first milking post partum and to estimate the yield of individual immunoglobulin from individual quarters of the glands.

(ii) An estimate of absorption efficiency of absorption of different classes of colostral immunoglobulins by calves fed from a nipple feeder.

(iii) An estimation of the efficiency of absorption of colostral immunoglobulin under natural conditions using data obtained from (ii).

CHAPTER II

MATERIALS AND METHODS

2.1 Animals

2.1.1 Cows and calves

Eighteen multiparous Friesian cows which calved in autumn were used in Experiment A; their newborn calves were used in Experiment B. Another twenty cows which calved in spring and their newborn calves were used in Experiment C. These animals were part of the town milk herd of Massey University No. 1 Dairy Unit.

2.1.2 Rabbits and guinea pigs

New Zealand white rabbits and guinea pigs bred and maintained in the Massey University Small Animal Production Unit were used for the raising of antisera.

2.2 Chemicals

All the chemicals used in the experiment for the preparation of solutions and buffers were of analytical quality.

2.3 Isolation of Immunoglobulins

2.3.1 Source of immunoglobulins

(a) Bovine serum

Blood for the preparation of sera was obtained by venepuncture from cows when required. The blood was allowed to clot at

room temperature and then remained overnight at 4°C. The serum was then expressed by centrifugation 2500 g for 20 minutes.

(b) Bovine colostrum

Bovine colostrum used for the isolation of IgG1 and other analytical purposes was obtained from the first milking post partum. Colostral whey was prepared as follows. The colostrum at 4°C was first centrifuged at 2000 g for 30 minutes in order to separate the fat. Commercial rennet was then added to the fat free colostrum at a rate of 1:100 (Selman et al, 1971) and the mixture incubated at 37°C for 15 minutes. The resultant curd was broken into small pieces and the whey expressed by centrifugation at 12350 g for 20 minutes.

(c) Sheep serum

Sheep serum was obtained from the local Freezing Works. The blood was collected in clean buckets and its serum expressed in a similar manner to that of bovine serum (page 29).

2.3.2 Fractionation methods

(a) Salt fractionation

Fractions containing gamma globulins from sera and colostral whey were prepared by precipitation with ammonium sulphate at a final concentration of 33% as described by Campbell et al (1970). The procedure was repeated twice in order to produce relatively pure gamma globulin fractions which were subsequently further purified by gel chromatography. A detailed flow diagram is outlined in Figure 2.

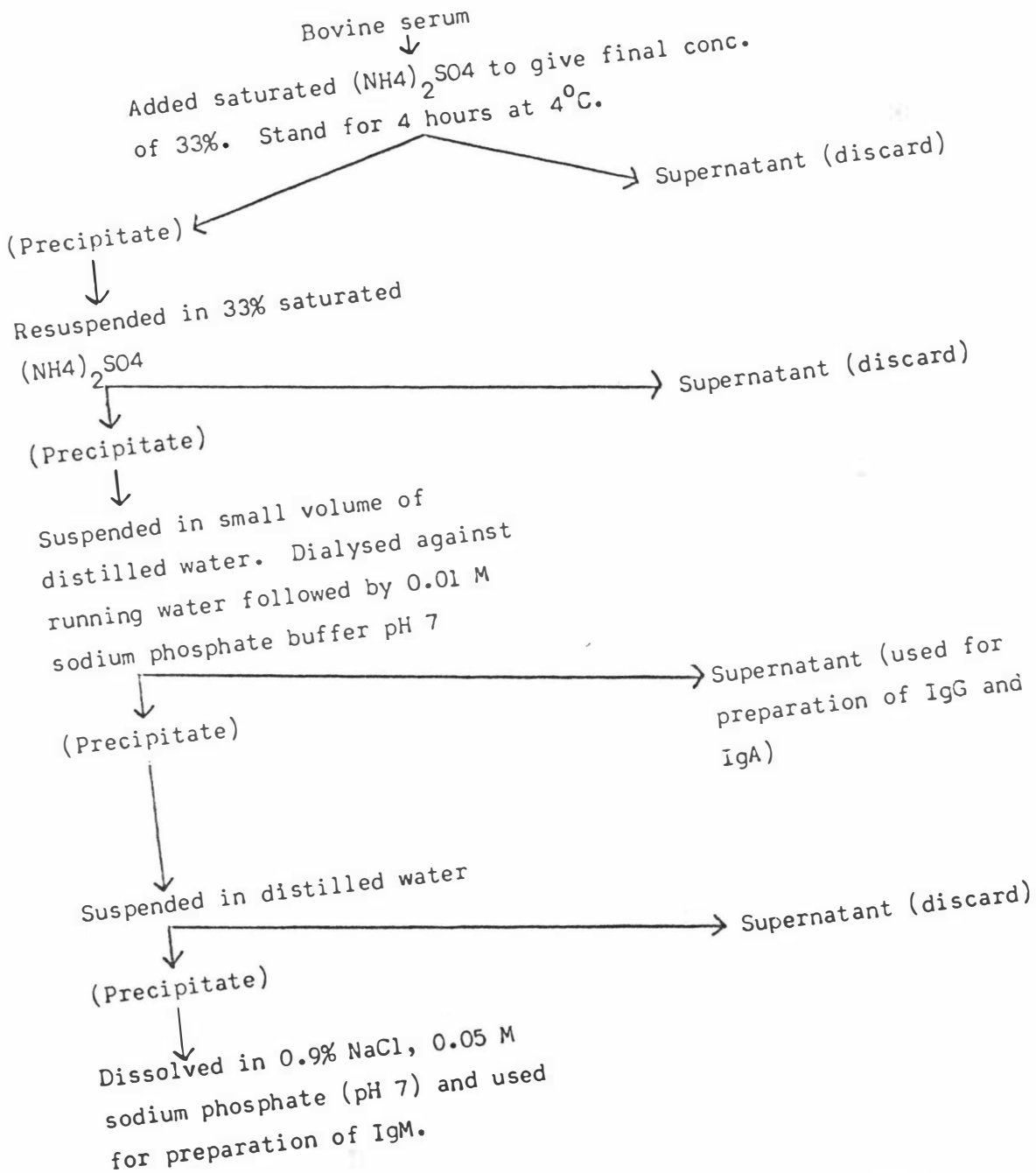


Figure 2. Flow diagram outlining the isolation procedure for preparation of different bovine immunoglobulins.

(b) Gel chromatography

This was performed on Sephadex G200 (Pharmacia) column (3.0 x 60 cm) equilibrated with 0.05M sodium phosphate buffer pH 7 and containing 0.9% sodium chloride and 0.02% sodium azide. The gel was prepared following the method described in Work and Work (1969). The flow rate was maintained at 20 ml per hour and fractions of 5 ml were collected using a fraction collector. The protein in the eluent was determined at 280 nm.

(c) Ion exchange chromatography

Anion exchange chromatography was performed on either DEAE Sephadex A50 (Pharmacia) column (2.2 x 60 cm) equilibrated with 0.02M sodium phosphate buffer (pH 8) or on DEAE cellulose (2.2 x 60 cm) column equilibrated with 0.05M sodium phosphate buffer (pH 8) containing 0.9% sodium chloride. A continuous gradient was produced by slowly increasing the molarity of sodium chloride and sodium phosphate in the eluting buffer. This was achieved by connecting a siphon from the flask containing the limiting buffer into a second flask containing 500 ml of the eluting buffer and equipped with a stirrer. This flask was connected to the column via a pump. This effectively provided a linear gradient of increasing molarity as determined by the conductivity of the eluent.

2.3.3 Methods of assessing the purity of the protein fractions

(a) Immunoelectrophoresis

Micro immunoelectrophoresis was performed on microscopic slides with barbital buffer (pH 8.6) using the LKB apparatus. The method

used was similar to that recommended in the manufacturer's handbook.

(b) Double diffusion

Double immunodiffusion was carried out in 1% agar in 0.9% sodium chloride on glass microscopic slides, as described by Campbell et al (1970).

(c) Radial immunodiffusion

This was carried out as described in section 2.5.4.

2.3.4 Preparation of individual immunoglobulins

(a) Bovine IgG1

Bovine IgG1 was prepared from colostral whey with slight modification, by the method of Brandon et al (1971). Salt precipitation of colostral whey with ammonium sulphate was carried out and repeated twice as described earlier. After centrifugation, the precipitate was dissolved in small amounts of distilled water and dialysed in running water followed by several changes of 0.01 M sodium phosphate buffer (pH 8). The preparation was then centrifuged and a suitable amount of the supernatant which was rich in IgG1 was fractionated on DEAE cellulose with a continuous gradient from 0.01 M to 0.05 M phosphate in 0.5 M sodium chloride, pH 8. The fractions in the descending side of the second peak were concentrated by negative pressure dialysis and further purified on a Sephadex G200 column. Pure IgG1 was recovered from the fractions in the largest peak. This shown shown in Figure 3a.

(b) Bovine IgG2

This was prepared from bovine serum by a method

similar to that described by Porter (1972) and Brandon et al (1971). Bovine serum was first dialysed overnight in distilled water. After centrifugation the supernatant was equilibrated with 0.02M sodium phosphate buffer (pH 8) and chromatographed on DEAE Sephadex A50 column eluted in the same buffer. The fall through fraction which was rich in IgG2 was concentrated and further fractionated on a Sephadex G200 column. Pure IgG2 was obtained in the fractions of the largest peak.

(c) Bovine IgM

Relatively pure IgM fractions were prepared as follows. Bovine serum was first subjected to salt preparation and the protein precipitate obtained was dissolved in equal volume of water. The preparation was dialysed in distilled water and then in sodium phosphate buffer (0.01 M, pH 7). After centrifugation the supernatant was retained for later use in isolation of serum IgA to be described on page 36. The precipitate was resuspended in distilled water and again centrifuged. The precipitate obtained was then dissolved in 0.05 M sodium sulphate buffer (pH 7) containing 0.9% sodium chloride, and fractionated on a Sephadex G200 column. The ascending limb of the first peak was then collected, concentrated and applied to a continuous gradient DEAE cellulose column. The third peak was collected and recycled on G200 for further purification (Figures 3b, 3c and 3d).

Figure 3. Immunoelectrophoretic and immunodiffusion analyses of variious bovine immunoglobulins purified from serum or colostrum. The symbols used are:

G1 = IgG1

G2 = IgG2

M = IgM

A = IgA

BS = whole bovine serum

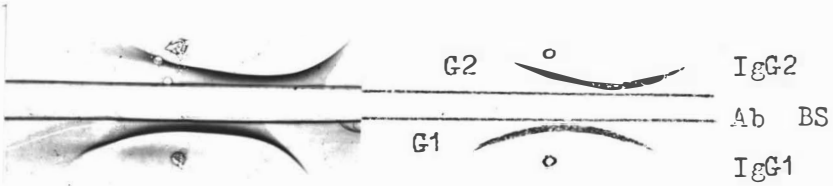
C = colostrum

Ab preceding any of the above denotes antiserum to that protein.

Ab IgM* refers to monospecific antiserum to bovine IgM provided by Professor Lascelle (page 38).

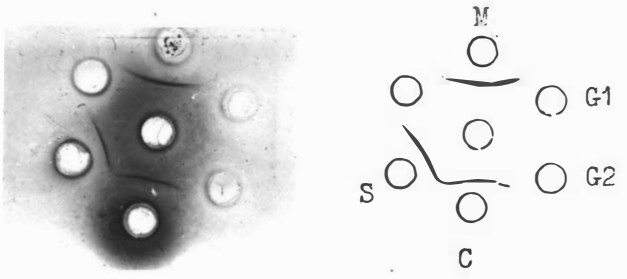
Anode Cathode

3a

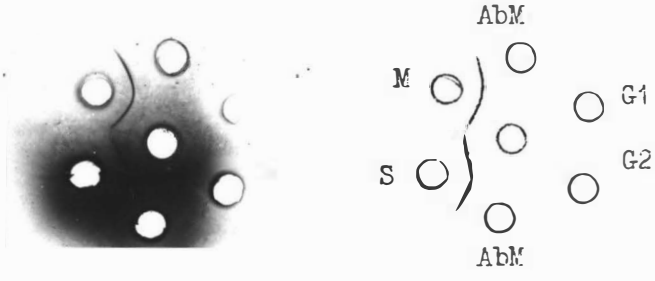


3b

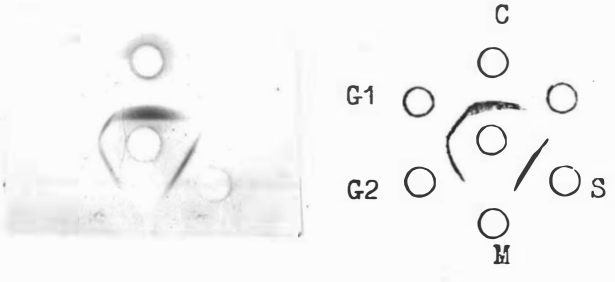
3c



3d



3e



3f

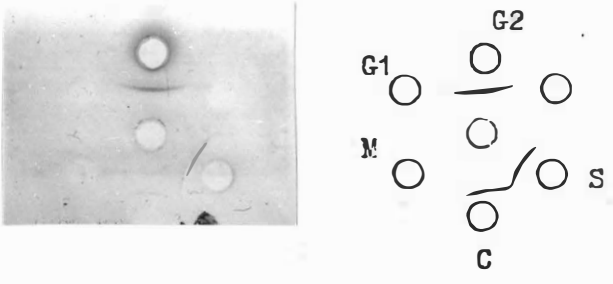


Figure 3

In later preparations, the IgM fraction was isolated by gel filtration of serum euglobulin on Sepharose 6B column. The IgM fraction was obtained by diluting the serum with six volumes of distilled water. The precipitate formed was collected and redissolved in 0.9% sodium chloride solution and the sample applied on to the column. The fractions preceding the largest peak were collected, concentrated and recycled three times in the same column to give pure IgM.

(d) Bovine IgA

IgA used for the preparation of antisera was prepared from serum by modification of the method of Butler and Maxwell (1972). The supernatant obtained during the preparation of IgM (page 34) was used as a source of serum IgA. This was first dialysed in the starting buffer before fractionating on DEAE cellulose column. The fraction from the descending limb of the second peak were collected and concentrated and filtered on a Sephadex G200 column. The fraction immediately after the first peak and preceding the second peak were then recycled on the same column. Selected fractions from the first peak were found to be rich in IgA but contained traces of IgG. This fraction was used in the preparation of antiserum to IgA.

Several further attempts to fractionate IgA from samples of colostral whey, lymph and milk had not yielded the quantity of reasonably pure IgA required for construction of IgA standard curve. These failures are however not surprising, due to the low level of this immunoglobulin in the samples used. Other workers have also expressed difficulties in obtaining IgA in reasonable quantity and

purity (Butler and Maxwell, 1972). Because of this, it was considered as an alternative, that the quantity of IgA would be best expressed as a relative to the concentration of IgA in the serum, which vary from 0.1 to 1.2 mg/ml (Table 2). It is also pointed out by Butler (1971a), Mach and Pahud (1971) and Butler et al (1972) that both forms of IgA, i.e. dimeric IgA and SIgA occur in the serum. Thus the standard curve was constructed using dilutions of bovine serum. The resultant slope of the curve was then rechecked with dilutions of colostral whey to confirm its similarity. In view of this, the quantity of IgA in the present study is expressed as relative to serum IgA (RSA).

(e) Sheep IgG2

Pure sheep IgG2 was kindly provided by Dr D.D.S. Mackenzie.

