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PASSIVE TRANSFER OF DELAYED-TYPE HYPERSENSITIVITY WITH
BOVINE COLOSTRUM

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PASSIVE TRANSFER OF DELAYED-TYPE HYPERSENSITIVITY
WITH BOVINE COLOSTRUM

by

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ABSTRACT

Passive transfer of delayed-type hypersensitivity (DTH) from mother to neonate via colostrum has been hypothesized but not substantiated in the bovine species. This study demonstrates that bovine colostrum contains soluble factors, transfer factor and Ichorin, which can passively transfer DTH across the species barrier.

Calves skin tested for Brucella abortus before and after ingestion of colostrum obtained from mothers vaccinated for B. abortus demonstrate substantial delayed-hypersensitivity lesions after colostrum feeding. Tuberculin controls were negative in both groups.

Attempts were made to isolate antigen-specific biological activity from transfer factor and Ichorin isolated from the blood of calves taken at birth, before colostrum ingestion, and at 48 hours postpartum. Results show that at 48 hours postpartum, calves possess transfer factor and Ichorin in their blood which is capable of interspecies passive transfer of DTH to B. abortus. Similar activity is seen with the transfer factor isolated from the calves at birth.

These results indicate that soluble factors present in the blood and colostrum of a sensitized mother may be passively transfered to the neonatal calf via colostrum thereby possibly sensitizing the calf to specific antigens.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

In 1922, Smith and Little (83) noted that a large majority (75-80%) of calves deprived of colostrum failed to survive. They concluded that the colostrum-deprived calf lacked something which resulted in systemic invasion by intestinal bacteria and its multiplication in various organs. It was later shown that, unlike the human, the bovine neonate does not receive maternal antibody via the placenta but instead, the maternal antibody is passively transferred by colostrum ingestion and absorption. The absorption of colostral antibody occurs by pinocytotic activity of the gut epithelium in the bovine neonate and lasts for only 12 to 48 hours postpartum (73), at which time, closure of the epithelial cells occur (71).

Whereas the passive transfer of humoral immunity by colostrum has been very well documented, the passive transfer of cell-mediated immunity by colostrum still needs to be substantiated. Although cells were identified in colostrum more than a hundred years ago (16), they have received little attention until recently. The significance and function of colostral white cells and their products is still controversial.

The focus of this study is to determine whether bovine colostrum contains factors which can passively transfer antigen-specific delayed-type skin test hypersensitivity (DTH) to the neonatal calf after oral ingestion of colostrum. The study is divided into four main objectives: 1) to determine if bovine colostrum contains a component which is biologically and physicochemically similar to bovine serum Ichorin, described by Olson and Drube (68) to passively transfer DTH with antigen specificity across the species barrier, 2) to determine whether bovine colostral white cells contain a component biologically and physicochemically comparable to blood transfer factor (TF), a dialyzable leukocyte extract also shown to passively transfer DTH, 3) to determine if bovine colostrum contains the necessary components for an immune response by comparing colostrum and blood samples obtained immediately postpartum with reference to total white cell counts, white cell differentials, and quantity of antibody, and 4) to determine if antigen-specific DTH is passively transferred to neonatal calves via whole colostrum with the subsequent appearance of antigen-specific Ichorin and transfer factor in the peripheral blood of the colostrum-fed calf.

Immunological Components of Bovine Colostrum

Extensive work has been done on the quantitation of total and class specific immunoglobulin levels present in whole bovine colostrum. This research has been summarized in Table 1. Bovine colostrum obtained immediately postpartum contains maximum levels of gamma globulin as compared to subsequent milkings (85) but these levels vary considerably between individual animals (22).

TABLE 1

TOTAL AND CLASS SPECIFIC COLOSTRAL IMMUNOGLOBULIN LEVELS

Total Ig	IgG	IgG1	IgG2	IgM	IgA	Ref #
46.5[a]	43.3			3.2		39
39.0	34.1			4.9		72
85.2		75.0	1.9	4.9	4.4	57
39.4		33.8	3.6		2.0	8
102.0		79.2	5.2	10.7	6.5	5
103.3		88.2	2.5	9.2	3.4	75
42.9		34.0	3.5	3.9	1.5	87
48.0	36.0			5.1	6.9	73
147.6	39.5			8.1		61
51.3	36.6			5.0	9.7	22
92.3	63.2			12.4	16.2	85

[a]Values indicate concentration in mg/ml.

Colostrum has also been shown to contain large numbers of viable leukocytes. Table 2 summarizes the total cell counts and cell differentials from human and bovine colostrum obtained by various researchers. Colostral cell counts show a significant amount of variability within and between women and cows. Total cell counts, percent macrophages, and percent polymorphonuclear cells from bovine colostrum appear to have seasonal and diurnal variation as well as significant variability among the quarter samples (25,82). The numbers of lymphocytes in colostrum appear to remain somewhat stable, showing less variability under most of the conditions listed above (82). Of the lymphocyte population found in human colostrum, 31-65% (mean value of 50.2%) of the lymphocytes appear to be T-cells as determined by indirect immunofluorescence and 35.6% $\pm$ 8.8% of the lymphocytes demonstrate E-rosetting (14). It appears that B-cells comprise about 34% of the colostral lymphocyte population as determined by immunofluorescence of surface immunoglobulins (7,14).

Passive Transfer of Immunity by Colostrum

As described in the introduction, passive transfer of humoral immunity from dam to neonate occurs postpartum by absorption of colostral immunoglobulins in the small intestine of the neonate. Early work by Little and Orcutt (55), McAlpine and Rettger (58), and McDiarmid (59) show that colostrum-deprived calves are always negative for antibody to *Brucella abortus* using agglutination and complement fixation tests despite positive tests for antibody in their mothers. These early researchers also demonstrated that calves from dams which

TABLE 2

COLOSTRAL WHITE CELL COUNTS AND DIFFERENTIALS

Species	WBC X 10^6/ml	% lymphocytes	% neutrophils	% macrophages	Ref #
Human	0.6-11 (3.37)[a]	1-39 (9.6)	2-87 (33)	11-94 (57)	52
	0.1-14 (2.8)	2-23 (6.6)	9-69 (44.3)	28-84 (49.1)	30
	3	10			27
	0.5-3 (2.1)	10	7		81
	0.3-10	20			67
	0.88-13.9 (4.97)	1.6	57.7	40.6	31
	1.5-4.1 (2.1)		9.5		64
	0.11-12 (3.3)	5.2-8.9	40-60	30-47	12
		2-23 (11)			70
	0.2-6				47
Bovine	0.6				60
		2-4	50-65	8-17[b]	
				23-31[c]	53
	2.7				33
	0.62				25
	5.6				65

[a]Parenthesis represents mean value

[b]Non-vacuolated macrophage

[c]Vacuolated macrophage

were immune to Brucella abortus will show specific antibody in their blood within 2 hours after colostrum ingestion (58,59,55). The antibody titer continues to increase, peaking at about 24 hours postpartum, and then declines gradually (usually logarithmically) over the first 4-6 months, depending upon the titer present in the ingested colostrum (58,59,69).

The extent of the passive transfer of humoral immunity by colostrum in neonatal calves is apparent in the serum protein profile of calves at various times after colostrum ingestion. Results of a recent study comparing total protein, gamma globulin, and class specific immunoglobulin levels in the serum of colostrum-fed and colostrum-deprived calves during the first 96 hours postpartum is shown in Table 3. Only trace amounts of serum gamma globulin and class specific immunoglobulins were present in colostrum-deprived calves during this time period. Immunoglobulin-producing cells have been observed in the calf as early as 59 days of gestation for IgM and 145 days for IgG (79). Trace quantities of IgM and IgG can be detected in the fetal serum as early as 130-135 days of gestation (79). The levels of these immunoglobulins usually remain very low until parturition with subsequent colostrum ingestion, although some congenital infections may stimulate precocious immunogenesis (77).

The passive transfer of cell-mediated immunity by colostrum has been much more difficult to substantiate. Studies have shown that mothers with positive delayed-type skin test reactions to tuberculin purified protein derivative (PPD) have PPD-reactive lymphocytes in their

TABLE 3

SERUM PROTEIN PROFILES IN NEONATAL CALVES (10)

Hours After Birth	Total Serum Protein (g/100ml ± SEM) Colostrum-Fed Calves	Total Serum Protein (g/100ml ± SEM) Colostrum-Deprived Calves	Serum γ-globulin Colostrum-Fed Calves	Serum Ig (mg/100ml) Colostrum-Fed Calves IgG	IgM	IgA
0	3.77 ± .35	4.30 ± .30	0	0	0	0
6	4.60[a] ± .42	3.77 ± .16	0.86 ± .13	1175	142	147
12	5.14[a] ± .37	4.10 ± .24	0.96 ± .17	1152	291	320
24	5.60[a] ± .21	4.47 ± .18	1.36 ± .12	1629	291	320
48	6.13[a] ± .25	4.50 ± .14	1.33 ± .16	1385	309	272
72	6.15[a] ± .33	4.31 ± .07	1.20 ± .20	1500	300	190
96	6.60[a] ± .28	4.48 ± .21	1.22 ± .16	1579	304	142

[a]$P < 0.010$

blood, colostrum, and early milk, as opposed to mothers with negative skin test reactions (78). Human infants breast-fed by skin test positive mothers have positive intradermal skin test reactions as well as PPD reactive lymphocytes in their perpheral blood (78,63,66,67), whereas infants bottle-fed or breast-fed by skin test negative mothers showed no such reactivity. Controversy exists as to whether this passive transfer is the result of transfer factor or other soluble components present in the colostrum being absorbed by the infant and thus providing protective sensitization (52,63,74) or if the colostral leukocytes can actually penetrate the gut epithelium to directly transfer immunologic information to the infant (63,44,3).