(f) Sheep serum immunoglobulin preparation

This was prepared by salt precipitation as described on page 30. The protein precipitate obtained after the second precipitation was dialysed overnight against running water. The supernatant was taken and its protein content was determined using an extinction coefficient (E1% 280 nm) of 13.7 for IgG. Sodium chloride was then added to give a final concentration of 0.9 g/100ml. Aliquots of approximately 30 ml containing 2 gm of gamma globulin were frozen until required.

2.4 Antisera

2.4.1 Preparation

Antisera to bovine immunoglobulins were prepared by injecting the rabbits or guinea pigs with the appropriate protein fractions

emulsified with Freund's incomplete adjuvant. Three injections were given at two weekly intervals. Each fraction for injecting rabbits contained either 5 mg IgG1, 5 mg IgG2, 2 mg IgM or 1 mg IgA. 1 mg IgG2 was injected into the guinea pigs.

Two weeks after the last injection, the rabbits and guinea pigs were bled either from the ear veins or by cardiac puncture. Several bleedings at two weekly intervals were carried out after which another injection was given.

Monospecific anti-bovine IgA and anti-IgM were also kindly provided by Professor A.K. Lascelles of the Dairy Research Foundation, Canberra, Australia. These were extensively used to assess the purity of our preparations of antisera and antigens.

2.4.2 Adsorption of antisera

(a) Anti bovine IgG2

Rabbit antisera to bovine IgG2 failed to show spurring of IgG1 and IgG2 specific determinants (Figure 4a). This was in accordance with the result reported by Butler and Maxwell (1972). It was then decided that the rabbit antisera to bovine IgG2 be used for quantitative determiation of total IgG (IgG1 + IgG2). Nevertheless this antisera had to be extensively adsorbed with IgM to render it monospecific.

Guinea pig antisera to bovine IgG2 on the other hand, showed spurring to both IgG subclass determinants as shown in Figure 4b, and was suitable for production of anti IgG2 when appropriate adsorbed with IgG1. This was done by repeated adsorption with soluble IgG1

which removed the line corresponding to IgG1 while leaving the IgG2 line partially intact (Figure 4c).

(b) Anti bovine IgM

Antiserum to bovine IgM required adsorptions with precolostral calf's serum and IgA to render it monospecific (Figures 4g, 4h, 4i and 3c). The resultant antiserum when reacted with whole serum in double immunodiffusion showed a single line of complete identity with anti IgM provided by Professor Lascelles (Figure 3d).

(c) Anti bovine IgA

Antiserum to bovine IgA required adsorptions with both precolostral calf's serum and IgG2 (Figures 4d and 4e). The resultant antiserum also showed a single line of complete identity with anti IgA provided by Professor Lascelles when reacted with whole serum and colostrum in double immunodiffusion (Figure 4f).

(d) Anti bovine IgG2

Rabbit antiserum to bovine IgG2 (provided by Dr D.D.S. Mackenzie) was exhaustively adsorbed with bovine serum to remove cross reaction to bovine IgG in the antiserum (Figure 4j).

2.5 Analytical Methods

2.5.1 Fat content in the colostrum

Fat percentage in the colostrum were assessed in triplicate using the method of Fleet and Linzel (1964). Colostral samples were drawn by capillary action into haematocrit tubes immediately after milking. Whenever this was not possible, the sample was first heated

Figure 4. Immunoelectrophoretic and immunodiffusion analyses of antiserum to bovine IgG2, IgA, IgM and sheep IgG2.

The symbols used are:

G1 = IgG1

G2 = IgG2

G = IgG (IgG1 + IgG2)

A = IgA

M = IgM

BS = whole bovine serum

C = colostrum

SS = whole sheep serum

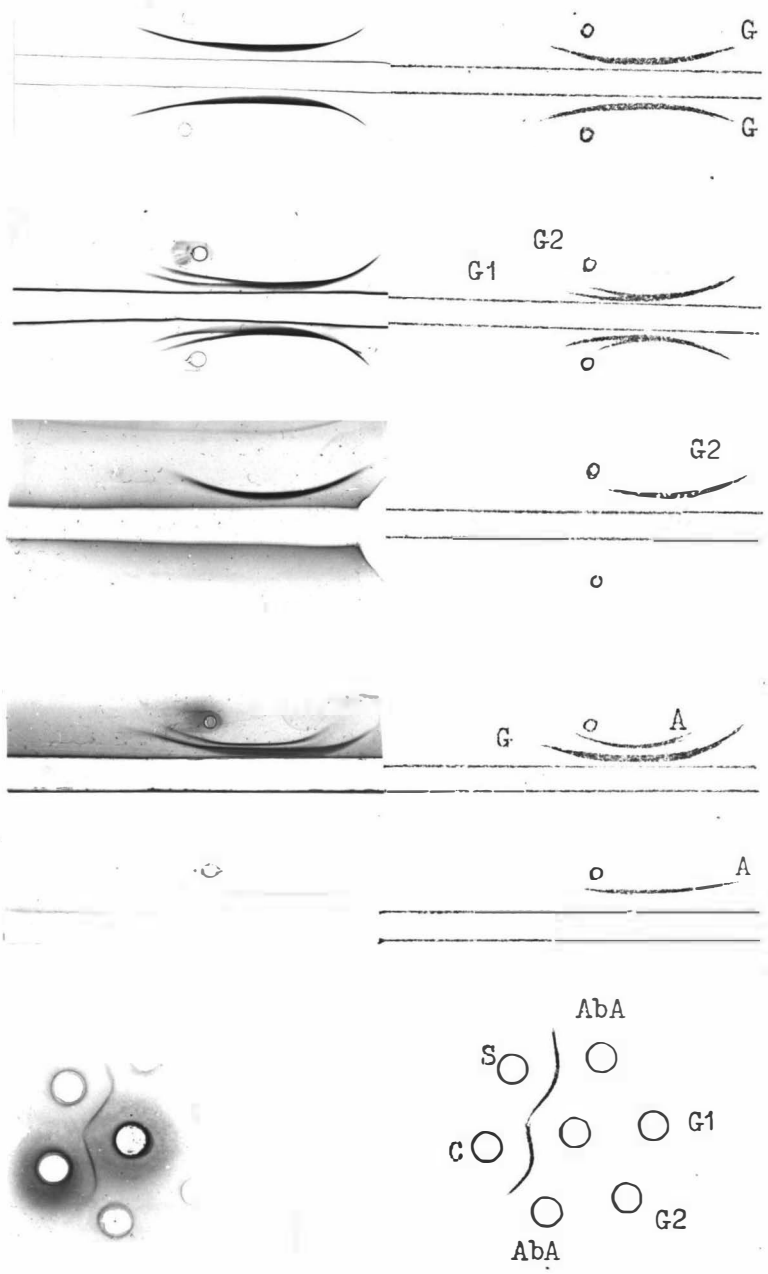
SG2 = sheep IgG2

Ab preceding any of the above denotes antiserum to that protein.

R or GP denotes that the antiserum was raised in rabbits or guinea pigs.

*Ab IgA refers to monospecific antiserum to bovine IgA provided by Professor Lascelles (page 38)

Anode Cathode



BS
R Ab IgG2 (adsorbed with IgM)
C

IgG
GP Ab IgG2
BS

IgG2
GP Ab IgG2 adsorbed with IgG1
IgG1

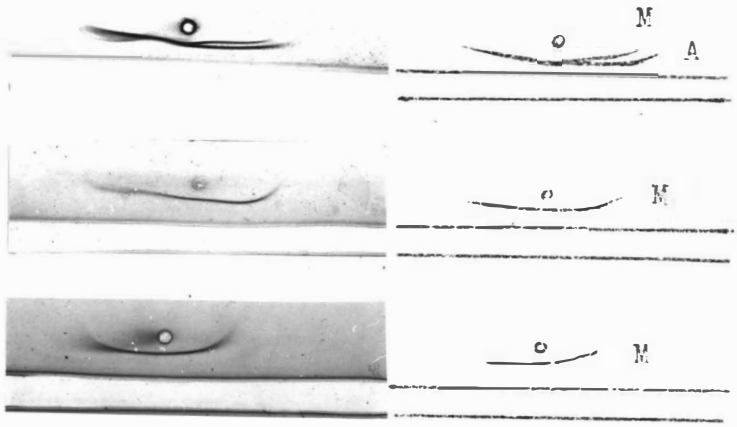
BS
R Ab IgA (unadsorbed)

BS
R Ab IgA (adsorbed with IgG)

Centre well
Ab IgA*

Figure 4

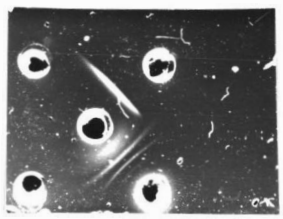
Anode Cathode

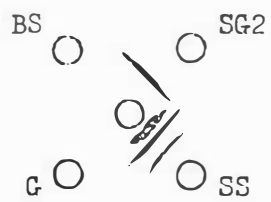


4g BS
Ab IgM (unabsorbed)

4h BS
Ab IgM (absorbed with IgA)

4i C
Ab IgM (absorbed with IgA)





4j Centre well
Ab sheep IgG2

Figure 4 - continued

to 37°C and thoroughly mixed before the sample was drawn. The tubes were then sealed and spun for 15 minutes in a haematocrit centrifuge at 10,000 g and the fat percentages were obtained by measuring the fat layer from a haematocrit chart.

2.5.2 Estimation of casein content in the colostrum

The casein content in the colostral samples was estimated using the dye binding method of Dolby (1961). Protein content of the fat free colostrum and total protein in the colostral whey were estimated and the casein content obtained by the difference. Details of the method were as follows.

To 0.05 ml of the protein sample was added 1 ml of 0.9% saline solution. 5 ml of dye solution containing 0.062% amido black was added, mixed, left for 15 minutes and then centrifuged at approximately 1,500 g. 0.2 ml of the supernatant was then diluted with 4 ml of distilled water and its optical density (O.D.) measured at 615 mu. Determination of the O.D. was also made on the blank which was prepared by adding 1 ml saline to 5 ml of the dye solution and carried through in a similar manner. The difference in the O.D. value, $O.D._{blank} - O.D._{sample}$ was then used to determine the protein content of the unknown directly from a standard curve. The latter was prepared in a similar manner using dried skin milk of known protein content. An example of the standard curve is shown in Appendix 17, Figure 2.

2.5.3 Estimation of colostral immunoglobulin using Biuret reaction

This was estimated from the difference between the total

protein content in the colostral whey and its albumin content. The Biuret reaction method was used as described by Gornal and coworkers (1949). The standard curve was prepared using commercial bovine albumin. An example of this is shown in Appendix 17, Figure 1.

2.5.4 Radial immunodiffusion

Quantitative determination of immunoglobulins in the colostrum and serum samples was carried out using the method of single radial immunodiffusion of Mancini et al (1965), and Fahey and McKelvey (1965). 1% agar (Noble Difco) was prepared in sodium phosphate buffer (0.01 M pH 8) containing 0.9% sodium chloride and stored in volumes of 20 ml. When required the agar was melted and allowed to cool to 50°C in water bath. Accurate amounts of appropriate antisera were then added and mixed thoroughly. The antibody containing agar was then poured onto a level glass plate (7 x 15 cm) enclosed in a perspex frame and allowed to harden at room temperature. Antigen wells of 2.5 mm diameter were later cut 1.5 cm apart. The wells were subsequently filled with 10 ul of suitably diluted colostrum or serum samples. Reactions were then allowed in a humid chamber at room temperature for 24 hours for quantitation of IgG and IgA and 48 hours for IgM. The ring diameter was subsequently determined using a Partigen reader (Hoechst), and converted to the actual concentration in mg/ml using the appropriate standard curve.

The standard curve was prepared using known concentrations of each antigen. Examples of these are given in Appendix 17, Figures 3, 4, and 5).

2.6 Statistics

Statistical evaluations were based on t-test, analysis of variance and the method of 'Least Square Deviation' as described in Snedecor and Cochran (1967).

CHAPTER III

EXPERIMENTAL PROCEDURES AND RESULTS

3.1 Experiment A

A survey of the yield of colostrum and its immunoglobulin content at first milking post partum.

3.1.1 Procedure

Eighteen multiparous Friesian cows which calved during the autumn months had their calves removed before they suckled. The cows were then milked as soon as possible. Most of the cows were milked within two to six hours of parturition. Each quarter was milked independently using a quarter sample bucket. A transparent section in the milk line allowed observation of milk flow from each quarter, so that individual milking cups could be removed as milk flow from that quarter stopped. In eleven of the cows, when all the four quarters had been milked, the cow was injected with 5 i.u. of oxytocin (Orasthin forte, Hoechst) in sterile saline through its jugular vein, and milking was resumed until completion. The volume of the colostrum from each quarter was recorded and a representative sample of about 50 ml was taken after it had been thoroughly mixed. The colostrum from the four quarters was then bulked and a further sample drawn. The bulked colostrum was stored at -10^{o}C in plastic bucklets until needed for feeding the calves.

Colostral samples were analysed within three to four hours of collection for fat percentage, casein and immunoglobulin content

as described earlier (pages 39 and 41).

3.1.2 Results

The increase in colostrum yields following injections of oxytocin was high in most cows (Appendix 1b), possibly because of the stress which was noticeable in these cows, affecting milk letdown prior to the injection. The mean and standard error of this increase was $27.5 \pm 2.5\%$ of the total yield. It might be expected that the other cows which were not injected with oxytocin would have yielded a similar increase if similarly injected.

The total volumes of the colostrum from the four quarters, the immunoglobulins and fat concentrations are presented in Appendices 1a, 2, 3, 4, 5, and 6 and are summarised in Table 4. The means and standard errors of the total yields of colostrum and immunoglobulins are given in Table 5. There was a considerable variation between cows in the yield which ranged from 1.9 to 13.8 litres. The calculated ranges of the total immunoglobulins in the colostrum were: IgG, 217-691 g; IgG2, 7.4-46.3 g; IgA, 7.6-80.9 (RSA) and IgM, 10.5-76.3 g.

Volume differences between the four quarters were however not significantly different, although the mean of the total from the front quarters was less than from the rear quarters ($P < .05$). The means of the fat percentage and concentrations of the four immunoglobulins were also not significantly different between the four quarters. But again there were wide variations between the cows. Nonetheless almost all cows produced high concentrations of immunglobulins

TABLE 4. - Summary of the yields of fat % and concentrations of immunoglobulins IgG, IgG2, IgA and IgM in colostrum from the four quarters of 18 cows obtained at first milking post partum. Values presented are means ± standard errors. Full data are presented in Appendices 1, 2, 3, 4, 5 and 6.

Quarter	Front Left	Front Right	Rear Left	Rear Right	Sig Diff.
Volume (l)	1.30±0.20	1.31±0.21	1.87±0.25	1.72±0.24	NS
Range	0.27-3.05	0.40-3.51	0.61-3.85	0.50-3.83	
Fat %	7.3 ± 0.8	7.2 ± 0.9	6.5 ± 0.8	6.4 ± 0.8	NS
Range	1.5 -13.5	1.0 -13.5	1.0 -14.0	1.0 -14.0	
Total IgG (mg/ml)	82.1± 9.4	85.1± 8.3	88.7± 8.1	88.4± 7.9	NS
Range	33.0-128.9	31.0-154.2	30.0-151.3	30.0-144.5	
IgG2 (mg/ml)	4.82±0.39	4.81±0.43	4.82±0.41	4.95±0.41	NS
Range	1.63-7.88	1.88-8.68	1.48-8.68	1.63-8.08	
IgA (RSA)*	10.3± 1.4	10.2± 1.5	9.6 ± 1.4	11.1± 1.7	NS
Range	2.4 -24.3	1.4 -26.1	1.6 -26.1	1.8 -26.0	
IgM (mg/ml)	7.24±0.72	7.15±0.77	7.20±0.83	7.40±0.82	NS
Range	1.47-12.50	1.66-12.00	1.08-12.00	1.28-12.20	

*RSA = relative to concentration of IgA in bovine serum.

NS = Non significant ($P > 0.05$)

TABLE 5 - The means and standard errors and the ranges of the total yield of colostrum (litres) and immunoglobulins IgG (g) IgG2 (g), IgA (RSA)* and IgM (g) in the colostrum of 18 obtained at first milking post partum.

	Mean ± S.E.	Range
Total volume (l)	6.20±.63	1.90 - 13.80
Total IgG (g)	411±44	217 - 691
Total IgG2 (g)	23.4±3.0	7.4 - 46.3
Total IgA (RSA)*	42.6±4.9	7.6 - 80.9
Total IgM (g)	35.0±4.8	10.5 - 76.3

* = relative to concentrations of IgA in bovine serum.

in their colostrum which ranged from 30.0-151.3 mg/ml for IgG, 1.48-8.68 mg/ml for IgG2, 1.4-26.1 RSA/ml for IgA and 1.08-12.5 mg/ml for IgM. The details of the ranges of the different immunoglobulins from the four quarters are shown in Table 4. Of the eighteen cows investigated, only three produced colostrum containing less than 50 mg/ml of immunoglobulins. None had less than 30 mg/ml. Similarly, most of the colostrum had high levels of fat which were greater than 3% and up to 14% (Appendix 2). The levels of casein were also high (Appendix 7).

It was also evident from the data listed in Appendices 1, 3, 4, 5 and 6 that cows producing a smaller colostrum yield tended to have a higher concentration of immunoglobulins in the colostrum. This is indicated by the high and significant negative correlations between these parameters as shown in Table 6.

Significantly high correlations also exist between the concentrations of different immunoglobulins in the colostrum (Table 7).

TABLE 6 - Correlations between the volumes of colostrum and the concentrations of immunoglobulins IgG, IgG2, IgA and IgM in the colostrum of 18 cows obtained at first milking post partum.

Correlations between colostral volume and:	
IgG	r = -0.58***
IgG2	r = -0.46***
IgA	r = -0.49***
IgM	r = -0.41**

NS = Non significant ($P > 0.05$)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

TABLE 7 - Correlations between the concentrations of different immunoglobulins in the colostrum from the four quarters, and from the bulked colostrum samples of 18 cows, obtained at first milking post partum.

	Correlation (r) in quarter samples	Correlation (r) in bulked samples
Correlations between IgG and:		
IgG2	0.65***	0.65**
IgA	0.72***	0.75***
IgM	0.72***	0.77***
Correlations between IgG2 and:		
IgA	0.51***	0.63**
IgM	0.63***	0.67**
Correlation between IgA and:		
IgM	0.46***	0.52*

NS = Non significant ($P > 0.05$)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

3.2 Experiment B

Estimation of the apparent efficiencies of absorption of bovine colostral immunoglobulins and sheep IgG2 by newborn calves.

The nineteen presuckled calves which were removed from their dams following parturition were used in this experiment. The calves were intended to be fed with the previously available cow's colostrum at a rate of either 4 or 8 g of immunoglobulin (Ig) per kg body weight at birth. The volume of colostrum required to feed each calf was calculated using information from the preliminary assessment of immunoglobulin content in the colostrum by Biuret reaction (page 41). The calculation was as follows:

Volume (ml) of colostrum per kg body weight (B.W.) required.

$$\text{For 4 g Ig/kg B.W.} = \frac{4 \times 100}{\gamma G} \times \frac{100}{(100-F-C^*)} \text{ ml.}$$

$$\text{For 8 g Ig/kg B.W.} = \frac{8 \times 100}{\gamma G} \times \frac{100}{(100-F-C^*)} \text{ ml.}$$

where:

γG = concentration of immunoglobulin in the colostrum in g/100 ml. (g%)

F = fat percentage in the colostrum.

C* = corrected casein percentage in the colostrum.

This was derived as follows:

$C^* = \frac{(100 - F)C}{100}$ where c is the casein content assessed from the fat free colostrum.

3.2.1 Procedure

Each calf was first weighed to the nearest 0.5 kg, sampled and placed in an individual pen. The calculated amount of thawed colostrum was then offered to each calf from a nipple feeder (Appendix 9). Ten of the calves were also given 4 g of sheep serum immunoglobulin (Appendix 14). All calves were only fed colostrum once and within 2 to 6 hours of post natal life. Subsequently, only whole milk was given at approximately 5% of body weight per day. A second and a third blood sample were taken at 24 and 48 hours after feeding colostrum.

3.2.2 Estimation of colostral immunoglobulin ingested and absorbed by the calves

The concentration of the colostral immunoglobulin and sheep serum IgG2 actually fed and absorbed by the calves was analysed from the colostrum and serum samples respectively. Each class of immunoglobulin was estimated using the method of radial immunodiffusion, described earlier (page 42). The mass of immunoglobulin absorbed into the blood was then calculated by multiplying the increase in the serum immunoglobulin concentration at 24 hours after feeding (ΔIg 24), by the plasma volume which was assumed to be at 7% of body weight at birth (McEwan et al, 1968) i.e.

$$\text{g Ig absorbed/kg B.W.} = \Delta Ig_{24}\,(\text{mg/ml}) \times \text{B.W. (kg)}$$

An estimate of the apparent efficiency of absorption was then calculated as a proportion of the amount of immunoglobulin absorbed into the blood

to the amount fed.

3.2.3 Result

(a) Fat and casein content in the colostrum

During the initial stage of the experiment, the casein content of seven colostrum samples used to feed the calves was not estimated but was assumed to be at 5%, based on the data of Roy's (1969). Later the casein content was determined by the method of dye binding (Dolby, 1961). These results and those of fat are presented in detail in Appendix 7. The means and standard errors for fat and casein are 6.0-0.6% and 5.1-0.4% respectively. The latter thus lend support for the earlier assumption which was 5%.

(b) Immunoglobulin in the colostrum

The concentrations of colostral immunoglobulin in the whey determined by the Biuret reaction are listed in Appendix 7. The concentrations of the immunoglobulins in the same colostrum when determined by the more specific method of radial immunodiffusion are given in Appendix 8. From the results it may be seen that the two methods did not give similar results. Almost all estimations based on the Biuret reaction method had overestimated the values obtained by radial immunodiffusion. This overestimate varies from 7.8% to 68% and has a mean and standard error of $32 \pm 6\%$. As a result of this anomaly, and since feeding doses of colostrum to calves was based on the estimation of immunoglobulin by Biuret method, the intended doses of 4 and 8 g immunoglobulin per kg body weight were not reconciled. This, however, did not affect the design of the experiment as will be discussed later. The reasons for the differences in estimation between

TABLE 8 - The means $\pm$ standard errors and the ranges of the amounts of bovine colostral immunoglobulins IgG, IgG2, IgA, IgM and sheep IgG2 ingested, and the amounts absorbed into the blood at 24 hours post feeding, and the apparent efficiencies of absorption of the immunoglobulins by 19 newborn calves. The colostrum and sheep IgG2 were fed to the calves from a nipple feeder at 2 to 6 hours post natal life.

	No. of Calves	Ig ingested (g/kg B.W.)	24-hour serum Ig (mg/ml)	Efficiency of absorption (%)
Total IgG	19	4.404 ± 0.29	20.75 ± 2.22	33.3 ± 2.7
Range		2.43-6.56	5.4-49.2	15.2-54.6
IgG2	19	0.229 ± 0.01	0.902 ± 0.07	26.8 ± 3.5
Range		0.102-0.389	0.36-1.74	12.8-46.3
IgA*	19	0.464 ± 0.05	2.03 ± 0.26	32.8 ± 3.1
Range		0.10-1.03	0.65-4.60	16.6-70.0
IgM	19	0.354 ± 0.03	1.569 ± 0.13	36.0 ± 3.8
Range		0.119-0.555	0.77-2.90	17.7-73.5
Sheep IgG2	8	$29.7 \pm 3.3 \times 10^{-3}$	0.077 ± 0.01	18.5 ± 1.6
Range		$14.0\text{-}45.0 \times 10^{-3}$	.040-0.120	12.2-25.5

* RSA = relative to concentration g IgA in bovine serum

Biuret and immunodiffusion method will be disscussed.

(c) <u>The apparent efficiency of absorption of colostral immunoglobulins and sheep IgG2 by the newborn calves</u>.

The data showing the amount of colostral immunoglobulins and sheep IgG2 fed to and absorbed by the calves are listed in detail

in Appendices 10, 11, 12, 13 and 14 and summarised in Table 8. Analysis of variance showed that the coefficient of absorption of colostral IgG (33.3±2.7%), IgG2 (26.8±3.5%), IgA (32.8±3.1%), and IgM (36.0±3.8%) were not significantly different. Sheep IgG2 was also absorbed by at least eight of the ten calves which were given this protein. However, the absorption efficiency was lower than of the colostral immunoglobulins ($P < .05$). Sheep IgG2 was not positively detected in the remaining two calves.

The relationship between the increase in the concentration of colostral immunoglobulin in the calves' sera and the amount of the corresponding immunoglobulin fed are shown in Figures 5a, b, c, d and e. The correlation coefficients of these relationships are given in Table 9. It can be seen that a tendency occurred for the amount of immunoglobulin absorbed to increase with the amount fed. The apparent efficiency of absorption, however, remains the same.

TABLE 9 - Correlations and regression coefficients between the increase in the serum immunoglobulins concentrations at 24 hours after feeding colostrum (mg/ml), and the amounts of corresponding immunoglobulins ingested (g/kg B.W.) in 19 newborn calves fed with colostrum within 2 to 6 hours after birth.

Immunoglobulins	correlations (r)	Regression ± S.E. coefficient
IgG	0.56*	4.26*** ± 1.53
IgG2	0.64**	2.63* ± 0.78
IgA	0.75***	3.71** ± 0.77
IgM	0.47*	1.78^{NS} ± 0.86
Sheep IgG2	0.81*	2.47* ± 0.60

Figure 5a - The relationship between the amounts of colostral IgG ingested (g/kg B.W.) and the amounts absorbed into the blood (mg/ml) at 24 hours post feeding in 19 newborn calves. The colostrum was fed to the calves from a nipple feeder at 2 to 6 hours post natal ages.

Figure 5b - The relationship between the amounts of colostral IgG2 ingested (g/kg B.W.) and the amounts absorbed into the blood (mg/ml) at 24 hours post feeding in 19 newborn calves. The colostrum was fed to the calves from a nipple feeder at 2 to 6 hours post natal ages.

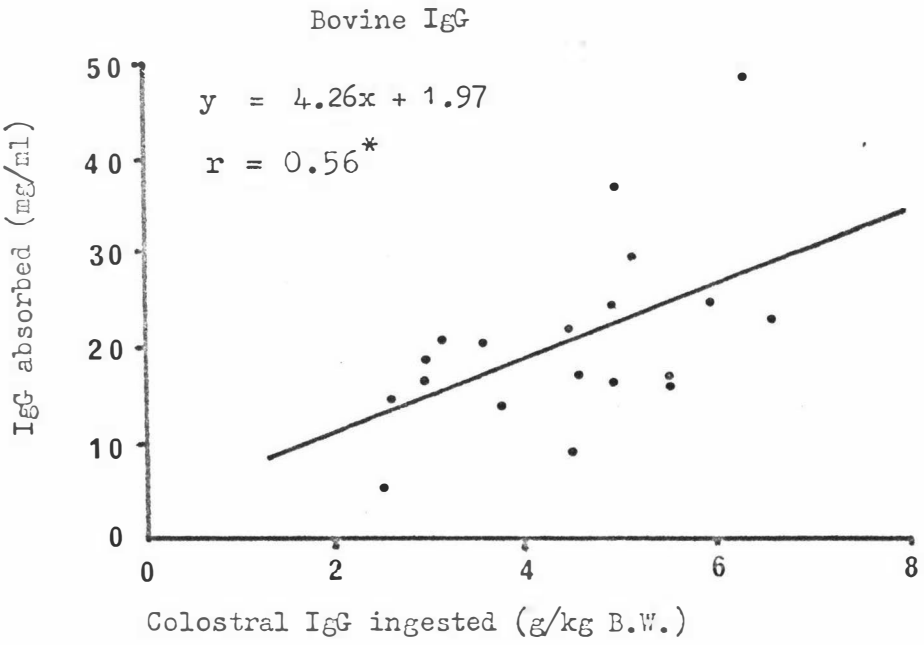


Figure 5a

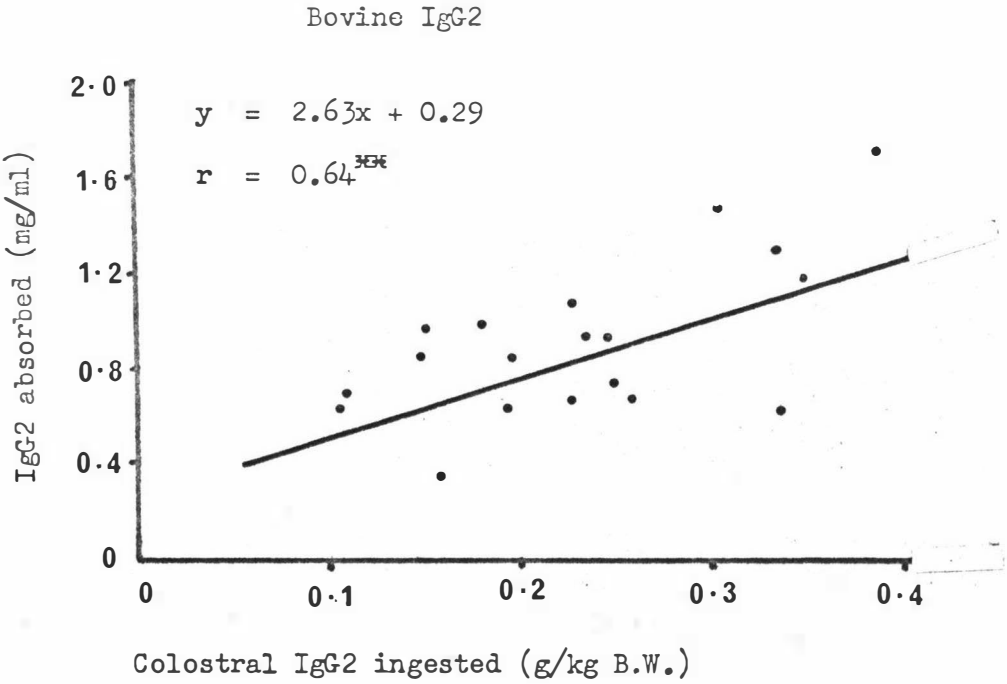


Figure 5b

Figure 5c - The relationship between the amounts of colostral Iga ingested (RSA*/kg B.W.) and the amounts absorbed into the blood (RSA*/ml) at 24 hours post feeding in 19 newborn calves. The colostrum was fed to the calves from a nipple feeder at 2 to 6 hours post natal ages.

RSA* = relative to concentration of bovine serum IgA.