The blood indices of colostrum-fed and colostrum-deprived calves do differ. Colostrum-fed calves have significantly more white blood cells at 6, 12, and 24 hours postpartum than colostrum-deprived calves, due to a transient and statistically significant ($P < 0.05$) neutrophilia (10,45,56). No significant differences were reported between the number of peripheral blood lymphocytes from colostrum-fed and colostrum-deprived calves (10,45,56).

Delayed-Type Hypersensitivity

The first description of delayed-hypersensitivity reaction was probably that of Jenner (1798) when he described the reaction which occured following the injection of antigen into the arm of a patient who had recovered from cowpox (84). In 1891, Koch observed that if tubercle bacilli were injected into an animal previously infected with *Mycobacterium tuberculosis*, the skin at the inoculation site became indurated and erythematous within 24 to 48 hours. This reaction became known as the "Koch phenomena" (84). By 1925, Zensser distinguished delayed-hypersensitivity from the other forms of hypersensitivity and recognized that this reaction could take place without antibody (88, 89). Landsteiner and Chase, however, laid the groundwork for the current understanding of delayed-hypersensitivity as a part of cellular-immunity by establishing the principle of passive transfer of delayed-type hypersensitivity using living cells (46).

The initial stage of delayed-type hypersensitivity is the binding of antigen by a small number of specific antigen-reactive T-lymphocytes which is followed by the production and release of soluble mediators or lymphokines, whose overall function is to amplify the initial cellular response. This is accomplished by recruitment and induced mitogenesis of T- and B-lymphocytes, attraction of granulocytes to the area, as well as the attraction, localization, and activation of macrophages at the inoculation site. The cutaneous response of erythema and induration is evident within 12 hours and a maximum response occurs by 24 to 48 hours, with a massive accumulation of mononuclear cells at the inoculation site. The erythema is due to increased vasodilation accompanied by increased vascular permeability while the induration is the result of the cellular infiltration.

Other types of dermal hypersensitivity reactions may sometimes be confused with "true" delayed-type hypersensitivity. Table 4 describes various hypersensitivity reactions and their differentiating characteristics. The topical appearance of the skin test site is not

TABLE 4

COMPARISON OF VARIOUS DERMAL HYPERSENSITIVITY REACTIONS

	Delayed-Type Hypersensitivity	Jones-Mote Hypersensitivity	Arthus Reaction	Anaphylactic Reactions	Shwartzman Reaction
Older Terms	Type IV Tuberculin-Type Hypersensitivity	Cutaneous Basophil Hypersensitivity	Type III Hypersensitivity	Type Hypersensitivity	--
Major cell type at the Inoculation site	Mononuclear (29)[a]	Basophils (29)	Neutrophils (24)	Eosinophils (24)	Granulocytes (4)
Time after Inoculation for maximum reaction	24-72 hours (29)	24 hours (29)	4-10 hours (24)	0-20 minutes (4)	6-48 hours (4)
Topical appearance of skin reaction	Erythema and Induration (29)	Erythema with little or no induration (29)	Erythema and Edema (24)	Wheal and Flare (24)	Hemorrhagic Necrosis (4)
Initiation of Reaction	Antigen (29) and Antigen specific reactive T-cells (46)	Protein AGs in IFA[b] and Immune complexes[c] (29)	Immune Complexes (24)	Homocytotropic Antibodies (24)	Endotoxin (4)
Passive Transfer	Cells (46), Cell-Extracts (48), Ichorin (68)	Cells only (24)	Serum IgM, IgG (4)	Serum IgE or reaginic antibody (4)	none (4)
in vitro: Lymphocyte Transformation	Increased (29)	Increased (29)	--	--	--
in vitro: Macrophage Migration	Strongly positive (29)	Weakly Positive (29)	--	--	--

[a]Parenthesis indicate reference number

[b]Incomplete Freund's Adjuvant

[c]Antigen-Antibody complexes

the best indicator for differentiation, especially between Jones-Mote hypersensitivity and tuberculin-type delayed-hypersensitivity therefore slides made from transverse sections of the skin test area should be screened to determine which cell type predominates in the cell lesion.

Transfer Factor

Transfer factor has been generally defined as a component found in dialyzable leukocyte extracts which is capable of transferring delayed-type hypersensitivities (DTH) from immune donors to non-immune recipients. The first experiments which uncovered the ability of cellular extracts to passively transfer DTH were by Crepea and Cooke (13) and Jeter et al. (37) in animals and by Lawrence et al. (48,49) in humans. Lawrence (50) then termed this biologically active component as transfer factor.

Comparative studies on the activity of transfer factor have shown that bovine transfer factor could be used to transfer DTH across the species barrier to rhesus monkeys, dogs, rabbits (42,43), guinea pigs (54), and man (35,6,34). The dermal reactivities to various antigens were transferred by systemic treatment in these studies. In addition, bovine transfer factor is capable of antigen-specific passive transfer of DTH within the bovine species (36,43,42,41). There appears to be no genetic restriction governing the biological activity of the transfer factor component.

Table 5 lists various biochemical properties of transfer factor. Transfer factor, produced by T-lymphocytes, appears to contain inosine, ribose, a peptide, and a phosphodiester bond (38). Presumably

TABLE 5

BIOCHEMICAL PROPERTIES OF TRANSFER FACTOR (TF)

1. Bovine TF is a dialyzable factor with an estimated molecular weight of 1100 (43).
2. The composition of TF appears to be polypeptide-polynucleotide (1). Bovine TF does possess a ribose-reacting material (43).
3. TF has a p I of 1.6 (38).
4. Bovine TF has a 260/280 nm ratio of 2.
5. TF is heat labile and can be inactivated by heating at 56^{o}C for 30 minutes (1).
6. TF appears to contain inosine--probably as IMP (38).

the property of antigen-specificity is encoded within the peptide component (38).

In addition to the antigen-specific properties of passive transfer with TF, nonspecific properties have also been reported. These nonspecific antigen-independent activities of transfer factor have been summarized by Klesius (40) as follows: increased chemotactic activity for monocytes and neutrophils, enhanced or inhibited migration of neutrophils, increased accumulation of cyclic GMP in monocytes, enhanced formation of T-cell rosettes, and augmented or suppressed blastogenic responses to antigens and mitogens. These effects are thought to be the result of small, active molecules such as cyclic nucleotides, prostaglandin 1, serotonin, histamine, ascorbate, nicotinamide, certain amino acids, and purines (40). The biological effects of these nonspecific components on the antigen-specific transfer factor moiety is still controversial.

Ichorin

Ichorin, a small molecular weight component of plasma or sera capable of interspecies passive transfer of antigen-specific DTH, has recently been reported and termed by Olson and Drube (68). Ichorin has been obtained from the sera of guinea pigs, sheep, and cattle and is capable of passive transfer of dermal reactivity to a number of bacterial, viral, fungal, and chemical antigens (68). Ichorin isolated from a heifer vaccinated against Brucella abortus possessed the ability to passively transfer DTH to homologous antigen in the guinea pig (68).

Ichorin appears to have a molecular weight range of 500-10,000, possesses a high 280/260 nm ratio, and is negative to a Limulus amebocyte lysate test (68). Although Ichorin is fluram and orcinol positive, the moiety responsible for antigen-specificity is presently unknown.

Passive transfer of DTH to tuberculin sensitivity with serum has been reported in the past (11,13,15,23,28,76,86,89) but many other attempts to transfer DTH with serum were not successful (2,9,15,17,23, 26,32,51). Until the discovery of the Ichorin component of serum, passive transfer of DTH with serum had not been substantiated.

CHAPTER 2

MATERIALS AND METHODS

Sample Collections

Twelve pregnant Holstein-Freisian cows from two separate herds were used in this study. At parturition, the newborn calf was separated from the dam prior to suckling. Each cow was completely milked out using a portable milker after 5 ml samples for cell analysis were obtained from each teat. The 5 ml samples were placed into containers containing 0.1 ml of 15% w/v EDTA. A minimum of four liters of colostrum was acquired: two liters for calf feedings, a 5 ml sample in EDTA for total cell count and differential analysis, and the remainder for preparation of transfer factor and Ichorin. Colostrometer readings were obtained to determine the approximate immunoglobulin level (21). After milking each dam, 500 ml of blood was collected via the jugular vein, into a container holding 10 ml of 15% w/v EDTA. An additional 5 ml of blood was collected into an EDTA vacutainer.

Prior to feeding the calf, 250 ml of venous blood was collected into 5 ml of 15% w/v EDTA. One EDTA vacutainer of blood was also obtained from each calf as described above. Calves were then fed one liter of their dams colostrum followed by the second liter at 12 hours postpartum. Subsequent feedings consisted of one liter of pooled colostrum at 12 hour intervals. At 48 hours postpartum, each calf was bled again as described above.

Pooled colostrum for calf feedings was obtained prior to sample collections and frozen in 1 or 2 liter aliquots which were thawed 12-36 hours prior to feedings. Fresh and thawed pooled colostrums were kept at 4°C until feeding time. Calves were only fed pooled colostrum obtained from their dams herd. The pooled batches of colostrum from the two herds had similar total immunoglobulin levels of 50 mg/ml based upon colostrometer readings (21).

All the blood samples and the 5 ml colostrum samples were placed on ice immediately after collection and transported to the laboratory. Bleeding and milking apparati were sterilized by autoclaving prior to collection of the samples to minimize contamination by bacteria.

Depending on the amount of colostrum collected on the first milking, 2-8 liters of colostrum was used for the preparation of transfer factor and Ichorin. EDTA (15% w/v) was added to the colostrum at a concentration of approximately 20 ml per liter of colostrum. Colostrum was transported to the laboratory at a temperature of about 25-30°C. This was done to minimize the cell trauma induced by cooling the colostrum for transport then later reheating the colostrum prior to its separation with a milk clarifier.

Processing of the Colostrum

White cell counts were performed on the 5 ml colostrum samples using a method described by Smith and Goldman (81) with the following modifications. One ml of the colostrum was diluted 1:10 in saline, mixed, and centrifuged at 400 X g for 15 minutes at 4°C. The fat layer was carefully removed and the supernatant liquid poured off. Cells were then resuspended in 0.5-1.0 ml of saline and counted on a hemocytometer. Counts were corrected according to the dilution factor. Microscope slides for differential counts were made and stained using Wrights-Geimsa stain.

The 2-8 liters of colostrum saved for transfer factor and Ichorin preparations were filtered through a layer of cheesecloth prior to being placed into a small, portable milk clarifier (DeLaval Model #619). Cells and other solids not removed by the cheesecloth remain in the clarifier bowl while the supernatant colostrum passed through. The cells were carefully removed from the clarifier bowl by reaming the inside of the bowl with a small, sterilized rubber spatula. The cell material was placed into a small graduated beaker and the approximate volumn was recorded. White cell counts and slides for differential analysis were done using 1 ml of the cell material diluted 1:2 in saline before using the procedure described above for colostral cell counts. The cell material was then used for the preparation of transfer factor.

The supernatant colostrum from above was collected in one liter volume centrifuge containers with 2.5 gms of rennet (Sigma R-3376 Lot #99C-0348). The samples were mixed, incubated at 37°C for one hour then centrifuged at 4000 X g for 30 minutes at 4°C. The whey, positioned below the fat layer, was removed by suction. A one ml aliquot of whey was frozen for immunoglobulin analysis while the remaining whey was lyophilized and stored at -20°C until used for the preparation of Ichorin.

Processing of the Blood

White cell counts and differential counts were performed on the vacutainer samples using standard manual procedures. The large blood samples were centrifuged at 4000 X g for 40 minutes at 4°C. Plasma was removed by suction and saved. The buffy coat was carefully removed and respun as before. Once again, the plasma was suctioned off, the buffy coat removed and the total buffy volume was recorded. White cell counts and differential counts were performed on the buffy coat. The buffy coat material was then used for preparation of transfer factor.

A one ml sample of the plasma was frozen for immunoglobulin analysis. The remaining plasma was then lyophilized and stored at -20°C until used in preparation of Ichorin.

Preparation of Transfer Factor

The colostral cell mixture and blood buffy coats were diluted 1:2 with sterile 2X dH_2O and mixed vigorously. The samples were frozen and thawed ten times then centrifuged to remove all cell debri. The samples were then placed into size #8 dialysis tubing (VWR Scientific #25725-204) and vacuum dialyzed at 25 psi for 48 hours at 4°C (34). The dialysate was collected and acetone was added to a final concentration of 90%. The suspension was mixed for 5 minutes, then centrifuged at 650 X g for 30 minutes at room temperature. The white precipitate containing the transfer factor was allowed to air dry prior to resuspension in 2X dH_2O. The samples were then sterilized using .45 μ Millipore filters, lyophilized, and stored at -20°C until used.

Preparation of Ichorin

One gram portions of the whey from the experimental parturent cows, their plasma, and the 48 hour calf plasma were reconstituted in 15 ml of 2X dH_2O and placed upon Geon-Pevikon matrixes as described by Fahey and McLaughlin (19) with modifications described by Olson and Drube (68). Electrophoresis of the first separation was performed using Na-barbital Na-acetate buffer (0.05M and pH 8.2) at 450 v and 50 mA constant current. The matrix was then cut, separating the gamma globulin region. The globulin protein was eluted from the gel and analyzed by spectrophotometric means and immunoelectrophoresis (68). The gamma globulin was desalted using a UM05 Amicon filter and lyophilized. One gram of the gamma globulin from each preparation was reconstituted as described above, placed upon a second Geon-Pevikon matrix, and electrophoresed using a 0.02M buffer (pH 8.2) at 450 v and 10 mA constant current. The alpha-albumin region of the second separation was shown by Olson and Drube (68) to contain the biological activity. It was filtered through PM10 and UM05 Amicon filters to obtain the Ichorin. Samples were lyophilized and stored at -20°C until use.

One gram of the newborn (0 hour) calf plasma was reconstituted as above and placed on the first Geon-Pevikon matrix. The block was fractionated and the protein eluted and analyzed as described. Since the newborn calf plasma contains little or no gamma globulin, material from the beta, alpha, and albumin protein regions were collected, desalted through UM05 Amicon filters, lyophilized, and stored at -20°C until they could be analyzed for biological activity.

Antigen Preparation

Brucella abortus strain 19, live vaccine (Jen Sal serial #571) was reconstituted to 5 ml with diluent, then incubated for 4 hours at 70°C to kill the organism. No growth was observed in culture tubes after one week of incubation at 37°C. The vaccine preparation was then dialyzed, lyophilized, and stored at -20°C until use.

Human Tuberculin, purified protein derivative (PPD), purchased in tablet form (Parke, Davis NDC 71-1296-1 Lot #995953A), was stored at -20°C until use.

Endotoxin Assay

The presence or absence of gram-negative bacterial endotoxin in the skin test antigens, transfer factor, and Ichorin preparations was determined using Limulus Amebocyte Lysate (Sigma "E-toxate" 210-7 Lot #41F-0495). The procedure described by Sigma (80) and by Olson and Drube (68) was used with the following modifications. The transfer factor and Ichorin preparations were reconstituted to 500 and 50 μg/ml with pyrogen-free saline. The *Brucella abortus* skin test antigen was prepared in dilutions ranging from 2 mg/ml to 50 μg/ml and the human PPD skin test antigen was diluted to 1 μg/ml with saline. Lipopolysaccharide B *Esherichia coli* (Difco Laboratories 3920-25 Control

#700397) was used as a positive control with dilutions ranging from 100 ng/ml to 0.1 pg/ml. Saline was employed as a negative control. All preparations and reagents were prepared either in new, pyrogen-free glassware or in glassware soaked overnight in a 1% solution of E-toxa-Clean (Sigma #210-3) then rinsed and treated as described by Sigma (80).

Each vial of E-toxate was reconstituted with 0.1 ml of pyrogen-free water (Sigma #210-7) and gently mixed. Ten µl of the test samples or control was placed into 6 X 50 mm test tubes followed by the addition of 10 µl of E-toxate. The tubes were gently mixed and incubated for a total of 18 hours. The tubes were inspected after 1, 2, 3, 4, and 18 hours for changes in viscosity or gel formation. Results were recorded as follows: 3+=solid gel formed within 2 hours, 2+=weak, soft gel formed within 2-4 hours, 1+=no gel but an increase in viscosity, opacity, or starchy granules which tend to adhere to the wall of the tube when tilted within 18 hours or less, and negative was reported with the absence of any of the above signs (46).

RNA and Protein Assays

The Orcinol assay, originally described by Mejbaum (62) was used to obtain an accurate estimate of RNA (ribose) in the transfer factor and Ichorin samples. The lyophilized test samples were diluted to a concentration of 500 µg/ml with saline. The standard, 2-deoxy-D-ribose (Schwarz Bioresearch #6901P), was diluted to concentrations ranging from 10 µg/ml to 100 µg/ml. One ml of standard or test sample was added to 1 ml of 0.1% $FeCl_3$ in concentrated HCl, followed by 0.1 ml of Orcinol reagent (100 mg/ml Orcinol (Calbiochem 4971 Lot #63434) in 95% alcohol). Tubes were loosely covered and gently boiled for 40 minutes. Once cooled to room temperature, samples were read on a spectrophotometer at 670 nm. Concentrations were derived using Beer's law.

Protein content in the transfer factor and Ichorin was assayed using fluorescamine (Fluram) which detects primary amines (20). Samples were diluted to 1 mg/ml with saline. L-glutamate (Nutritional Biochemical Corp. #9741 Lot #800502) was used as the standard with dilutions ranging from 10 mg/ml to 3.125 μg/ml. Forty μl of sample or standard was added to 3 ml phosphate buffer (0.02M Na_2HPO_4/NaH_2PO_4 pH 8.2) and vortexed while adding 1 ml Fluram (Roche Diagnostics 43026 Lot #0101-00, 30 mg/100 ml spectrophotometric grade acetone). After 20-40 minutes incubation at room temperature, samples were read on a fluorescent spectrophotometer (λ excitation =390 nm and λ emission=475 nm). Protein concentration was determined using Beer's law.

Immunoglobulin (Ig) Analysis

Single radial immunodiffusion was used to measure immunoglobulin concentration in the plasma and whey samples. The procedure described by Fahey and McKelvey was employed (18). Antisera and standards were prepared as described by Fleenor and Stott (22) for IgG, IgM, and total Ig.

Interspecies Sensitization and Skin Testing

Transfer factor and Ichorin preparations were reconstituted to 500 µg/ml and 50 µg/ml with pyrogen-free saline, and the calf 0-hour plasma fractions (beta, alpha, and albumin) were reconstituted to 500 µg/ml, also with pyrogen-free saline. Seventy-five Albino guinea pigs (800 gms average body weight) were shaved on the abdominal area and injected intradermally with 0.1 of the above preparations (transfer factor and Ichorin dilutions were injected in duplicate). Saline was injected as a negative control.

After 24 hours, each site was skin tested intradermally with 0.1 ml of 500 µg/ml or 50 µg/ml of the *Brucella abortus* antigen or 1 ug/ml of the PPD skin test antigen. Sites were observed at 6, 24, and 48 hours. Measurement of erythematous induration was recorded as follows: 0-4 mm = negative, 5-7 mm = 1+, 8-10 mm = 2+, 11-13 mm = 3+, and 14 mm or greater = 4+.

Various transfer factor and Ichorin preparations were previously shown to react optimally at the dilutions prepared above when skin testing with 500 and 50 µg/ml of *B. abortus* antigen. Each concentration of *B. abortus* was tested against each concentration of transfer factor or Ichorin.