Figure 5d - The relationship between the amounts of colostral IgM ingested (g/kg B.W.) and the amounts absorbed into the blood (mg/ml) at 24 hours post feeding in 19 newborn calves. The colostrum was fed to the calves at 2 to 6 hours post natal ages.

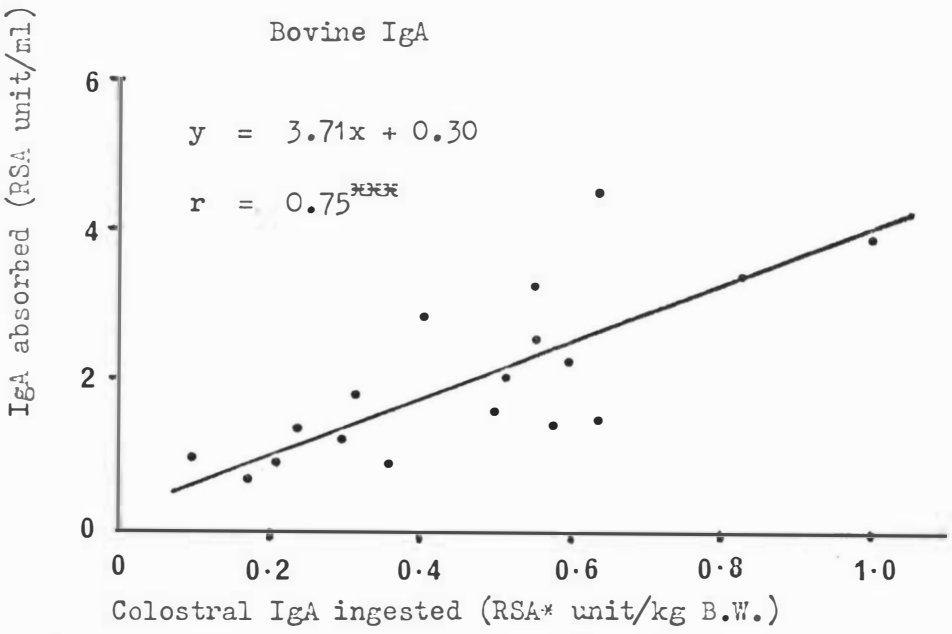


Figure 5c

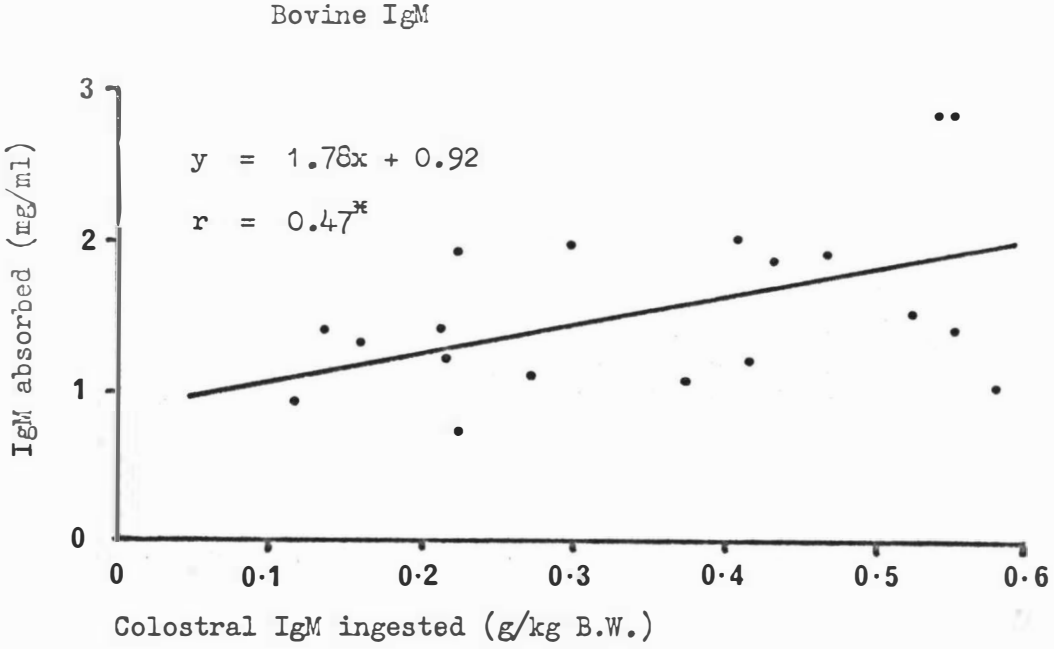


Figure 5d

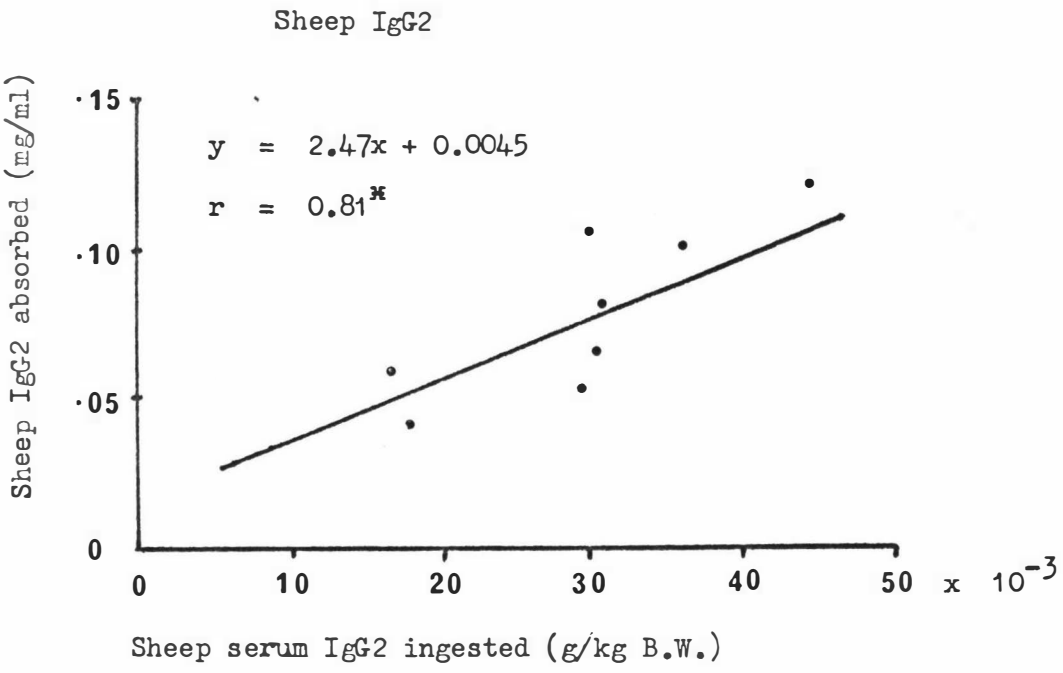


Figure 5e

The relationship between the amounts of sheep serum IgG2 ingested (g/kg B.W.) and the amounts absorbed into the blood (mg/ml) at 24 hours post feeding in 8 newborn calves. Sheep IgG2 was fed to the calves with bovine colostrum from a nipple feeder at 2 to 6 hours post natal age.

TABLE 10 - Correlations between the absorption efficiency of the different immunoglobulins in 19 newborn calves fed with colostrum within 2 to 6 hours after birth.

Correlation between absorption efficiency of total IgG and:		
	IgG2	r = 0.67**
	IgA	r = 0.68**
	IgM	r = 0.57*
Correlation between absorption efficiency of IgG2 and:		
	IgA	r = 0.56*
	IgM	r = 0.84***
Correlation between absorption efficiency of IgA and:		
	IgM	r = 0.50*

NS = Non significant ($P > 0.05$)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

3.3 Experiment C

(i) Determination of the apparent efficiency of absorption of sheep IgG2 by calves left with their dams

(ii) Estimation of the colostral immunoglobulins absorbed by these calves

Following determination of absorption efficiencies of colostral immunoglobulins and sheep IgG2 by calves when fed from a nipple feeder (Experiment B), a survey was carried out to determine the absorption of sheep IgG2 when the calves were allowed to suckle their dams.

3.3.1 Procedure

Twenty newborn calves born in spring months were used. The calves were weighed soon after birth. 4 grams of sheep serum immunoglobulin was then infused slowly by using a 50 ml syringe fitted with plastic tubing (Appendix 15). This eliminates losses through dripping, while allowing suckling.

Following dosing with sheep immunoglobulin, the calves were allowed to suckle their dams and remain with them for two days. Blood samplings were carried out at 24 and 48 hours.

3.3.2 Estimation of sheep IgG2 absorbed and colostral immunoglobulins in the calves' sera

The estimations of colostral immunoglobulins IgG, IgG2, IgA IgM and sheep IgG2 in the calves' sera were carried out using immunodiffusion method as described in page 42.

3.3.3 Results

The results of this experiment are given in Appendices 15 and 16 and summarised in Tables 11 and 12. The relationship between the amounts of sheep IgG2 fed and the amount absorbed at 24 hours post feeding is shown in Figure 6. Seventeen of the twenty calves absorbed detectable amounts of sheep IgG2, while in the remaining three the amounts absorbed were less than 0.04 mg/ml and were not easily determined with certainty. The implication of this will be discussed.

The mean ($26.0\pm1.4\%$) of the apparent absorption efficiency of sheep IgG2 from this experiment was higher than that ($18.5\pm1.6\%$) from

the group in Experment B ($P < 0.01$), indicating a better absorption by calves left with their dams. However this efficiency was not significantly different to the apparent absorption efficiencies of other bovine colostral immunoglobulins in Experiment B.

The amounts of colostral immunoglobulins absorbed by the calves left with their dams in this experiment is shown in Appendix 16. The means and standard errors of the 24-hour serum levels of the different immunoglobulins are shown in Table 12. Large individual variations in the serum levels of immunoglobulins was apparent. The ranges were from 6.9-102.2 mg/ml for IgG, 0.54-2.62 mg/ml for IgG2, 0.51-7.9 RSA/ml for IgA, and 0.29-4.20 mg/ml for IgM. Seventeen of the calves had serum IgG levels of well over 20 mg/ml. Of the other three, one had IgG level of less than 10 mg/ml, while the other two had intermediate levels of between 10 and 20 mg/ml (Appendix 16).

TABLE 11 - Means $\pm$ standard errors and ranges of the amounts of sheep IgG2 fed and absorbed into the blood at 24 hours post feeding and the apparent efficiency of absorption of this immunoglobulin by 17 newborn calves fed within two hours after birth and then left with their dams for 48 hours.

	Dose ($g/kg_3 B.W.$) $\times 10$	IgG2 absorbed (mg/ml)	absorption efficiency (%)
	25.8 ± 1.9	0.096 ± 0.007	26.0 ± 1.4
Range	16.6 - 50.2	.06 - .18	14.8 - 34.2

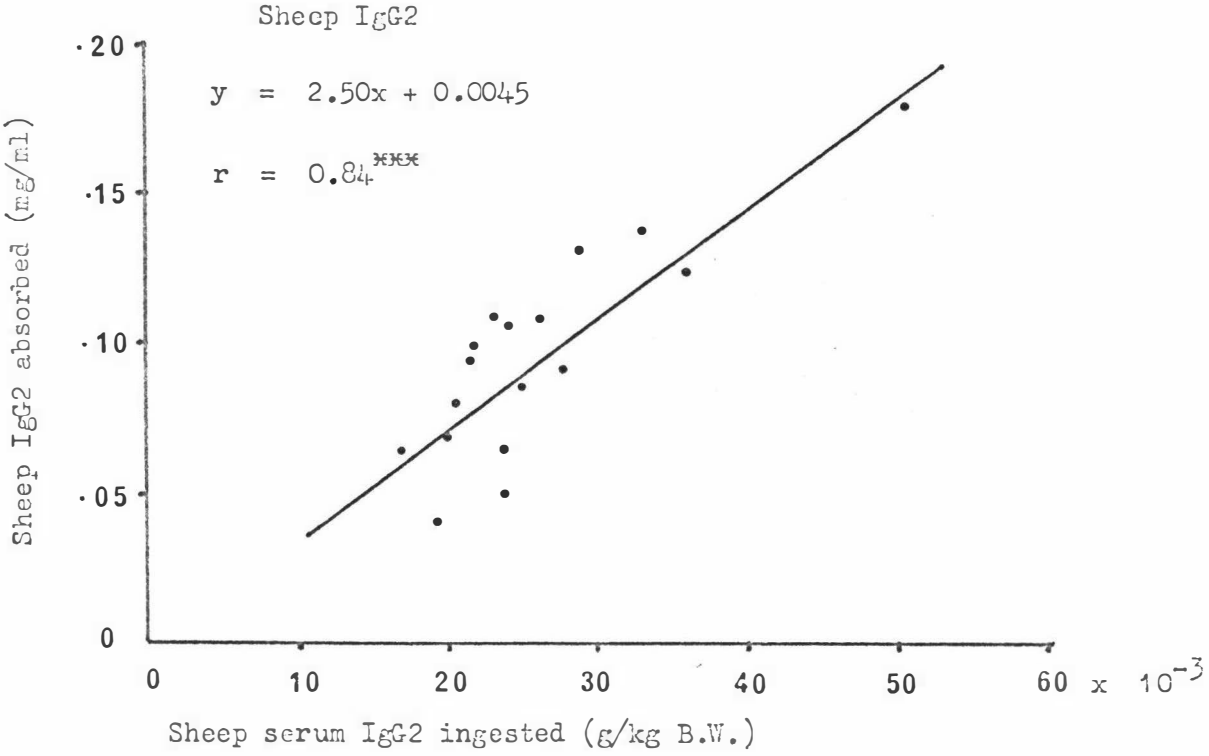


Figure 6

The relationship between the amounts of sheep serum IgG2 ingested (g/kg B.W.) and the amounts absorbed into the blood (mg/ml) at 24 hours post feeding, in 17 newborn calves which were left with their dams for two days. The calves were given sheep IgG2 within 60 minutes of birth.

TABLE 12 - The 24-hour increase in the serum immunoglobulins concentrations in 20 calves left with their dams for 48 hours after birth. The data given are means ± standard errors and the ranges.

	Means ± S.E. (mg/ml)	Range (mg/ml)
Total IgG	37.1 ± 5.3	6.9 - 102.2
IgG2	1.35 ± 0.12	0.54 - 2.62
IgA	3.2 ± 0.4*	0.51 - 7.9*
IgM	2.17 ± 0.26	0.29 - 4.20

* Relative to concentration of IgA in bovine serum

It is also if interest to note that calf 2.05 did not apparently absorb colostral immunoglobulins during the first 24 hours of life, but only obtained immunoglobulins during the next 24 hours (Appendix 16). It had, however, absorbed sheep IgG2 during the first day. This observation contrasted to all the other calves which obtained their colostral immunoglobulins only in the first day of life and which did not show further increase of immunoglobulins in the 48-hour serum samples over the 24-hour levels.

TABLE 13 - Correlations between the different immunoglobulins concentrations in the 24-hour serum samples of 20 newborn calves which were left with their dams for 48 hours after birth.

	Correlation coefficient (r)
Correlation between IgG and:	
IgG2	0.64**
IgA	0.86***
IgM	0.86***
Correlation between IgG2 and:	
IgA	0.78***
IgM	0.72***
Correlation between IgA and:	
IgM	0.79***

NS = Not significant ($P > 0.05$)

* = $P < 0.05$

** = $P < 0.01$

*** = $P < 0.001$

CHAPTER IV

DISCUSSION

The discussion of the results in this study falls mainly into two sections. The first will deal with discussion of the analysis of immunoglobulins, in particular the reasons for the disparity in the values of the concentrations of immunoglobulins estimated by the Biuret reaction method and values obtained by radial immunodiffusion method, and the analytical problems associated with estimation of sheep IgG2 absorbed by the calves. In section 4.2, which forms the main part of this chapter, the results obtained from the present experiments will be discussed in relation to the relevant work reported in the literature.