Skin Testing of Colostrum-Fed and Colostrum-Deprived Calves

Nine calves were separated from their dams immediately postpartum, prior to suckling. Three of these calves (Group A) were skin tested at birth with six concentrations of *Brucella abortus* antigen

(5, 10, 25, 50, 100, and 200 $\mu g/0.1$ ml) and 1 $\mu g/ml$ of the PPD skin test antigen. The skin tests were administered intradermally with 0.1 ml of antigen on the left side of the neck. The calves in this group were deprived of colostrum for 24 hours. Skin test readings were obtained at 6, 12, 24, 36, and 48 hours after injection.

The six remaining calves were fed one liter of their dam's colostrum at birth and one liter at 12 hours postpartum, followed by 12 hour feedings using pooled colostrum obtained from the same herd. Total immunoglobulin level in all the colostrum sources used for feeding was estimated using the colostrometer (16). Three calves (Group B) were skin tested as described above, at 48 hours postpartum and three calves (Group C) were skin tested as above, at 72 hours postpartum.

Serum samples were obtained from each calf every 12 hours starting at 0 hours postpartum. Total immunoglobulin level in these samples was determined using the method described previously.

CHAPTER 3

RESULTS

Immunologic Status of the Test Animals

Twelve multiparturent cows and their newborn calves were selected for use in this study. The dams appeared normal with no gross indication of infection and all gave birth naturally with no severe complications.

White cell counts and cell differentials were performed in duplicate on blood and colostrum samples obtained from each cow immediately postpartum. This data is shown in Table 6. A large amount of variability is seen between the cows with respect to the total white cell counts obtained from the colostral samples. The predominant cell type in both the blood and colostral samples was the polymorphonuclear cell, which constituted approximately 60% of the white cell population in both sample groups. Data on the lymphocyte population indicate that the blood contains a slightly larger percentage of lymphocytes than the colostral samples. It is interesting to note that a positive correlation is indicated in this data between the blood and colostrum lymphocyte percentages giving a coefficient of correlation of 0.69. No correlations are apparent with the data on the other cell types and no significant differences are seen between the two herds. Additional data representing cell counts and differentials obtained from colostral samples taken from individual quarters are presented in the appendix.

TABLE 6

WHITE CELL COUNTS AND DIFFERENTIALS IN PARTURENT COWS

Cow #	Herd #[a]	WBC X $10^4/mm^3$ Colostrum	WBC X $10^4/mm^3$ Blood	% PMNs[b] Colostrum	% PMNs[b] Blood	% Macrophages[c] Colostrum	% Macrophages[c] Blood	% Lymphocytes Colostrum	% Lymphocytes Blood
7193	1	1.800[d]	0.685	69	60	4	5	27	35
4351	1	0.185	1.360	68	68	16	4	16	28
4915	1	0.105	1.220	56	53	16	1.5	28	45.5
9588	1	0.205	2.090	52	59	24	1	24	40
9433	1	0.235	1.480	60	69	24	0.5	16	30.5
9200	1	0.550	1.040	56	66	24	0	20	34
9848	1	0.280	1.480	64	66.5	16	0.5	20	33
9349	1	0.115	0.375	64	58	12	2	24	40
1020	2	0.265	1.620	52	68.5	32	3.5	16	28
713	2	0.135	0.885	56	49	16	0	28	51
762	2	0.110	1.610	56	70.5	16	0	20	29.5
774	2	1.040	1.400	56	55	28	2	16	43
$\bar{X}$		0.422	1.270	59.1	61.9	19	1.7	19.9	36.5
S.D.[e]		0.505	0.464	5.8	7.2	7.7	1.7	5.0	7.4
% C.V.[f]		199.7	36.5	9.8	11.6	40.5	100	25.1	20.3

[a]Herd #1 represents animals from a local commercial dairy whereas Herd #2 represents animals from a smaller University dairy.

[b]Polymorphonuclear cells

[c]Monocytes in the blood

[d]Mean of duplicate counts

[e]Standard deviation

[f]Coefficient of variation

The use of the portable milk clarifier to remove white cells from colostrum resulted in a 15% recovery of viable white cells. Of these viable cells, a mean of 20% were classified as lymphocytes. Table 7 shows the total number of lymphocytes acquired from the colostrum as compared to the number isolated from the blood samples from each cow. These cell samples were used in the preparation of transfer factor.

White cell counts and differentials were also run in duplicate on blood samples obtained from calves at birth, prior to colostrum ingestion, and samples obtained at 48 hours postpartum. These results are shown in Table 8. No significant differences are seen between the two sample times with respect to the cell counts. Table 9 shows the total number of lymphocytes isolated from each blood sample of the calf which transfer factor preparations were made. Once again, no significant differences were noted.

Immunoglobulin levels were recorded for colostral whey and blood plasma samples obtained from each dam at parturition. Data is shown in Table 10. Overall, the total immunoglobulin levels were higher in the whey samples than the corresponding plasma samples. Also shown are data obtained from the calves at 0 and 48 hours postpartum. Newborn calves are usually born with little or no immunoglobulin in their blood as is shown here. The data for the 48 hour samples confirm that passive transfer of humoral immunity via the colostrum occured in all calves.

TABLE 7

TOTAL NUMBER OF LYMPHOCYTES ISOLATED FROM BLOOD AND COLOSTRUM[a]

Cow #	Lymphocytes X 10^{10} Colostrum	Blood
7193	7.44	9.66
4351	0.99	10.40
4915	1.56	20.10
9588	--[b]	44.60
9433	2.54	14.40
9200	1.49	13.60
9848	1.89	10.20
9349	0.46	5.52
1020	0.58	19.40
713	1.64	11.70
762	0.84	15.00
774	5.42	11.20

[a]Total number of lymphocytes = lymphocytes/ml X volume (ml)

[b]Inadequate amount obtained

TABLE 8

WHITE CELL COUNTS AND DIFFERENTIALS IN NEONATAL CALVES BEFORE AND AFTER COLOSTRUM

Calf #	Herd #	WBC X $10^3/mm^3$ 0 hour	WBC X $10^3/mm^3$ 48 hour	% PMNs 0 hour	% PMNs 48 hour	% Monocytes 0 hour	% Monocytes 48 hour	% Lymphocytes 0 hour	% Lymphocytes 48 hour
1 (7193)[a]	1[b]	6.95[c]	4.75	69	53	1	2	30	45
2 (4351)	1	10.50	10.10	58	53	2	4	40	43
3 (4915)	1	9.35	--	49	--	3	--	48	--
4 (9588)	1	13.50	11.10	50.5	66.5	0.5	2.5	49	31
5 (9433)	1	6.60	6.20	56.5	62.5	1.5	1	42	36.5
6 (9200)	1	11.40	16.20	68	66.5	1	4	31	29.5
7 (9848)	1	14.60	10.40	74	78	0	0.5	26	21.5
8 (9349)	1	3.40	3.05	61	41.5	0	1.5	39	57
9 (1020)	2	9.25	12.00	66	44	0	1	34	55
10 (713)	2	7.35	6.55	68	65.5	0	0	32	34.5
11 (762)	2	11.90	9.80	58.5	71	0	1	41.5	28
12 (774)	2	14.00	9.30	78	66	0	0.5	22	33.5
$\bar{X}$		9.90	9.04	63	56	0.8	1.6	36	38
S.D.		3.41	3.69	8.9	12.4	1.0	1.4	8.4	11.2
% CV		34.4	40.9	14.1	22.1	123.7	85.1	23.4	29.4

[a]Parenthesis indicate cow #

[b]Herd #1 represents cows from a commercial dairy and Herd #2 represents cows from a smaller University Dairy

[c]Mean of duplicate counts

TABLE 9

TOTAL NUMBER OF LYMPHOCYTES ISOLATED FROM NEWBORN AND 48 HOUR CALF SAMPLES[a]

Calf #	Lymphocytes X 10^{10} 0 Hour Samples	48 Hour Samples
1 (7193)[b]	3.61	0.14
2 (4351)	5.72	8.81
3 (4915)	8.90	--[c]
4 (9588)	3.32	4.45
5 (9433)	2.13	1.70
6 (9200)	6.54	5.92
7 (9848)	4.68	7.56
8 (9349)	2.11	2.81
9 (1020)	2.65	11.00
10 (713)	2.73	4.39
11 (762)	4.89	4.30
12 (774)	3.12	2.73

[a]Total number of lymphocytes = lymphocytes/ml X volume (ml)

[b]Parenthesis indicates corresponding cow #

[c]No sample obtained

TABLE 10

IMMUNOGLOBULIN CONCENTRATIONS IN THE TEST SAMPLES

Cow #	Total Immunoglobulins mg/ml Colostral Whey	Total Immunoglobulins mg/ml Blood Plasma	Calf #	Total Immunoglobulins mg/ml 0 hour Blood Plasma	Total Immunoglobulins mg/ml 48 hour Blood Plasma
7193	87.8	11.0	1	0.0	24.6
4351	ND[a]	19.2	2	0.0	23.6
4915	51.9	19.0	3	0.0	ND
9588	ND	25.4	4	3.2	18.8
9433	70.2	13.5	5	0.0	33.9
9200	63.1	22.6	6	3.4	30.6
9848	72.8	17.2	7	0.0	25.0
9349	70.2	13.4	8	0.2	14.4
1020	51.4	14.3	9	0.1	16.3
713	26.4	15.2	10	1.3	26.4
762	33.2	30.6	11	0.0	10.6
774	43.6	30.0	12	0.0	26.4

[a]No data

Biological Activity of Transfer Factor From Blood and Colostrum

Transfer factor preparations, obtained from the lymphocyte populations of blood and colostrum were compared for ability to passively transfer skin test reactivity to guinea pigs. Eight of the test cows were known to be vaccinated with *Brucella abortus* strain 19 at 3 and 9 months of age and were skin test negative to bovine tuberculin. The four remaining test cows were also skin test negative to bovine tuberculin but sensitivity to *Brucella abortus* antigen was unknown and not determined.

Optimum results were obtained with the guinea pigs locally sensitized with 50 μg/.1 ml of the transfer factor preparations obtained from colostrum and blood lymphocytes then skin tested 24 hours later with 50 μg/.1 ml of *Brucella abortus* or 1 μg/ml of the PPD antigen. The results of this study are shown in Fig. 1. Signs of erythematous induration were measured at 6, 24, and 48 hours post-skin test injection. Results of the 6 hour readings indicate very little reactivity to either *Brucella abortus* or PPD whereas the 24 and 48 hour readings signify that guinea pigs sensitized with the transfer factor preparations showed delayed-hypersensitivity responses to the *Brucella abortus* antigen but not the PPD antigen. There are no obvious differences in this specific reactivity between the colostral transfer factor and the blood transfer factor or between animals from the two herds.

Histological examination of the 48 hour skin test lesions revealed mononuclear cell infiltration at the sites tested with the

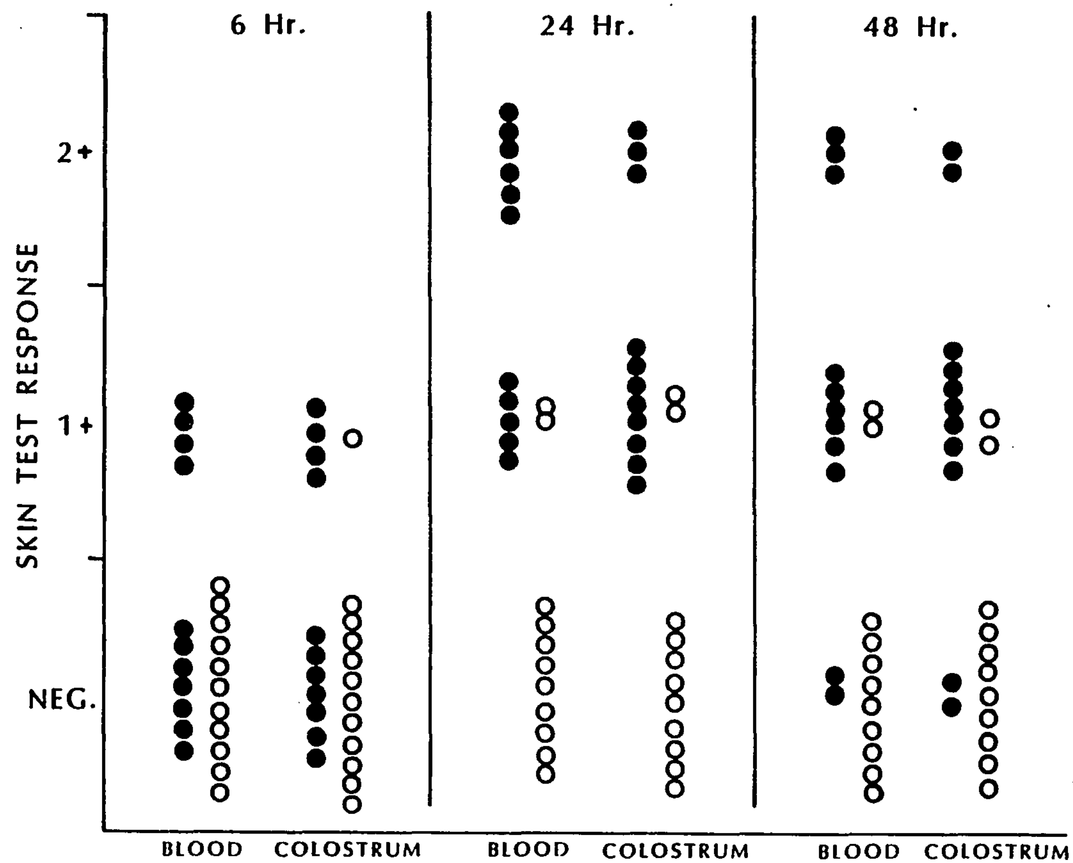

Fig. 1. Comparison of Skin Test Reactivity after Sensitization using Transfer Factor from Blood and Colostrum (Open circles represent PPD antigen and closed circles represent <u>B. abortus</u> antigen)

Brucella abortus. No such cell infiltration was seen with the sites skin tested with the PPD antigen.

Biological Activity of Ichorin from Blood and Colostrum

Ichorin preparations, obtained from blood plasma and colostral whey, were compared for their ability to passively transfer skin test reactivity to guinea pigs. Test samples were obtained from the twelve parturient cows described previously.

Optimal results were obtained with the local sensitization of the guinea pigs with 50 μg/.1 ml of the Ichorin preparation followed 24 hours later with skin test injections of 50 μg/.1 ml of Brucella abortus antigen or 1 μg/ml of PPD antigen. The results shown in Fig. 2 illustrate that antigen-specific transfer of reactivity occured to the Brucella abortus antigen but not with the PPD antigen. These results indicate that the colostral whey Ichorin and the blood plasma Ichorin have similar biological activity.

Histological examination of the 48 hour skin test lesions showed mononuclear cell infiltration at the sites tested with the Brucella abortus. Little or no cell infiltration was seen with the sections from sites skin tested with PPD.

Isolation of Transfer Factor and Ichorin from Calves

Lymphocytes isolated from the blood samples taken from calves at birth and 48 hours postpartum were used for the preparation of transfer factor. The biological activity of this transfer factor was

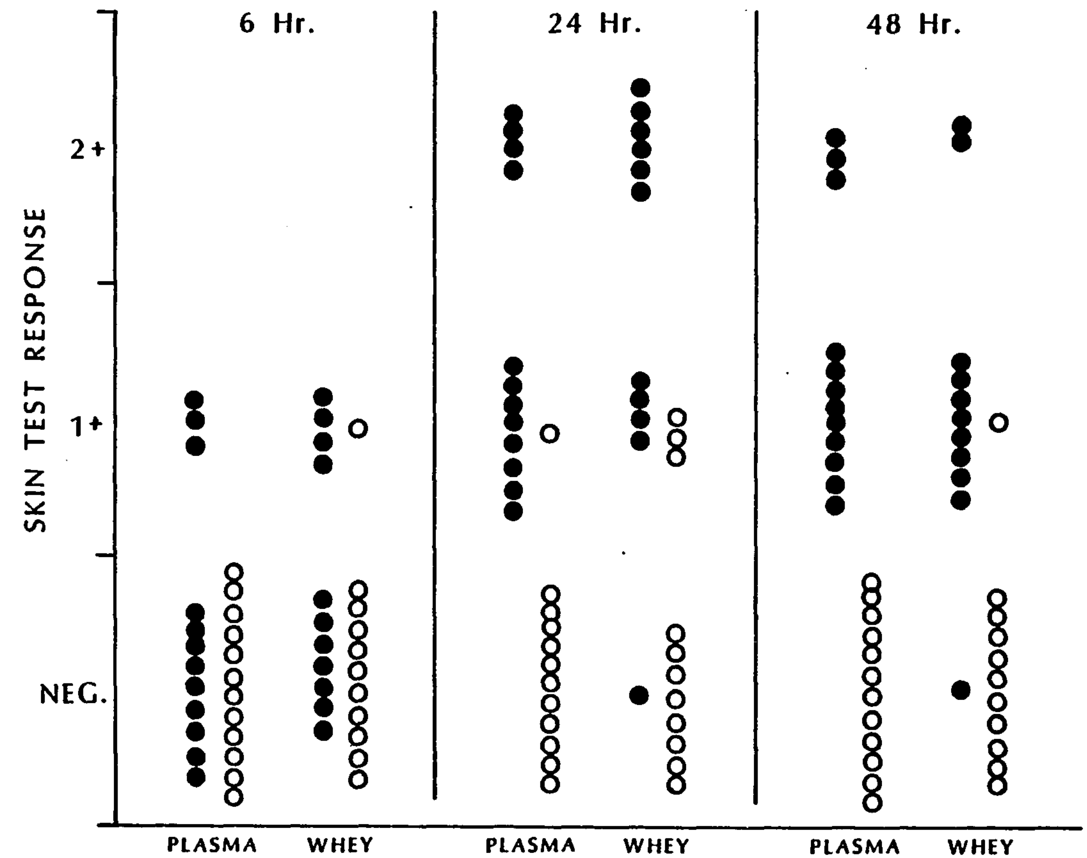

Fig. 2. Comparison of Skin Test Reactivity after Sensitization using Ichorin from Plasma and Whey (Open circles represent PPD antigen and closed circles represent B. abortus antigen)

tested by local injection of the preparation into guinea pigs followed by skin testing with *Brucella abortus* and PPD antigens 24 hours later. The amount of erythematous induration for readings taken at 6, 24, and 48 hours post-skin test injection are shown in Fig. 3. Although some immediate hypersensitivity reactions are seen in both test groups at 6 hours, antigen-specific delayed-type hypersensitivity responses are seen with both of the test groups at 24 and 48 hours post-skin test. No visual differences are seen between the test samples taken before and 48 hours after colostral feeding or between calves fed colostrum from either herd.

Also shown in Fig. 3 are the results of interspecies transfer of DTH using Ichorin isolated from the blood plasma obtained from calves at 48 hours postpartum after colostrum ingestion. The results indicate that *Brucella*-specific passive transfer of DTH occured with these Ichorin samples. Since a newborn calf deprived of colostrum has essentially no gamma globulin, the other protein fractions obtained from the blood plasma of these newborns were tested for biological activity similar to the testing of the transfer factor and Ichorin samples. Although the albumin and alpha 1 protein appeared to possess some reactivity to *Brucella abortus*, the results obtained are insufficient to substantiate that Ichorin-like activity is indeed present in these sample proteins. The data presented by Olson and Drube (68) indicate that biological activity is only seen in the gamma globulin protein fractions and are not seen when albumin, alpha 1 and beta protein was tested.