4.1 Analysis of Immunoglobulins

4.1.1 Relationship between the values of colostral immunoglobulins obtained by the Biuret reaction and from radial immunodiffusion.

Comparison of the data on the values of colostral immunoglobulin obtained by the Biuret reaction (Appendix 7) and those by the more specific method of radial immunodiffusion (Appendix 8) reveals outstanding anomalies. This is featured by the strikingly high values of immunoglobulin concentration obtained by the Biuret reaction method relative to the more specific method of radial immunodiffusion. The results obtained by the Biuret reaction were 32 $\pm$ 6% (mean $\pm$ S.E.) greater than the results obtained by radial immunodiffusion. The relationship between the values obtained by the two methods was also significantly correlated ($r = 0.91$ ($P < .001$), indicating a possible

intrinsic methodical error in estimation. It is suggested that the reason for the higher results obtained from the Biuret reaction was probably due to the use of bovine serum albumin (BSA) for the construction of the standard curve (Appendix 17, Figure 1). A standard curve determined later using sheep IgG revealed an obvious difference in gradient to that for BSA. In a later investigation, it was shown that estimation of protein concentration using a BSA standard curve would overestimate bovine immunoglobulin concentration by 27% (Mackenzie, D.D.S., personal communication).

This would explain the failure to achieve the feeding levels of 4 and 8 g/kg body weight originally proposed (Appendices 10, 11, 12 and 13). Except for this, the experiments have not been affected in any way. In addition the scatter of points indicating varying doses (Figures 5a, 5b, 5c and 5d) were inevitable, as the proportion of the individual immunoglobulins varied between the different colostrum samples.

4.1.2 Analysis of the concentrations of sheep IgG2 absorbed by the calves.

Analytical problems were also encountered in estimating sheep IgG2 particularly when assessing the amounts absorbed into the blood of the calves. This situation arose largely because insufficient sheep IgG2 was given to each calf. The amounts of sheep IgG2 given were barely adequate for it to be absorbed in measureable quantities particularly since the effectiveness of the antiserum to sheep IgG2 was reduced considerably by the repeated adsorption of the antiserum with bovine serum necessary to make it specific to sheep IgG2. The cross

reaction between the sheep IgG2 and bovine IgG2 when reacted with the original antiserum was quite extensive and a considerable amount of the antibody to sheep IgG2 was removed by the adsorption process. Moreover reading of the precipitin diameters was made difficult by the presence of non specific artifacts in close proximity of the precipitin ring. As a result the method of estimation was subjected to some error, particularly at the lower range of absorption.

The method of estimation, therefore, did not allow determination of absorbed sheep IgG2 at concentrations of less that 0.04 mg/ml with any certainty. In the five calves affected (Appendices 14 and 15) this represents absorption efficiencies ranging from 11% to 20% and less. Thus, these calves, which apparently did not absorb sheep IgG2, may have in fact absorbed it but at efficiencies of less than that calculated (Appendices 14 and 15).

4.2 Evaluation of the Results from the Present Experiments

4.2.1 Apparent absorption efficiencies of bovine colostral immunoglobulins by newborn calves.

Most experiments describing the efficiency of absorption of colostral immunoglobulins by newborn calves that have been reported in the literature have estimated immunoglobulin or total immunoglobulins by relatively inaccurate methods. These included the work of Bangam *et al* (1958), Balfour and Comline (1962), Hardy (1969), McEwan *et al* (1970a) and Kruse (1970b) mentioned in the Review. Kruse (1970b), basing his work on electrophoretic fractionation to estimate immunoglobulin and assuming plasma volumes of 5% of body weight and after taking into consideration immunoglobulin in both extravascular and intravascular space,

found a real efficiency of absorption of 20% when fresh colostrum was used. McEwan et al (1970a) obtained an apparent efficiency of 25% in a similar experiment in which individual plasma volumes of the calves were also measured. (The term 'apparent efficiency' is used because the estimate only includes immunoglobulins present in the serum and does not take into account immunoglobulins which circulate in other fluid compartments.)

More specific data were recently reported by Husband et al (1972; 1973). A notable feature of their data was that all the colostral immunoglobulins were absorbed at much higher apparent efficiencies than previously reported. The values obtained were: 44-46%, 48-59%, 56-71% and 87-95% for IgG1, IgG2, IgA and IgM respectively.

The data on the absorption efficiency of various immunoglobulins from the present studies (Table 8) were also expressed in terms of apparent efficiency so as to facilitate comparison with other work. As indicated in Table 8, all the colostral immunoglobulins were absorbed at a similar apparent efficiency, but did not attain the magnitude observed by Husband et al (1972; 1973). The lower values obtained in the present work, which in spite of similar approach to Husband et al, were difficult to explain, although the highest observed efficiencies obtained, i.e. 55%, 46%, 70% and 73% for IgG, IgG2, IgA and IgM respectively were within the range shown by them (Husband et al, 1972; 1973).

It is possible that the fairly low absorption efficiencies were a result of denaturation of immunoglobulins during freezing and

thawings. Close examination of the correlation coefficiencies between IgG and other immunoglobulins, both in colostrum and in serum (Tables 7 and 10), showed a decrease from the former to the latter, particularly for IgM. These decreases although not significant were probably indicative of some denaturation of the immunoglobulins, particularly IgM. This is not surprising in view of the larger size of IgM in relation to other immunoglobulins. Thus denaturation may explain the non-significant relationship between IgM ingested and subsequently absorbed by the calves (Figure 5d and Table 9).

Nevertheless denaturation *per se* was unlikely to have been the only reason for the low apparent efficiencies. This is evident from the work of Kruse (1970b) and McEwan *et al* (1970), who in spite of using fresh colostrum, only obtained efficiencies of absorption of about 20 to 25%. Admittedly, however, they used the non specific method of electrophoretic fractionation to determine immunoglobulin concentrations. Further recalculation of the data of McEwan *et al* (1970) by making allowance for the volume of fat and casein in the original colostrum fed to the calves which was assumed to be 17% of the total volume, the apparent efficiency of absorption was 31%. This was well in the range of the results obtained from the present work (Table 8).

The higher efficiencies observed by Husband *et al* (1972; 1973) may have been due to the feeding method employed. This involved repeated feeding of about one litre each of colostrum at one and four hours of life respectively. This may have had the effect of increasing the efficiency of absorption of ingested colostral immunoglobulins, as has been stated by Kaeckenbeeck *et al* (1961) (c.f. Selman *et al*, 1970a). In the present study all calves were fed with colostrum once only.

Thus on the basis of the above consideration, it is suggested that the efficiency of absorption of different immunoglobulins in the present study was partly reduced by feeding colostrum which had been frozen and by giving it at a single feeding. Moreover, the results of Husband et al (1972; 1973) indicating that immunoglobulins were absorbed at real efficiencies approaching 100% are unexpected. Thus Selman et al (1970; 1971) have suggested there are psychological effects due to mother deprivation in calves removed from their mothers which may depress absorption efficiency. They have shown that the serum immunoglobulin in calves which were allowed to remain with their dams after colostrum feeding (but muzzled) were significantly greater than the calves which were reared in pens. Similar amounts of colostrum were given to each group although no attempt was made to estimate the differences in the absorption efficiencies between the groups.

The results from the experiment on the apparent absorption efficiency of sheep IgG2 tend to confirm their observations. Calves which were left with their dams after feeding sheep IgG2 (Experiment C) absorbed significantly more efficiently ($26.0 \pm 1.4\%$) than those ($18.5 \pm 1.6\%$) left in the pens (Experiment B), ($P < 0.01$), indicating a superior absorption efficiency by calves which were allowed to remain with their dams. Nevertheless care is required in interpreting the results and it would be unwise to overemphasize the magnitude of the difference because of the possible errors which may arise from the low doses given to each calf mentioned earlier, and of using several different batches of sheep IgG2 when feeding the calves. The small

standard errors and the highly significant difference between the groups, however, means that the influence of the two factors are not great. More clear data is therefore required.

4.2.2 Relative apparent absorption efficiencies of bovine colostral immunoglobulins and sheep IgG2.

The reason for the lower apparent absorption efficiency of sheep IgG2 relative to bovine immunoglobulins in Experiment B is not apparent. The possibility exists, however, that the estimation of sheep IgG2 was low because of the reduced effectiveness of the anti-serum to sheep IgG2 as a result of extensive adsorption stated earlier. But low absorption of sheep IgG2 may have also been due to small amounts of sheep IgG2 fed *per se*. In newborn piglets, Pierce and Smith (1967a) showed that the amounts of bovine IgG absorbed into the blood was not proportional to the amounts fed when less than 2 g IgG was given. Thus only 1% was absorbed into the blood when 0.5 g IgG was fed, but the proportion absorbed increased to 5% when 1g IgG was fed and to 10% when 2 g was given. No further increase was noted as the amounts of IgG fed were increased. This showed that the efficiency of absorption of bovine IgG was not optimum when the amount of IgG given was less than 2 g. Examination of Appendices 14 and 15 reveals that most of the calves in the present study were given less than 2g of sheep IgG2. Thus and absorption of this protein may have behaved in the same manner to that reported by Pierce and Smith (1967a) for the absorption of bovine IgG in the piglets.

At the same time, the lower absorption of sheep IgG2 may have been an indication of some minor selection phenomenon between homologous and heterologous IgG. In newborn piglets it is known that some

selectivity occurs between homologous and heterologous IgG (Pierce and Smith, 1967a) and between human serum albumin and bovine IgG (Pierce and Smith, 1967b). In newborn calves, it has been stated that the intestine of few hours old calves can absorb many different kinds of proteins and nonprotein macromolecules non selectively (Deutch and Smith, 1957; Bangam et al, 1958; Pierce, 1961; Hardy, 1969a; b). Hardy (1969a; b) also demonstrated that labelled serum immunoglobulin and labelled PVP K60 were absorbed from the small intestine into the lymph of anaesthetized, unsuckled calves in euqal amounts. Thus although the data suggest that calves are able to absorb non selectively, no one has, however, attempted to determine the differences or similarities in absorption efficiencies of similar but not identical protein macromolecules from different species.

A third possibility is that some unaccounted loss of sheep IgG2 may occur by way of denaturation, or by way of unknown factors causing relatively greater rate of destruction of sheep IgG2 directly related to the method of preparation from sheep serum involving repeated precipitation with ammonium sulphate. This may result in more rapid loss of sheep IgG2 from the circulation of the calves, especially during the first day, and may therefore introduce an underestimation of the amounts of sheep IgG2 in the sera of the calves. This error will also become significant as the amounts of sheep IgG2 fed were small, thus lowering the calculated efficiency of absorption of sheep IgG2.

In view of the factors mentioned, firm conclusions cannot therefore be drawn. It remains to be verified that heterogenous IgG (sheep IgG2) is either absorbed by the calf's intestine at an equal

efficiency or at a lower efficiency relative to bovine immunoglobulins when they are given together.

4.2.3 The yield of colostrum at first milking post partum and the amounts of immunoglobulins absorbed by newborn calves left with their dams for two days.

The yields of immunoglobulins in the colostrum of the cows used in this experiment were considered adequate to meet the requirements of their calves (Appendices 1, 2, 3, 4, 5 and 6). Where the volume of the colostrum was small, it was more than compensated for by high immunoglobulin concentrations. The negative correlations between the volume and concentration of the different immunoglobulins (Table 6) were strong and significant. Thus in spite of considerable individual variations in volumes, the total amounts of immunoglobulins available were high and always greater than 200 g (Table 5). This is well in excess of the 100 g minimum requirement stipulated by Meyer and Steinbach (1961) (c.f. Kruse, 1970a). The results also contrasted with the findings of Kruse (1970a) who showed that about 12% of the cows investigated had less than 100 g of total immunoglobulin at the first milking and that the negative correlation between the volumes of the colostrum and their immunoglobulin concentrations were small and not significant ($r = -0.05$ to -0.27). The difference shown in this study compared to that of Kruse's is therefore likely due to breed differences.

The means of immunoglobulin concentrations in the colostrum in the present study were higher than those shown by Klaus et al (1969), Kruse (1970a), and Butler et al (1971), but were in agreement with data presented by Brandon et al (1971), Husband et al (1972) and Porter (1972).

In the present study very little difference between quarter volume yield and immunoglobulin concentrations were found (Table 4). The slightly lower volume yields from the front quarters were undoubtedly related to the relative size of the front and rear quarters (Schmidt, 1971). In spite of this, a calf, which for reasons of behavioural preference only suckled the front teats, was unlikely to remain hypogammaglobulinaemic, as the total immunoglobulins from the front quarters were generally sufficient for its requirements. Furthermore, behavioural studies have not indicated any evidence of such preference (Hafez, 1969). Selman et al (1970) showed that 91% of newborn calves started suckling from one of the anterior teats and tended to continue suckling from the same side of the dam in subsequent sucklings. They did not, however, show any calf which suckled only the anterior teats throughout observation periods of eight hours from birth. Thus it may be assumed that all calves were able to obtain large quantities of colostrum, regardless of suckling behaviour, except in special circumstances such as parent rejection, conditions surrounding the birth area which, due to slippery conditions delayed the calf's ability to stand and suckle, and also due to poor udder shape of the dam which made suckling difficult for the young calf (Selman et al, 1970a; b).

The generally high levels of serum immunoglobulins in calves left with their dams (Appendix 16) justified the above conclusion. Except for three calves which have serum IgG levels of less than 20 mg/ml, all calves have serum IgG levels of greater than 20 mg/ml and were comparable to Porter's (1972) observations for calves which were left with their dams. From the mean values shown in Table 12, it

may also be calculated that for an average 35 kg calf, the means and standard errors of immunoglobulins absorbed into the blood were 92.7 $\pm$ 13 g, 3.4 $\pm$ 0.3 g, 8 $\pm$ 1 RSA and 5.4 $\pm$ 0.6 g for IgG, IgG2, IgA and IgM respectively. On allowing for diffusion into extravascular pool, which was shown to be 1.2 times larger than the intravascular pool (McDougall and Mulligan, 1969) the calculated total amounts of immunoglobulins absorbed would be 203.9 $\pm$ 28.6 g, 7.5 $\pm$ 0.7 g, 17.6 $\pm$ 2.2 RSA for IgG, IgG2 and IgA respectively. Very little IgM is assumed to diffuse out into extravascular pool (Husband et al, 1972). Thus, the above serum levels of immunoglobulins which are considered 'high' for passive immunity, were amply provided for by the amount of immunoglobulin in the colostrum as shown in Table 5.

The levels of serum immunoglobulins considered adequate for protection against most diseases in very young calves vary. Penhale et al (1973, c.f. Logan et al, 1973) showed that the levels of serum immunoglobulins in calves that survived colisepticaemia had means of 7.5 mg/ml for IgG, 0.8 mg/ml for IgM and 0.22 mg/ml for IgA. Those dying of septicaemia had only 0.8 mg/ml of IgG, 0.2 mg/ml of IgM and 0.1 mg/ml of IgA. Based on these observations it may be seen that most of the calves in the present study were potentially resistant to most pathogens, although by no means completely protected from neonatal diseases.