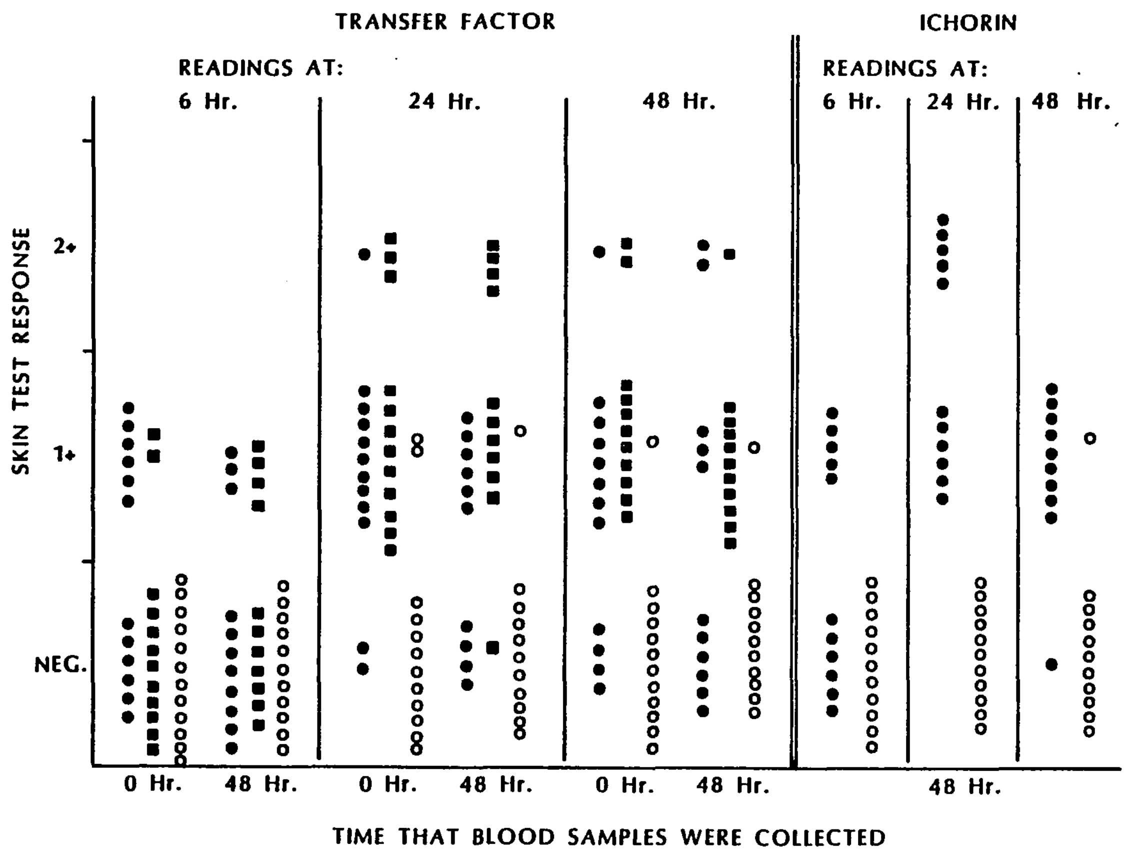

Fig. 3. Comparison of Skin Test Reactivity after Sensitization with TF or Ichorin from Calf Blood (Open circles represents PPD antigen and closed circle and square represents B. abortus antigen at 50 and 5 μg/0.1 ml respectively)

Skin Testing of Colostrum-Fed and Colostrum-deprived Calves

Nine calves, separated from their dams prior to suckling were equally divided into three test groups, as described previously. These calves were obtained from cows previously vaccinated at three and nine months of age with Brucella abortus strain 19 vaccine and which show negative skin test reactions to bovine tuberculin antigen. Group A calves were skin tested within an hour after birth with Brucella abortus and PPD antigens and were deprived of colostrum until after 24 hours postpartum. Groups B and C were fed colostrum every 12 hours with the two initial feedings being colostrum from the calf's mother. Group B calves were skin tested at 48 hours postpartum with the above antigens and Group C calves were skin tested at 72 hours postpartum.

Fig. 4 shows the skin test readings for each group at 24-48 hours after injection of the skin test antigens. At 6 and 12 hours post-injection, all groups showed strong edematous reactions to the Brucella abortus antigens. In this figure, the open circles represent the PPD skin test antigen whereas the closed squares, circles, and triangles represent the Brucella abortus skin test antigen at 50, 100, and 200 μg/0.1 ml respectively. Assays for endotoxin in the skin test antigens prior to use indicated no more than 10 pg/ml of endotoxin in the Brucella abortus antigens and less than 1 pg/ml of endotoxin in the PPD antigen.

At 24-48 hours post-injection, the skin test reactions are reduced from the earlier readings, but no obvious differences can be detected between the three groups. It appears that eventually the

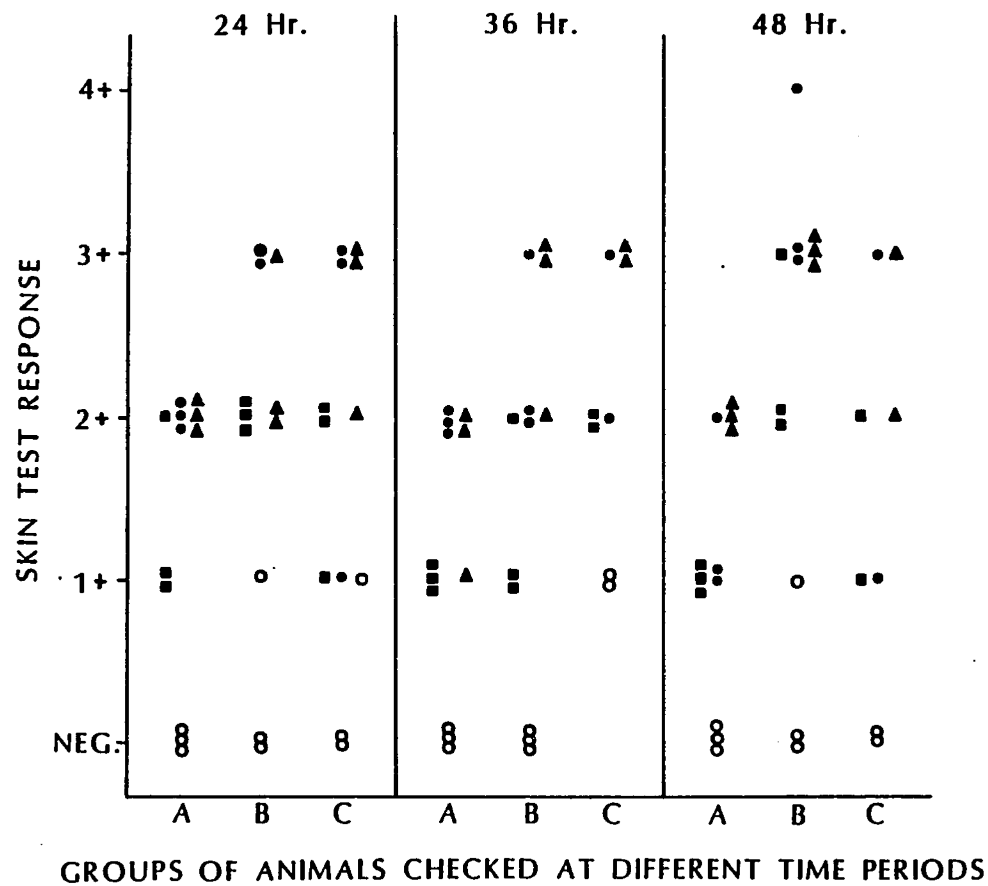

Fig. 4. Skin Test Reactivity in Colostrum-fed and Colostrum-deprived Calves

response to PPD returns to negative but skin test reactivity to the various concentrations of Brucella antigen remain at a 2+ to 4+ activity. Gross observations of the skin test lesions at this time indicated little or no edema. The lesions were indurated and some erythema was noted in the lighter skinned animals.

Serum immunoglobulin levels were recorded for the nine calves at 12 hour intervals throughout this study. Table 11 shows the level of total immunoglobulin present in the calf's serum at birth and at the time of injection of the skin test antigens. These levels did not change significantly during the course of the skin test readings and therefore are not shown here. Although the Group A calves were fed for the first time after the 24 hour reading, no significant increase in their immunoglobulin level had occured by the time of the 48 hour reading, possibly indicating that gut epithelial cell closure to macromolecules had already occured.

Of interest in Table 11 is the fact that although calf 3 of Group A was born with a reasonable amount of total immunoglobulin, this did not seem to affect the calf's skin test reactivity in comparison to the other members of Group A. Although calf 1 of Group B has the lowest level of total immunoglobulin, this calf reacted equal to or better than calves 2 and 3, which had significantly higher levels of immunoglobulin present in their serum at the time of skin testing. These results seem to indicate that the level of humoral immunity acquired by the calf from its mother does not correlate to the acquired

TABLE 11

IMMUNOGLOBULIN LEVELS OF CALVES AT BIRTH AND TIME OF SKIN TESTING

	Calf	Total Immunoglobulin Level mg/ml At birth	At Time of Skin Test	Mother's Colostrum
Group A	1	0.028[a]	0.028	--
	2	0.016	0.016	--
	3	6.508	6.508	--
Group B[b]	1	0.021	25.519	30
	2	0.025	84.580	90
	3	4.345	58.530	65
Group C[b]	1	0.092	16.994	60
	2	0.044	16.989	30
	3	0.002	28.582	55

[a]Mean of duplicate values obtained from serum

[b]Pooled colostrum for remaining feedings had a total immunoglobulin level of 55 mg/ml

skin test responses, but further studies must be done to substantiate these results.

Table 11 also shows that the level of immunoglobulin present in the mothers colostrum correlates to the level of immunoglobulin present in the calves serum after colostrum ingestion (calf 1 of Group C was an exception). Overall, the colostrum-fed calves (Groups B and C) show normal passive transfer of humoral immunity as the result of colostrum ingestion.

Chemical Properties of Transfer Factor and Ichorin Preparations

The protein and RNA content of the transfer factor and Ichorin preparations were evaluated using the Fluram and Orcinol assays. The grand mean for each test group is shown in Table 12 and the complete test sample data is shown in the Appendix. Attempts to correlate the amount of protein to amount of RNA in these samples indicate that a positive correlation may exist in the transfer factor preparations obtained from blood lymphocytes and Ichorin preparations from the 48 hour calf plasma. No correlation is present in the data from the transfer factor preparations obtained from colostral lymphocytes or with Ichorin preparations from colostral whey. The colostral factors gave low, negative coefficients of correlation. Also, no correlation was seen in the data from Ichorin samples obtained from the blood of the parturent mothers. Overall, these results do indicate that both protein and RNA are indeed present in the biologically active transfer factor and Ichorin preparations tested in this study.

TABLE 12

PROTEIN AND RNA CONTENT OF TRANSFER FACTOR AND ICHORIN PREPARATIONS

	Protein (μg/ml)	RNA (μg/ml)
Transfer Factor Preparations		
Colostrum - dam	20.5 ± 15.9	13.5 ± 5.9
Blood - dam	18.0 ± 14.2	38.8 ± 31.5
Blood - Calf 0 Hour	14.7 ± 12.6	26.2 ± 31.9
Blood - Calf 48 Hour	6.2 ± 5.0	18.5 ± 21.8
Ichorin Preparations		
Colostrum - dam	0.58 ± 0.46	6.1 ± 2.1
Blood - dam	0.72 ± 0.47	3.8 ± 2.6
Blood - Calf 48 Hour	0.62 ± 0.81	4.6 ± 2.6

The Limulus Amebocyte Lysate assay for the presence of endotoxin was used to test each of the transfer factor and Ichorin preparations. All of the transfer factor and Ichorin preparations had less than 1 pg/ml of endotoxin activity and the majority gave negative test results. No correlation between the presence of endotoxin and the skin test results was noted.

CHAPTER 4

DISCUSSION

Although researchers have postulated the presence of soluble factors in colostrum which are capable of passive transfer of delayed-type hypersensitivity responses (52,63,74), no attempt to isolate these factors have been reported. The results shown previously indicate that transfer factor activity can be isolated from dialyzable extracts of colostral leukocytes. This colostral transfer factor is orcinol and fluram positive and shows little or no endotoxin activity using Limulus Amebocyte Lysate assay.

In addition, colostral whey appeared to possess an active moiety which also appears to produce interspecies passive transfer of antigen-specific delayed-hypersensitivity responses. This Ichorin, isolated from colostrum, is orcinol and fluram positive but contains much less protein than the blood and colostral transfer factor preparations. The colostral Ichorin has a high 260/280 optical density ratio and has little or no endotoxin (less than 1 pg/ml), using the Limulus Amebocyte Lysate assay.

The immunologic status of colostrum and blood from multiparturent dams which were immunized against Brucella abortus at 3 and 9 months of age were compared. The lymphocyte population in colostrum is less than that of blood samples from the same animal, but a positive correlation may exist between the two. On the other hand, colostral whey has been shown to contain more total gamma globulin than blood serum or plasma.

Ichorin and transfer factor were isolated from the blood and colostrum of dams and shown to passively transfer delayed-type hypersensitivity reactions across a species barrier to guinea pigs. The magnitude of the skin test response was not as great as reported in other studies (68). The reason for this may be twofold: 1) the donors had not been recently immunized as is the normal laboratory procedure and 2) the passive transfer of material was not done intraperitoneally, rather, a smaller amount of material was injected intradermally and later skin tested at the same site. This procedure was initiated to accommodate the larger numbers of samples and antigen dilutions to the restricted number of guinea pigs.

By comparing the dermal reactivity of colostrum-deprived calves to that of colostrum-fed calves indicate that colostrum is a source of passive transfer of skin-test reactivity. However, transfer factor obtained from calves at birth and at 48 hours postpartum were compared and showed no differences in biological or chemical activity. The biological activity of Ichorin isolated from calves at 48 hours postpartum was similar to the calf transfer factor.

This study has posed some interesting questions concerning the immunologic status of the newborn calf. It has shown that colostrum contains factors capable of interspecies transfer of antigen-specific delayed-type hypersensitivity responses and that these factors are present in the blood of colostrum-fed calves. The intraspecies transfer using colostrum may indicate that the colostrum-fed calf acquired antigen-specific responsiveness from whole colostrum ingestion, whereas the colostrum-deprived calf showed no reactivity. However, the point was not resolved due to the extremely high sensitivity of newborn calf skin tissues toward the initial injection of Brucella antigen.

This study and others may serve to show the overall importance of colostrum ingestion to the immune status of the neonatal calf. Further studies should reveal whether the factors described here, as well as other factors present in colostrum, can convey protective immunity to the newborn.

APPENDIX A

WHITE CELL COUNT AND DIFFERENTIAL DATA

FROM QUARTER SAMPLES

TABLE A-1

TOTAL WHITE CELL COUNTS FROM INDIVIDUAL QUARTER SAMPLES

Cow #	Herd #	WVC X $10^3/mm^3$ Left Front	Left Rear	Right Front	Right Rear	$\bar{X}$	S.D.	% C.V.
7193	1	5.50[a]	6.00	3.00	10.00	6.125	2.90	47.3
4351	1	1.95	1.25	3.50	1.50	2.05	1.01	49.2
4915	1	0.80	1.20	1.10	0.50	0.90	0.32	35.1
9588	1	3.70	2.10	1.75	5.50	3.26	1.72	52.6
9433	1	2.25	3.25	3.10	2.25	2.71	0.54	19.8
9200	1	9.15	2.95	2.45	4.75	4.825	3.05	63.2
9848	1	1.90	3.10	1.20	1.85	2.01	0.79	39.4
9349	1	2.50	1.65	1.25	1.05	1.61	0.64	39.9
1020	2	--[b]	6.65	2.95	2.40	4.00	2.31	57.8
713	2	0.90	20.00	0.75	0.65	5.575	9.44	169.3
762	2	1.40	2.05	0.75	0.85	1.26	0.60	47.4
774	2	9.05	13.20	7.25	14.90	11.10	3.55	32.0

[a]Value is average of duplicate testing

[b]Dry quarter

TABLE A-2

PERCENT POLYMORPHONUCLEAR CELLS FROM
INDIVIDUAL QUARTER SAMPLES

Cow #	Herd #	Left Front	Left Rear	Right Front	Right Rear	$\bar{X}$	S.D.	% CV
7193	1[a]	78[b]	79	70	66	73.25	6.29	8.59
4351	1	68	76	64	78	71.50	6.61	9.24
4915	1	76	66	60	70	68.00	6.73	9.90
9588	1	70	72	76	80	74.50	4.43	5.95
9433	1	64	68	68	72	68.00	3.27	4.80
9200	1	80	68	60	68	69.00	8.25	11.95
9848	1	68	52	60	60	60.00	6.53	10.89
9349	1	76	68	56	52	63.00	11.02	17.48
1020	2	--[c]	72	60	72	68.00	6.93	10.19
713	2	52	64	60	52	57.00	6.00	10.53
762	2	56	64	60	68	62.00	5.16	8.33
774	2	56	64	64	48	58.00	7.66	13.21

[a]Herd #1 represents cows from a local commercial dairy and Herd #2 represents cows from a small University dairy.

[b]Mean of duplicate counts

[c]Dry quarter

TABLE A-3

PERCENT MACROPHAGES FROM INDIVIDUAL QUARTER SAMPLES

Cow #	Herd #	Left Front	Left Rear	Right Front	Right Rear	$\bar{X}$	S.D.	% CV
7193	1[a]	8[b]	3	14	4	7.25	4.99	68.8
4351	1	12	8	6	12	9.50	3.00	31.6
4915	1	8	14	8	14	11.00	3.46	31.5
9588	1	10	16	12	10	12.00	2.83	23.6
9433	1	16	24	12	16	14.50	5.80	40.0
9200	1	4	20	20	12	14.00	7.66	54.7
9848	1	16	20	12	16	16.00	3.27	20.4
9349	1	8	20	32	36	24.00	12.65	52.7
1020	2	--[c]	8	20	12	13.30	6.11	45.9
713	2	28	8	16	24	19.00	8.87	46.7
762	2	20	20	20	16	19.00	2.00	10.5
774	2	20	16	16	20	18.00	2.31	12.8

[a]Herd #1 represents cows from a local commercial dairy and Herd #2 represents cows from a smaller University dairy.

[b]Mean of duplicate counts

[c]Dry quarter

TABLE A-4

PERCENT LYMPHOCYTES FROM INDIVIDUAL QUARTER SAMPLES

Cow #	Herd #	Left Front	Left Rear	Right Front	Right Rear	$\bar{X}$	S.D.	% CV
7193	1[a]	14[b]	18	16	30	19.5	7.2	36.9
4351	1	20	16	30	10	19.0	8.4	44.2
4915	1	16	20	32	16	21.0	7.6	36.1
9588	1	20	12	12	10	13.5	4.4	32.8
9433	1	20	8	20	12	15.0	6.0	40.0
9200	1	16	12	20	20	17.0	3.8	22.5
9849	1	16	28	28	24	24.0	5.7	23.6
9349	1	16	12	12	12	13.0	2.0	15.4
1020	2	--[c]	20	20	16	18.7	2.3	12.4
713	2	20	28	24	24	24.0	3.4	14.0
762	2	24	16	20	16	19.0	3.8	20.2
774	2	24	20	20	32	24.0	5.7	23.6

[a]Herd #1 represents cows from a local commercial dairy and Herd #2 represents cows from a small University dairy.