Another interesting feature of immunoglobulin intake by calves left with their dams was that most obtained their immunoglobulins only during the first day of life. A single calf (Calf 2.05) however, did not appear to absorb immunoglobulins until the second day of life

(Appendix 16), although absorption of sheep IgG2 by this calf was normal (Appendix 15). These observations showed that those calves which have had access to colostral immunoglobulins within the first day of life lost their capacity to absorb further immunoglobulins in the second day. The reason for this cessation of absorption after the first 24 hours of life was probably due to changes in the structure of absorptive cells after exposure to protein or immunoglobulins similar to that widely shown in other newborns (Staley et al, 1969; Clark and Hardy, 1971a; b; Simpson-Morgan and Smeaton, 1972).

Graves (1963) showed that adsorption of neutralizing antibody by newborn calves could be prevented by feeding skim milk or immune serum prior to feeding of colostrum. McCoy et al (1970) showed that the intestines of newborn calves become impermeable to colostrum immunoglobulins when colostrum was withheld and only given after 24 hours of life. However, calf 2.05 which presumably did not suckle during the first 24 hours, did not totally lose its ability to absorb colostral immunoglobulin and was able to obtained 18.5 mg/mlof IgG in its serum during the next 24 hours of life. This therefore contrasted to observations of Graves (1963) and McCoy et al (1970) that neither the presence of protein (sheep IgG2) in the calf's intestine prior to ingestion of colostrum nor withholding of colostrum prevented absorption of colostral immunoglobulins after 24 hours of life. But further absorption after the first day was prevented by the presence of large amounts of colostral immunoglobulins during the first day as indicated in the remaining 19 calves. Nevertheless, the nature of this observation warrants further study as it only occurred in isolation and involved a low level of sheep IgG2.

From the results of this study it may be seen that most calves were born with low intrinsic levels of IgG and IgM (means $\pm$ standard errors of 0.52 $\pm$ 0.18 and 0.07 $\pm$ 0.04 mg/ml respectively). Only one calf, however, had detectable amounts of IgA (Appendix 12). Thus in general the presuckling levels of serum immunoglobulins agree with that observed by other workers (Brandon et al, 1971; Merriman, 1971; Husband et al, 1972).

It has been reported that some calves which have had access to colostrum during the first day were unable to absorb immunoglobulin because of premature loss of absorptive capacity (Fey and Margadant, 1961 c.f. Kruse, 1970b; Gay et al, 1965; Gay 1965; Selman et al, 1970a). No such evidence was shown in the present study, although some calves (Calf 2.04, 2.10) had relatively low serum immunoglobulins at the end of the 48-hour period. However, further examination showed that these calves were capable of absorbing sheep IgG2 in comparable amounts to the other calves, thus suggesting that absorptive capacity in these calves was normal. Thus it may be concluded that the incidences of hypogammaglobulinaemia in some calves may not be due to the inability of the calves concerned to absorb immunoglobulin during the first 24 to 36 hours of life, but were more likely due to inadequate intake of colostrum and/or of low immunoglobulins contents. Factors which result in low intake of colostrum during these periods include parent rejection, condition of birth area and the shape of the udder of the dams as mentioned earlier. The time between birth and first suckling is also important. Serum immunoglobulin levels in calves vary inversely with the time post partum of first suckling (Smith et al, 1967; Kruse, 1970b; Selman et al, 1970a). In a simulation study the possibility of low

intake of colostrum by newborn calves due to one or more of the factors mentioned, always exists (Kruse, 1970c).

4.3 Conclusion

The present work provided data which suggest that the amount and composition of colostrum was adequate to meet the immunoglobulin requirement for passive immunity by calves during their early life. Further, most of the newborn calves in the present study did obtain a high level of serum immunoglobulins, although about 10% had intermediate to low levels.

From the data it is also shown that all the immunoglobulins were absorbed equally well with apparent efficiencies of 33.3%, 26.8%, 32.8% and 36.0% for IgG, IgG2, IgA and IgM respectively. Evidence is also given that the efficiency of absorption was greater in calves left with their dams than fed from a nipple feeder and kept isolated in pens. The results, however, did not allow accurate quantitation of the magnitude of these differences.

4.4 Suggestions for Further Work

Obviously questions regarding some aspects of calf immunology which this work was originally designed to resolve, remain unanswered, particularly the differences in absorption efficiency between calves allowed to remain with their dams and those removed from their dams. In view of this, a similar experiment would be best carried out in which these parameters should be accurately determined. This may involve the use of homologous immunoglobulins, such as from colostrum, or heterologous immunoglobulins. If the latter are used, it would be

advantageous to use those which would not cross react with a homologous immunoglobulin. The possibility of selective immunoglobulin should also be investigated.

Appendix 1a

Volumes of Colostrum (Litres)

The yield of colostrum (l) from the four quarters of 18 cows obtained at first milking post partum.

Quarter / Cow No.	Front Left	Front Right	Rear Left	Rear Right	Total
183	0.30	0.48	0.61	0.50	1.89
29	0.27	0.40	0.86	0.82	2.35
150	0.54	0.50	0.81	0.91	2.76
124*	0.59	0.51	1.02	1.00	3.12
121*	2.85	3.32	3.85	3.83	13.85
129*	2.01	1.38	2.55	2.85	8.79
25	1.05	0.98	1.84	1.87	5.74
87	1.10	1.35	2.05	2.38	6.88
93	0.76	0.73	1.33	1.34	4.16
119*	1.15	1.17	1.42	1.66	5.40
83	1.25	0.80	1.45	0.72	4.22
176*	1.10	1.20	1.00	0.54	3.84
27*	1.30	0.68	2.77	2.00	6.75
31*	1.66	1.89	2.05	2.03	7.73
44*	0.44	0.59	0.67	0.74	2.44
59*	1.38	1.51	2.71	2.48	8.08
104*	2.74	2.60	3.60	1.75	10.69
167*	3.05	3.51	3.23	3.48	13.27

* injected with oxytocin after the completion of initial milking.

Appendix 1b

The increase in the yield of colostrum (litres) after oxytocin injection and the total yields in the four quarters of 11 cows which were injected with oxytocin after completion of first milking.

Cow No. \ Quarter	Front Left	Front Right	Rear Left	Rear Right
124	ND 0.59	ND 0.51	ND 1.02	ND 1.00
121	0.53 2.85	0.65 3.32	0.71 3.85	0.75 3.83
129	0.38 2.01	0.18 1.38	0.55 2.55	0.45 2.85
119	ND 1.15	ND 1.17	ND 1.42	ND 1.66
176	0.55 1.10	0.45 1.20	ND 1.00	ND 0.54
27	0.50 1.30	0.13 0.68	0.85 2.77	0.70 2.00
31	0.12 1.66	0.17 1.89	0.15 2.05	0.18 2.03
44	0.19 0.44	0.20 0.59	0.28 0.67	0.35 0.74
59	ND 1.38	0.66 1.51	0.98 2.71	1.23 2.48
104	0.44 2.74	0.50 2.60	0.30 3.60	0.20 1.75
167	1.25 3.05	1.43 3.51	1.43 3.23	1.66 3.48

ND = not determined

Appendix 2

Fat Percentage in the Colostrum

The fat percentages in colostrum from the four quarters of 18 cows obtained at first milking post partum. The method of Fleet and Linzel (1964) was used to determined the fat content.

Quarter / Cow No.	Front Left	Front Right	Rear Left	Rear Right	Bulked
183	13.5	13.0	14.0	14.0	14.0
29	8.0	8.0	6.0	5.0	5.5
150	8.0	8.0	8.5	8.0	8.0
124	12.5	12.0	8.0	8.0	11.5
121	6.0	5.5	6.0	6.0	6.0
129	8.5	8.5	9.0	9.0	8.5
25	10.0	11.0	11.0	11.0	11.0
87	7.0	11.5	7.0	6.5	7.5
93	1.5	1.0	1.0	1.0	1.0
119	3.5	4.0	4.0	4.0	4.0
83	2.5	1.5	1.5	2.0	2.0
176	6.0	9.0	5.5	5.5	7.0
27	6.0	3.5	5.5	7.5	7.5
31	7.0	8.0	7.0	9.0	8.0
44	11.5	3.5	3.5	3.5	5.0
59	11.5	13.5	11.0	11.0	11.0
104	6.5	6.0	5.0	3.0	5.5
167	9.0	9.5	10.0	8.0	9.5

Appendix 3

Total IgG (IgG1 and IgG2) in the Colostrum

The concentrations of IgG (mg/ml) in the colostrum from the four quarters of 18 cows obtained at first milking post partum. Estimations of IgG were based on the method of immunodiffusion (page 42).

Quarter / Cow No.	Front Left (mg/ml)	Front Right (mg/ml)	Rear Left (mg/ml)	Rear Right (mg/ml)
183	128.9	154.2	151.3	144.5
29	120.0	137.0	101.0	120.0
150	98.0	81.0	115.0	132.5
124	130.0	132.0	140.0	122.5
121	74.0	65.0	71.0	65.0
129	78.5	73.5	77.5	69.0
25	94.0	102.5	110.0	100.0
87	95.0	98.0	120.0	127.5
93	96.5	74.0	69.0	82.5
119	106.0	106.0	98.0	77.5
83	72.5	67.5	61.5	63.0
176	97.0	105.0	114.0	112.0
27	47.0	31.0	49.5	50.0
31	33.0	34.5	30.0	30.0
44	111.0	109.0	111.0	102.5
59	71.0	61.0	81.5	82.5
104	65.0	60.0	50.0	72.5
167	38.5	40.0	46.5	37.5

Appendix 4

IgG2 in the Colostrum

The concentrations of IgG2 (mg/ml) in the colostrum from four quarters of 18 cows obtained at first milking post partum. Estimations of IgG2 were based on the method of radial immunodiffusion (page 42).

Quarter / Cow No.	Front Left (mg/ml)	Front Right (mg/ml)	Rear Left (mg/ml)	Rear Right (mg/ml)
183	5.55	5.15	5.55	4.85
29	6.16	6.66	5.76	6.56
150	4.24	4.14	3.94	4.54
124	6.76	6.77	7.38	7.38
121	3.67	3.21	3.62	3.83
129	4.54	4.14	4.34	4.95
25	6.97	6.97	5.77	6.37
87	4.79	5.10	5.10	5.35
93	4.95	6.06	5.66	5.45
119	3.23	3.13	3.23	3.74
83	3.11	2.75	2.75	2.39
176	5.66	5.45	6.06	6.16
27	4.44	3.74	4.24	4.24
31	1.63	1.88	1.48	1.63
44	7.88	8.68	8.68	8.08
59	6.56	5.76	5.76	6.36
104	4.49	4.79	5.10	5.10
167	2.14	2.19	2.29	2.19

Appendix 5

IgA in the Colostrum

The concentrations of IgA (RSA/ml) in the colostrum from the four quarters of 18 cows obtained at first milking post partum. Estimations were based on the method of radial immunodiffusion (page 42). The concentrations of IgA are expressed as relative to the concentrations of IgA in bovine serum.

Cow No. \ Quarter	Front Left	Front Right	Rear Left	Rear Right
183	20.7	26.1	26.3	26.0
29	24.3	23.4	19.1	31.0
150	10.3	12.1	14.2	12.1
124	18.0	16.5	16.0	18.0
121	5.6	5.6	6.1	6.3
129	4.2	4.3	5.2	5.4
25	6.6	7.8	5.6	6.6
87	11.2	9.1	9.1	11.2
93	12.7	12.1	10.7	11.0
119	11.1	11.1	9.4	12.1
83	2.4	1.4	1.6	1.8
176	5.6	6.6	7.2	7.2
27	4.9	2.8	3.9	4.6
31	5.4	5.4	3.6	4.2
44	14.0	11.0	10.2	13.0
59	12.3	12.6	11.7	11.7
104	11.7	11.5	8.0	11.5
167	5.2	4.7	4.8	5.2

Appendix 6

IgM in the Colostrum

The concentrations of IgM (mg/ml) in the colostrum from the four quarters of 18 cows obtained at first milking post partum. Estimations were based on the method of radial immunodiffusion (page 42).

Quarter / Cow No.	Front Left (mg/ml)	Front Right (mg/ml)	Rear Left (mg/ml)	Rear Right (mg/ml)
183	10.90	9.99	11.93	11.95
29	5.25	5.81	4.69	5.61
150	9.38	8.82	10.96	9.94
124	8.82	10.96	10.95	11.22
121	4.69	4.54	4.38	4.38
129	3.31	3.11	3.41	3.31
25	7.80	8.31	8.31	8.00
87	12.50	12.00	12.00	12.20
93	4.69	4.53	4.23	4.94
119	8.26	7.14	6.78	6.52
83	5.50	4.99	4.38	4.69
176	10.20	11.52	10.96	11.59
27	5.30	3.30	5.40	5.10
31	1.47	1.66	1.08	1.28
44	9.38	10.20	9.98	10.20
59	10.20	10.20	11.20	10.50
104	9.38	8.36	5.61	8.77
167	3.11	3.31	3.41	3.11

Appendix 7

The Fat, Casein and Immunoglobulin Content in the Colostrum fed to the Calves

The constituents of fat, casein and immunoglobulin in the colostrum from different cows used to feed the calves. The estimations of fat were based on the method of Fleet and Linzel (1964); the casein, by dye binding method (Dolby, 1961) and immunoglobulin by the Biuret reaction method of Gornal et al (1949). The intended amount of colostrum to be fed to each calf was calculated by using these three informations.

Col.of Cow No.	Fat % (F)	Casein % (C)	Total Ig% (in whey)	C*	Fat + C*	Whey % in col.
183	11.0	assumed to be 5%	19.6	4.4	15.4	84.6
29	5.5		18.6	4.7	10.2	89.8
150	8.0		16.0	4.6	12.6	87.4
124	11.0		24.2	4.4	15.4	85.6
121	6.0		4.3	4.7	10.7	89.3
129	6.5		5.7	4.5	13.0	87.0
87	7.5		17.6	4.6	12.1	87.9
119	4.0	5.0	12.4	4.8	8.8	91.2
83	2.0	3.0	8.4	2.9	4.9	95.0
176a	7.0	6.0	16.8	5.6	12.6	88.4
31	8.0	3.9	4.4	3.6	11.6	88.4
44	5.0	7.5	19.0	7.1	12.1	87.9
59	11.0	5.1	15.2	4.5	15.5	84.5
167	9.5	6.3	6.4	5.7	15.2	85.8
127	8.0	8.0	7.5	4.6	12.6	87.4
1	2.5	2.5	7.3	4.8	7.3	92.7
111a	5.0	5.0	15.8	4.7	9.7	90.3
111b	5.0	5.0	14.6	4.7	9.7	90.3
176b	7.0	4.0	14.2	3.7	10.7	89.3

C* = corrected caseing percentage in colostrum

where $C^* = \frac{(100 - F)\, C}{100}$

Appendix 8

The concentrations of IgG, IgG2, IgA and IgM in the colostrum used to feed the calves. Estimations were based on the method of radial immunodiffusion (page 42).

Col. of Cow No.	Total IgG (mg/ml)	IgG2 (mg/ml)	IgM (mg/ml)	IgA* (RSA/ml)
183	150.0	4.85	11.70	26.1
29	112.5	7.07	5.01	24.3
150	99.0	4.59	8.87	11.2
124	134.2	7.66	11.05	18.0
121	71.0	3.72	4.43	5.9
129	77.5	4.79	3.36	4.9
87	111.5	5.12	11.92	11.2
119	85.0	3.83	6.42	14.4
83	73.0	3.11	4.74	1.9
176a	115.0	5.15	11.00	7.2
31	35.5	1.73	1.82	5.4
44	102.5	8.68	10.60	15.2
59	80.0	6.29	10.50	12.1
167	56.0	1.94	3.26	5.2
127	53.0	3.39	2.22	5.4
1	55.0	3.43	5.40	5.4
111a	102.5	4.00	5.40	8.2
111b	105.0	3.80	5.76	8.3
176b	80.0	5.10	7.60	5.9

*RSA = relative to concentration of IgA in bovine serum.

Appendix 9

The volumes of colostrum, the sources of the colostrum and their calculated whey equivalent used to feed 19 newborn calves for estimations of absorption efficiencies of colostral immunoglobulins.

Calf No.	Birth weight (kg)	Vol. of Col. fed (l)	Source of col. (Cow No.)	Eq. vol. of whey in col. (l)
1.01	31.0	1.44	183	1.22
1.02	32.0	1.52	29	1.37
1.03	34.0	1.90	150	1.66
1.04	41.0	1.65	124	1.39
1.05	38.5	4.00	121	3.57
1.06	45.5	4.25	129	3.70
1.07	40.5	2.10	87	1.84
1.08	32.5	1.65	87	1.45
1.09	34.5	2.46	119	2.24
1.10	36.5	1.85	83	1.76
1.11	36.5	1.99	176a	1.76
1.12	37.5	1.23	176b	1.10
1.13	25.0	1.57	59	1.32
1.14	31.0	1.94	59	1.64
1.15	38.0	3.00	167	2.56
1.16	22.5	1.40	127	1.22
1.17	32.5	2.00	1	1.85
1.18	28.0	0.79	111a	0.72
1.19	39.0	1.19	111b	1.07

Appendix 10

The amounts of colostral IgG fed (g/kg B.W.) and absorbed into the blood (mg/ml) of 19 newborn calves fed with colostrum within 2 to 6 hours of post natal life. Apparent efficiencies of absorption were calculated by assuming plasma volumes of 7% of body weight and as described on page 50.

Calf No.	IgG fed g/kg B.W.	S_0 mg/ml	S_{24} mg/ml	S_{48} mg/ml	IgG_{24} mg/ml	Eff %
1.01	5.91	1.02	25.3	24.3	24.3	28.7
1.02	4.84	0.09	22.0	24.5	24.4	35.2
1.03	4.82	2.00	37.9	38.6	36.3	52.7
1.04	4.53	0.05	17.5	17.5	17.5	27.0
1.05	6.56	0.08	23.0	20.5	22.9	24.4
1.06	6.30	0.05	49.3	45.0	49.2	54.6
1.07	5.08	0.06	29.3	28.3	29.2	40.2
1.08	4.94	0.22	16.4	15.8	16.2	22.9
1.09	5.52	0.03	17.2	15.2	17.2	21.8
1.10	3.52	0.95	21.4	19.2	20.5	40.7
1.11	5.56	2.91	19.4	15.7	16.5	20.7
1.12	2.43	1.43	6.8	6.6	5.4	15.0
1.13	4.24	0.20	21.8	17.3	21.6	35.6
1.14	4.24	0.05	9.2	7.4	9.2	15.2
1.15	3.75	0.55	14.4	14.0	13.9	25.9
1.16	2.85	0.11	16.2	15.2	16.1	39.5
1.17	3.11	0.04	20.2	17.4	20.2	45.4
1.18	2.60	0.04	15.2	14.9	15.2	40.9
1.19	2.89	0.07	18.7	15.7	18.7	45.3

In this table and subsequent tables:

S_0 = Calf's serum samples taken at birth

S_{24} = Calf's serum sample taken 24 hours after colostrum feeding

S_{48} = Calf's serum sample taken 48 hours after colostrum feeding

Ig_{24} = The increase in the serum immunoglobulin level after 24 hours of colostrum feeding

Appendix 11

The amounts of colostral IgG2 fed (g/kg B.W.) and absorbed into the blood (mg/ml) in 19 newborn calves fed with colostrum within 2 to 6 hours of post natal life. Apparent absorption efficiencies were calculated by assuming plasma volumes of 7% of body weight and as described on page 50.

Calf No.	IgG2 fed g/kg B.W.	S_0 mg/ml	S_{24} mg/ml	S_{48} mg/ml	$\Delta IgG2_{24}$ mg/ml	Eff %
1.01	0.191	0.06	0.71	0.67	0.64	23.4
1.02	0.304	0.04	1.49	1.40	1.45	33.4
1.03	0.223	0.10	1.20	1.10	1.10	34.5
1.04	0.258	0.00	0.67	0.63	0.67	18.2
1.05	0.343	0.02	1.20	1.10	1.19	24.3
1.06	0.389	0.00	1.74	1.60	1.74	31.3
1.07	0.233	0.03	0.95	NM	0.92	27.6
1.08	0.227	0.00	0.63	0.63	0.63	19.4
1.09	0.248	0.00	0.72	0.71	0.72	20.3
1.10	0.150	0.00	0.99	0.91	0.99	46.3
1.11	0.249	0.88	1.78	1.60	0.90	25.3
1.12	0.152	0.12	0.49	0.47	0.36	16.6
1.13	0.333	0.03	1.32	1.26	1.30	27.3
1.14	0.333	0.02	0.63	0.56	0.61	12.8
1.15	0.144	0.00	0.84	0.80	0.84	40.8
1.16	0.182	0.03	1.03	0.98	1.00	38.4
1.17	0.194	0.00	0.82	0.76	0.82	29.6
1.18	0.102	0.02	0.63	0.63	0.61	41.8
1.19	0.104	0.02	0.67	0.60	0.65	43.7

Symbols used: see footnotes of Appendix 10.

Appendix 12

The amounts of colostral IgA fed (RSA*/kg B.W.) and absorbed into the blood RSA*/ml) in 19 newborn calves fed with colostrum within 2 to 6 hours of post natal life. Apparent absorption efficiencies were calculated by assuming plasma volumes of 7% of body weight and as described on page 50.

Calf No	IgA fed RSA*/kg BW	S_0 RSA/ml	S_{24} RSA/ml	S_{48} RSA/ml	ΔIgA_{24} RSA/ml	Eff %
1.01	1.03	0.00	3.98	2.87	3.98	27.0
1.02	0.82	0.00	3.45	3.00	3.45	29.5
1.03	0.55	0.00	3.30	2.30	3.30	42.0
1.04	0.58	0.00	1.40	1.08	1.40	16.9
1.05	0.55	0.08	2.52	1.64	2.52	32.0
1.06	0.40	0.00	2.92	1.15	2.92	51.0
1.07	0.51	0.00	2.12	1.65	2.12	29.1
1.08	0.50	0.00	1.64	1.04	1.64	23.0
1.09	0.59	0.00	2.28	1.18	2.28	27.0
1.10	0.10	0.00	1.00	0.69	1.00	70.0
1.11	0.35	0.00	0.92	0.32	0.92	18.4
1.12	0.17	0.00	0.65	0.35	0.65	26.7
1.13	0.64	0.00	4.60	3.80	4.60	50.0
1.14	0.64	0.00	1.52	0.65	1.52	16.6
1.15	0.35	0.00	0.86	0.92	0.92	18.4
1.16	0.29	0.00	1.32	0.88	1.30	31.4
1.17	0.31	0.00	1.80	0.95	1.80	40.6
1.18	0.21	0.00	0.92	0.41	0.92	30.6
1.19	0.23	0.00	1.40	0.52	1.40	42.6

*RSA = relative to concentration of IgA in bovine serum.

Symbols used: see footnote of Appendix 10.

Appendix 13

The amounts of colostral IgM fed (g/kg B.W.) and absorbed into the blood (mg/ml) in 19 newborn calves fed with colostrum within 2 to 6 hours of post natal life. Apparent absorption efficiencies were calculated by assuming plasma volumes of 7% of body weight and as described on page 50.

Calf No	IgM fed g/kg B.W.	S_0 mg/ml	S_{24} mg/ml	S_{48} mg/ml	ΔIgM_{24} mg/ml	Eff %
1.01	0.460	0.04	1.95	1.59	1.90	28.9
1.02	0.216	0.07	1.47	1.13	1.40	45.4
1.03	0.431	0.91	2.81	2.43	1.90	30.8
1.04	0.374	0.05	1.10	0.80	1.05	19.7
1.05	0.410	0.09	2.08	1.76	2.00	34.1
1.06	0.273	0.04	1.13	0.98	1.10	28.2
1.07	0.543	0.06	2.91	2.58	2.87	37.0
1.08	0.528	0.05	1.55	1.19	1.50	19.9
1.09	0.417	0.04	1.24	0.99	1.20	20.1
1.10	0.229	0.85	2.62	2.81	1.76	59.9
1.11	0.581	0.50	1.50	1.50	1.00	12.0
1.12	0.224	0.09	0.86	0.68	0.77	24.0
1.13	0.555	0.08	2.98	2.81	2.90	36.6
1.14	0.552	0.04	1.48	1.21	1.40	17.7
1.15	0.219	0.07	1.38	1.28	1.25	40.0
1.16	0.119	0.20	1.13	1.07	0.91	53.5
1.17	0.305	0.05	2.00	1.76	1.95	44.7
1.18	0.137	0.07	1.51	1.42	1.44	73.5
1.19	0.158	0.06	1.38	1.11	1.32	58.5

Symbols used: see footnote of Appendix 10.

Appendix 14

The amounts of sheep serum IgG2 fed (g/kg B.W.) and absorbed into the blood (mg/ml) of 10 newborn calves fed within 2 to 6 hours of post natal life. Apparent efficiencies of absorption were calculated by assuming plasma volumes of 7% of body weight and as described on page 50.

Calf No.	IgG2 given (g/kg B.W. $\times 10^{-3}$)	S_{24} (mg/ml)	Eff %
1.05	14.0	$<$ 0.04	$<$ 20
1.06	25.0	$<$ 0.04	$<$ 11
1.08	17.4	0.040	16.0
1.09	31.0	0.065	14.6
1.10	16.5	0.060	25.5
1.11	31.0	0.080	18.0
1.12	30.6	0.105	24.0
1.13	45.0	0.120	18.7
1.14	36.8	0.100	19.0
1.15	29.8	0.052	12.2

Symbols used: see footnote of Appendix 10.

Appendix 15

The amounts of sheep serum IgG2 fed (g/kg B.W.) and absorbed into the blood (mg/ml) at 24 hours post feeding in 20 newborn calves fed within 2 hours of post natal life and then allowed to remain with their dams for two days. Apparent efficiencies of absorption were calculated by assuming plasma volumes of 7% of body weight and as described on page 50.

Calf No.	B.W. (kg)	Sheep IgG2 fed (g/kg B.W. x 10^{-3})	IgG2 absorbed (mg/ml)	Eff % of absorption
2.01	40.0	21.5	0.095	30.9
2.02	31.5	35.8	0.125	24.4
2.03	34.5	33.0	0.136	28.8
2.04	35.5	24.2	0.088	25.4
2.05	36.0	23.9	0.065	19.0
2.06	43.5	19.7	0.070	24.9
2.07	40.0	21.5	0.096	31.3
2.08	45.0	19.1	0.040	14.7
2.09	38.0	22.5	0.110	34.2
2.10	36.0	23.6	0.105	31.1
2.11	36.5	23.6	0.050	14.8
2.12	32.5	26.3	0.110	29.3
2.13	30.0	28.6	0.130	31.8
2.14	22.5	50.2	0.180	25.1
2.15	30.5	27.8	0.090	22.7
2.16	41.5	20.7	0.080	27.1
2.17	32.0	16.6	0.063	26.6
2.18	36.5	19.5	< 0.040	< 14.0
2.19	38.0	16.0	< 0.040	< 17.0
2.20	32.5	20.6	< 0.040	< 13.0

Appendix 16

The 24-hour and 48-hour levels of serum IgG, IgG2, IgA and IgM in 20 newborn calves left with their dams for 48 hours.

Calf No.	IgG		IgG2		IgA		IgM	
	S_{24} (mg/ml)	S_{48} (mg/ml)	S_{24} (mg/ml)	S_{48} (mg/m)	S_{24} (RSA/ml)	S_{48} (RSA/ml)	S_{24} (mg/ml)	S_{48} (mg/ml)
2.01	42.0	39.9	1.74	1.70	2.85	1.90	1.70	1.66
2.02	47.8	44.2	1.74	1.93	3.45	2.50	4.10	3.86
2.03	24.5	21.3	0.77	0.74	1.72	1.19	1.28	1.26
2.04	9.5	8.1	0.58	0.54	1.30	0.85	0.67	0.56
2.05	2.0	18.5	0.03	1.09	0.07	1.32	0.08	1.55
2.06	34.3	31.7	1.97	1.93	5.40	3.60	2.85	2.62
2.07	19.1	20.1	1.32	1.32	2.92	2.40	0.62	0.56
2.08	27.5	25.7	2.01	1.84	2.35	1.41	2.52	2.52
2.09	27.1	22.1	1.40	1.32	3.58	2.35	1.59	1.50
2.10	6.9	ND	0.54	ND	0.58	ND	0.29	ND
2.11	43.2	ND	1.05	ND	1.72	ND	2.10	ND
2.12	32.6	ND	0.90	ND	1.30	ND	1.55	ND
2.13	55.6	ND	1.32	ND	3.90	ND	3.29	ND
2.14	76.2	ND	1.93	ND	6.20	ND	4.20	ND
2.15	22.7	ND	1.24	ND	2.00	ND	2.18	ND
2.16	21.1	ND	1.10	ND	2.10	ND	2.03	ND
2.17	52.2	49.2	1.03	0.88	4.75	3.20	2.69	1.97
2.18	102.2	82.2	1.93	1.80	7.95	5.70	4.20	3.50
2.19	16.0	14.9	0.63	0.61	1.32	0.75	0.77	0.67
2.20	62.0	58.4	2.62	2.43	7.10	6.00	3.30	3.04

ND* = Not determined, because samples were not taken.
Symbols used - refer footnote of appendix 10.

Appendix 17

Figure 1

An example of standard curve for determination of colostral immunoglobulin concentration by Biuret reaction method (Gornal *et al*, 1949) plotted using bovine serum albumin (BSA).

Figure 2

An example of standard curve for determination of colostral casein content by dye binding method (Dolby, 1961) plotted using skim milk powder. The skim milk contained 2% moisture and 40.3% protein (moisture free basis) of which 77% was casein.

APPENDIX 17

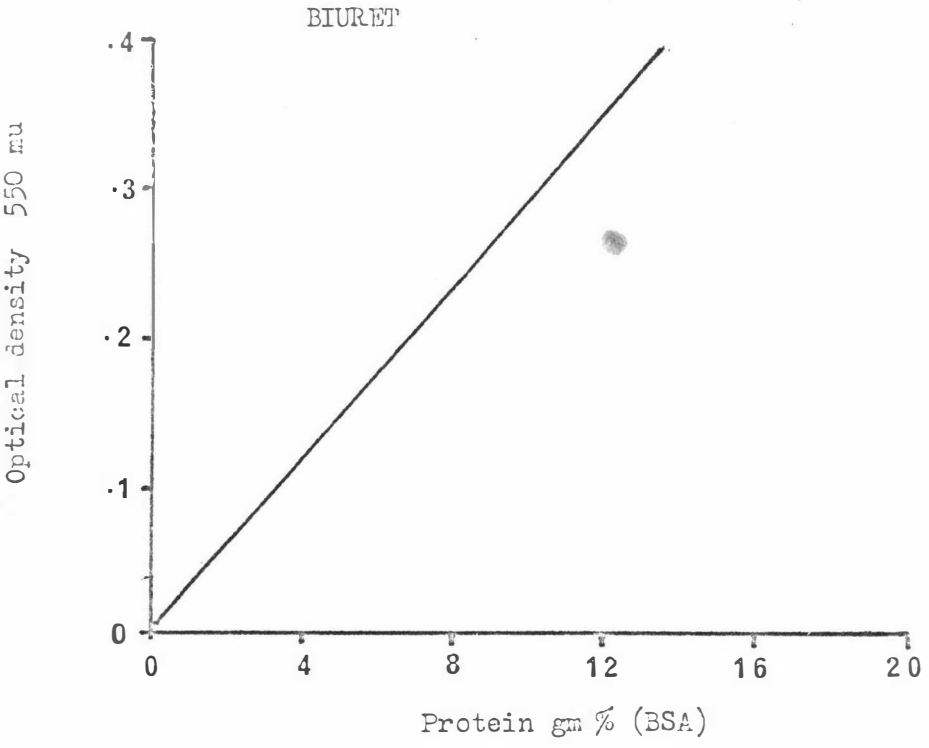


Figure 1

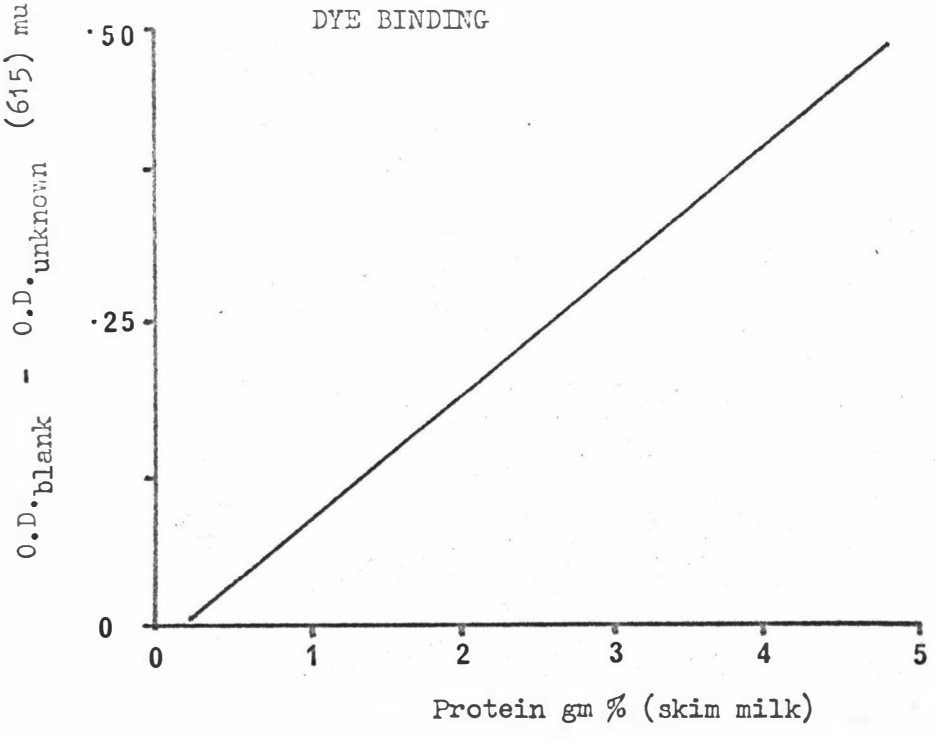


Figure 2

Figure 3

An example of standard curve for determination of IgG, IgG1, and IgM by radial immunodiffusion method, plotted on semi logarithmic graph paper.

Figure 4

An example of standard curve for determination of IgA in serum and colostrum samples, expressed as relative to concentration of serum IgA (RSA). The graph was plotted using dilutions of bovine serum and rechecked with dilutions of bovine colostral whey.

Figure 5

An example of standard curve for determination of sheep IgG2 by radial immunodiffusion method, plotted on semi logarithmic graph paper.

APPENDIX 17 - continued

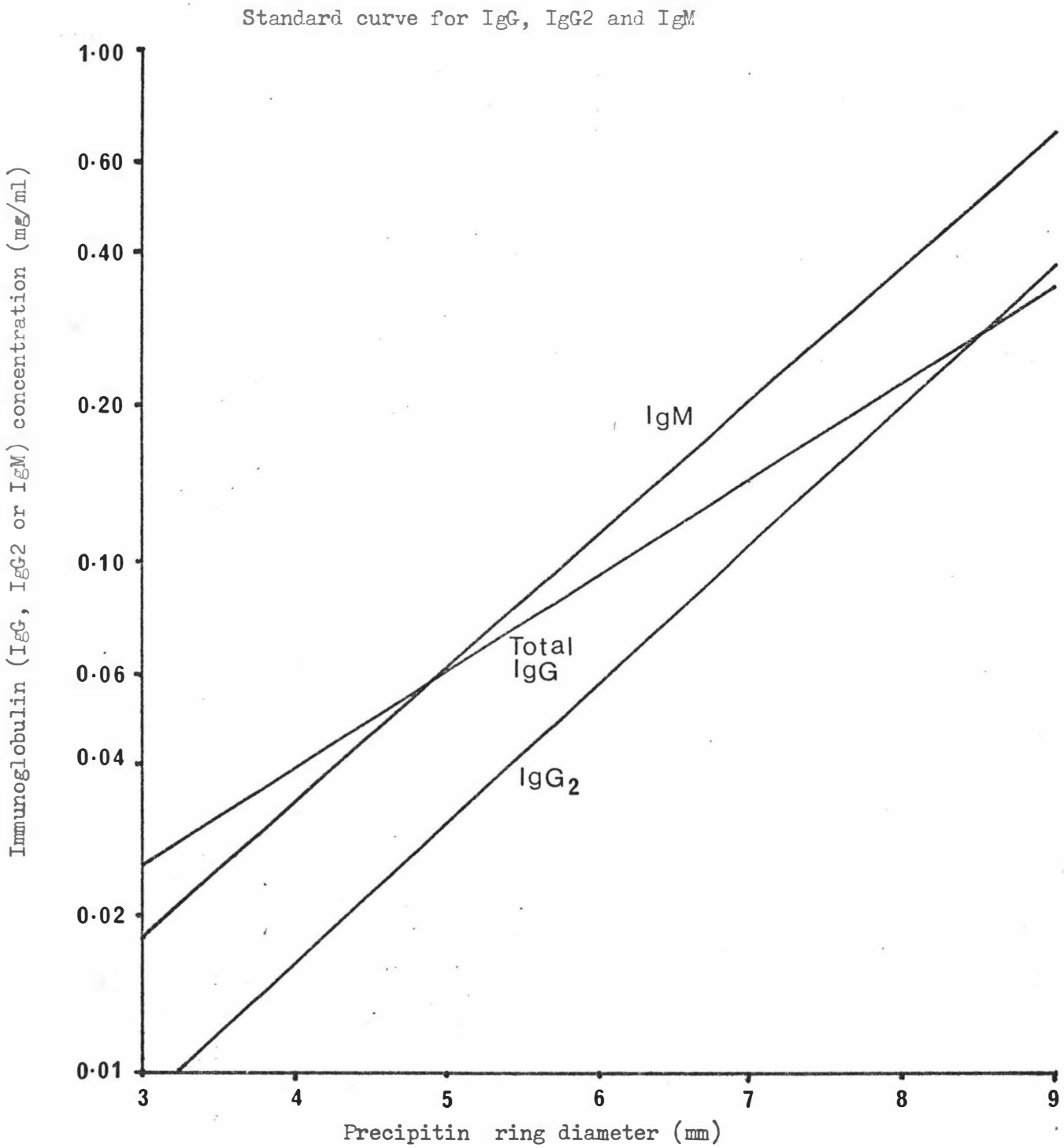


Figure 3

APPENDIX 17 - continued

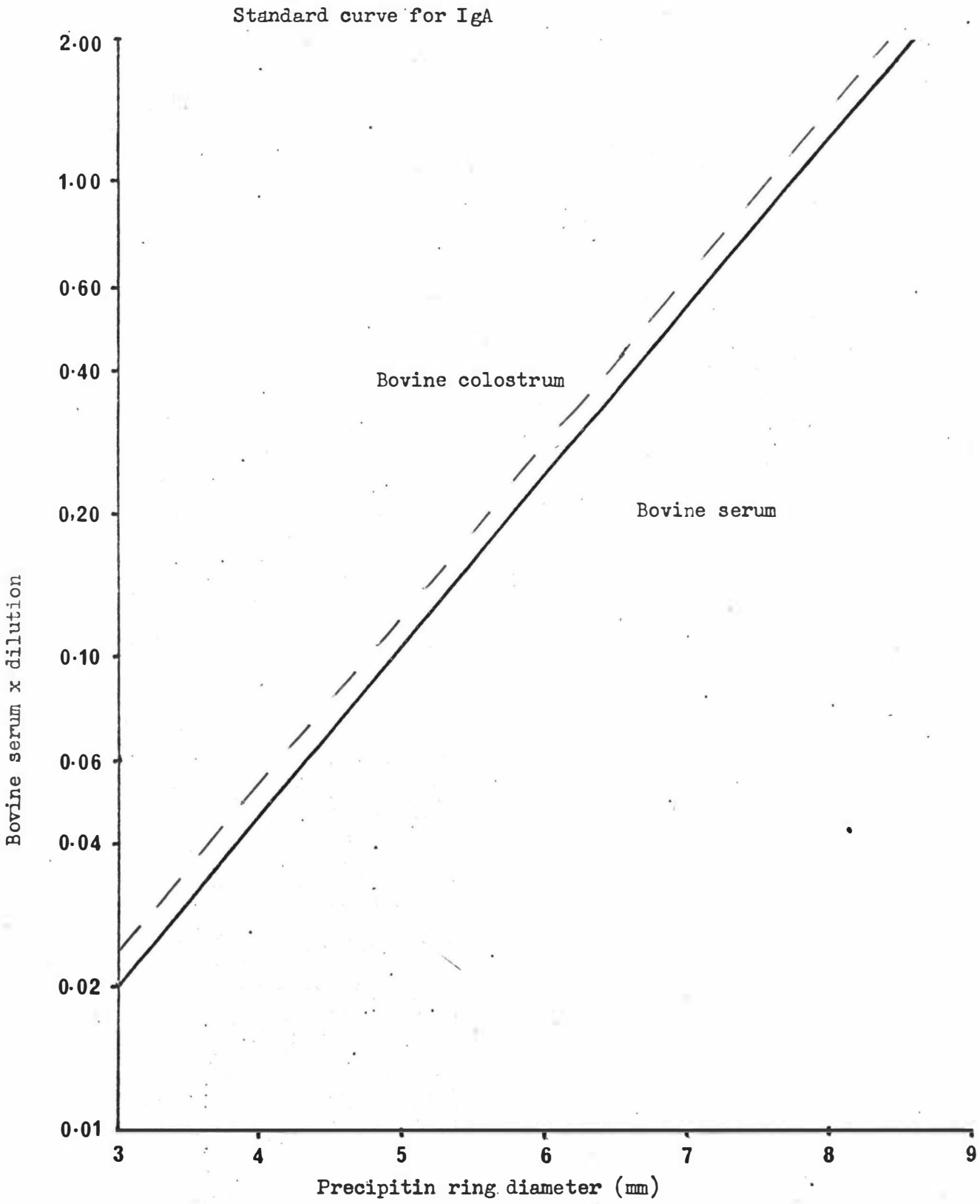


Figure 4

APPENDIX 17 - continued

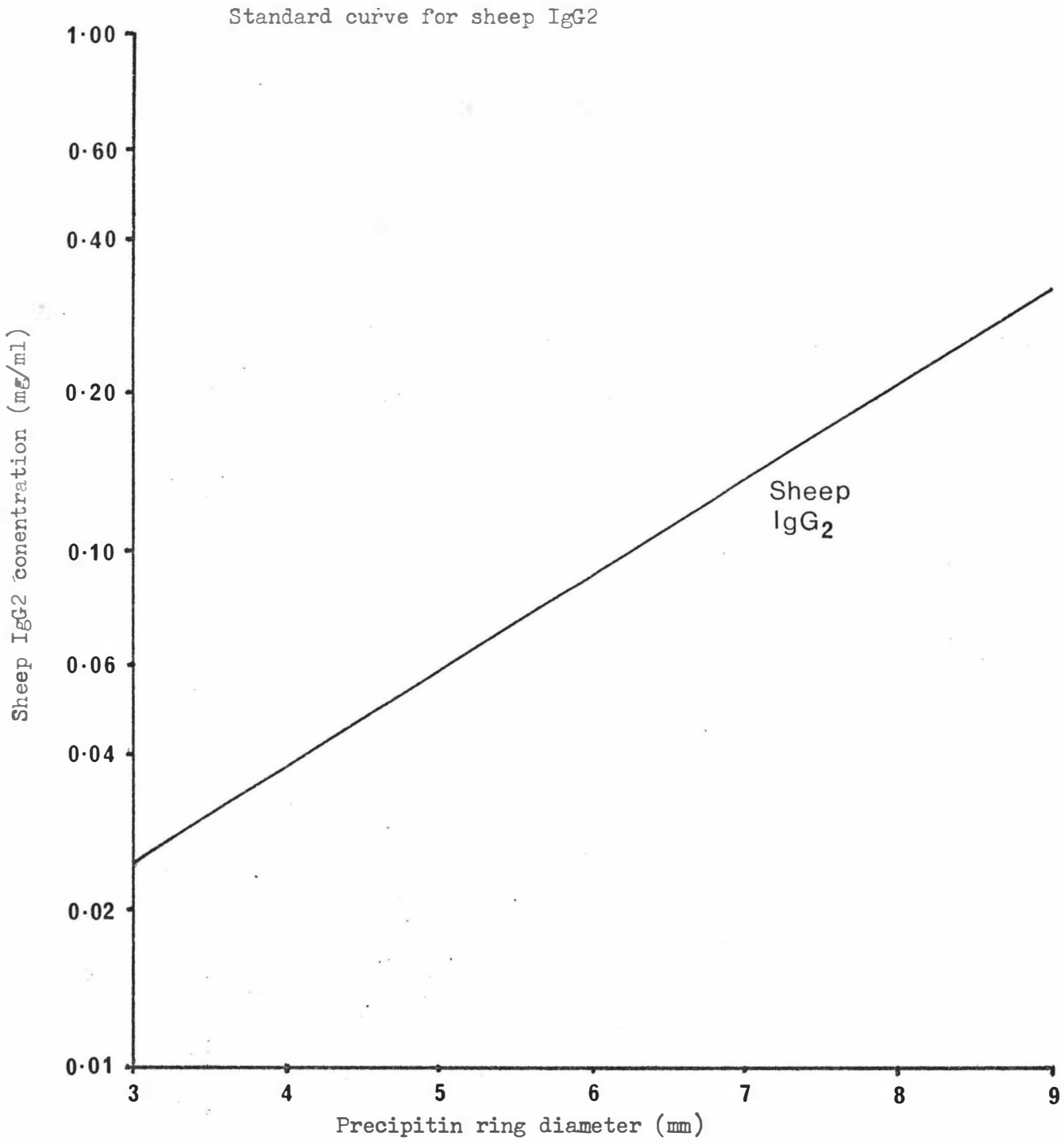


Figure 5

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