[b]Mean of duplicate counts

[c]Dry quarter

APPENDIX B

COMPLETE DATA ON CHEMICAL PROPERTIES OF ICHORIN AND TF SAMPLES

TABLE B-1

RNA AND PROTEIN CONTENT OF BLOOD AND COLOSTRAL TF AND ICHORIN

	Transfer Factor				Ichorin			
	Colostrum		Blood		Colostrum		Blood	
Dam #	Protein	RNA	Protein	RNA	Protein	RNA	Protein	RNA
7193	16.8[a]	8.8[a]	27.9	33.6	0.03	6.0	0.55	8.0
4351	19.9	21.2	1.3	12.8	0.85	4.0	0.60	2.5
4915	11.7	13.0	18.8	29.6	0.85	7.0	0.00	0.5
9588	ND[b]	ND	10.6	37.8	ND	ND	0.20	4.5
9433	20.7	4.4	26.1	29.4	0.93	4.5	0.28	0.5
9200	7.7	15.0	9.6	43.8	0.45	6.0	0.78	3.0
9848	60.0	13.0	ND	ND	1.10	4.5	0.80	6.0
9349	14.2	19.2	29.5	116.5	0.06	4.0	0.50	7.0
1020	4.6	15.0	49.6	76.0	0.93	8.0	1.60	0.5
713	37.8	4.4	3.7	13.0	0.01	6.0	0.95	5.5
762	9.7	13.0	8.0	17.0	1.10	6.0	1.40	3.0
774	22.4	21.2	13.2	17.0	0.02	11.0	0.93	5.0
$\bar{X}$	20.5	13.5	18.0	38.8	0.58	6.1	0.72	3.8
S.D.	15.9	5.9	14.2	31.5	0.46	2.1	0.47	2.6
% CV	77.6	43.7	78.9	81.2	79.3	34.4	65.3	68.4

[a]Values represent μg/ml

[b]No data

TABLE B-2

RNA AND PROTEIN CONTENT OF TF AND ICHORIN
FROM CALF BLOOD SAMPLES

	Transfer Factor				Ichorin	
	0 Hour		48 Hour		48 Hour	
Calf #	Protein	RNA	Protein	RNA	Protein	RNA
1 (7293)[a]	2.7[b]	10.8[b]	1.2	8.0	0.55	6.0
2 (4351)	6.4	10.8	2.3	3.0	2.95	2.0
3 (4915)	2.9	8.8	ND[c]	ND	ND	ND
4 (9588)	6.9	6.6	4.3	9.5	0.25	6.0
5 (9433)	21.6	17.2	3.4	4.0	0.05	3.0
6 (9200)	18.1	19.2	3.3	8.0	0.05	3.0
7 (9848)	8.2	15.0	6.6	10.5	0.35	5.0
8 (9349)	11.2	33.6	16.4	80.0	0.70	5.5
9 (1020)	26.6	113.5	8.9	28.0	0.58	6.0
10 (713)	47.1	63.9	5.1	13.0	0.80	10.0
11 (762)	11.1	11.0	2.3	17.0	0.33	0.5
12 (774)	13.9	4.5	14.0	23.0	0.23	3.5
$\bar{X}$	14.7	26.2	6.2	18.5	0.62	4.6
S.D.	12.6	31.9	5.0	21.8	0.81	2.6
% CV	85.7	121.8	80.6	117.8	130.6	56.5

[a]Parenthesis indicate Cow #

[b]Values represent μg/ml

LIST OF REFERENCES

1. Al-Sarraf, M., and L.H. Baker. 1979. Transfer factor. Cancer Treat. Rev. 6:209.

2. Bail, O. 1910. Uebertragung der tuberkulinempfindlichkeit. Immunitatsforsch. 4:470.

3. Beer, A.E., and R.E. Billingham. 1975. Immunologic benefits and hazards of milk in maternal perinatal relationship. Ann. Int. Med. 83:865.

4. Benveniste, J. 1978. Immediate Hypersensitivity. In "Immunology," (Bach ed.), pp. 321-322. Wiley Medical Publication, New York.

5. Brandon, M.R., D.L. Watson, and A.K. Lascelles. 1971. The mechanisms of transfer of immunoglobulin into mammary secretion of cows. Aust. J. Exp. Biol. Med. Sci. 49:613.

6. Burger, D., P.H. Klesius, A.A. Vanderbark, R.M. Vetto, and A.I. Swann. 1979. Transfer of keyhole limpet hemocyanin dermal-reactivity to man with bovine transfer factor. Cell. Immunol. 43:192.

7. Bush, J.F., and A.E. Beer. 1979. Analysis of complement receptors on B-lymphocytes in human milk. Am. J. Obstet. Gynecol. 133:708.

8. Butler, J.E. 1971. Review of the bovine immunoglobulins. Symposium: Bovine immune system. J. Dairy Sci. 54:1315.

9. Chase, M.W. 1945. The cellular transfer of cutaneous hypersensitivity to tuberculin. Proc. Soc. Exp. Biol. 59:134.

10. Clover, C.K., and A. Zarkower. 1980. Immunologic responses in colostrum-fed and colostrum-deprived calves. Am. J. Vet. Res. 41:1002.

11. Cole, L.R., and C.B. Favour. 1955. Correlation between plasma protein fractions, antibody titers and the passive transfer of delayed and immediate cutaneous reactivity to tuberculin, PPD, and tuberculopolysaccharides. J. Exp. Med. 101:391.

12. Crago, S.S., S.J. Prince, T.G. Pretlow, J.R. McChee, and J. Mestecky. 1979. Human colostral cells. I. Separation and characterization. Clin. exp. Immunol. 38:585.

13. Crepea, S.B., and R.A. Cooke. 1948. Study on the mechanism of dermatitis venenata in the guinea pig with a demonstration of skin-sensitizing antibody by passive transfer. J. Allergy. 19:353.

14. Diaz-Jouanen, E., and R.C. Williams, Jr. 1974. T and B lymphocytes in human colostrum. Clin. Immunol. Immunopath. 3:248.

15. Dienes, L. 1927. Local hypersensitiveness; transfer of local hypersensitiveness of tuberculous guinea pigs with blood serum. J. Immunol. 14:43.

16. Donne, A. 1844. "Cours de Microscopic." Vol 1 and 2. No. 1 Balliere, Paris.

17. Ehrenkranz, N.J., and B.H. Waksman. 1956. Failure to transfer tuberculin sensitivity passively with plasma fractions containing alpha globulin. J. exp. Med. 104:935.

18. Fahey, J.L., and E.M. McKelvey. 1965. Quantitative determination of serum immunoglobulins in antibody-agar plates. J. Immunol. 94:84.

19. Fahey, J.L., and C. McLaughlin. 1963. Preparation of antisera specific for 6.6 S γ-globulins, β2A-globulins, γ1-macroglobulins, and for Type I and II common γ-globulin determinants. J. Immunol. 91:484.

20. Felix, A.M., V. Toone, S. DeBernardo, and M. Weigele. 1975. Colorimetric amino acid analysis using fluorescamine. In "Peptides: Chemistry, Structure, Biology" Watler and Meinenhofer Publications, New York.

21. Fleenor, W.A., and G.H. Stott. 1980. Hydrometer test for estimation of immunoglobulin concentration in bovine colostrum. J. Dairy Sci. 63:973.

22. Fleenor, W.A., and G.H. Stott. 1981. Single radial immunodiffusion analysis for quantitation of colostral immunoglobulin concentration. J. Dairy Sci. 64:740.

23. Freund, J. 1925. On the role of the reticulo endothelial system in the tuberculin hypersensitiveness. J. Immunol. 11:383.

24. Frick, O.L. 1980. Immediate Hypersensitivity. In "Basic and Clinical Immunology" Lange Medical Publications, Los Altos, Ca.

25. Guidry, A.J., R.E. Pearson, M.J. Paape, and W.F. Williams. 1980. Relationship among leukocyte phagocytosis, milk immunoglobulins, and susceptibility to intramammary infection. Am. J. Vet. Res. 41:997.

26. Hanks, J.H. 1935. Mechanism of tuberculin hypersensitiveness; role of tubercle and of inflammatory reaction of early tuberculous lesion in production of tuberculin hypersensitiveness as determined by inducing "tuberculin type" of hypersensitiveness with egg white or horse serum. J. Immunol. 27:105.

27. Hanson, L.A. 1982. The mammary gland as an immunological organ. Immunol. Today. 3:168.

28. Helmholtz, H.F. 1909. Ueber passive uebertragung der tuberculinueberempfindlichkeit meerschweinchen. Immunitatsforsch. 3:370.

29. Himeno, K., K. Nomoto, A. Kuroiwa, S. Miyazaki, and K. Takeya. 1977. Relation between delayed skin reactivity and macrophage migration inhibition or lymphocyte transformation in tuberculin-type hypersensitivity and Jones-Mote hypersensitivity. Microbiol. Immunol. 21:99.

30. Ho, F.C.S., R.L.C. Wong, and J.W.M. Lawton. 1979. Human colostral and breast milk cells: a light and electron microscopic study. Acta. Paediatr. Scand. 68:389.

31. Ho, P.C., and J.W.M. Lawton. 1978. Human colostral cells: phagocytosis and killing of _E. coli_ and _C. albicans_. J. Ped. 93:910.

32. Hoxthausen, H. 1952. Experiments on passive transfer of eczematous allergy. J. Invest. Derm. 19:293.

33. Jensen, D.L., and R.J. Ekerhartl. 1975. Macrophages in bovine milk. Am. J. Vet. Res. 36:619.

34. Jeter, W.S., R. Kibler, T.C. Soli, and C.A.L. Stephens. 1979. Oral administration of bovine and human dialyzable transfer factor to human volunteers. _In_ "Immune Regulators in Transfer Factor" pp. 451-458. (Ed. by Kahn, Kirkpatrick, & Hill) Academic Press, New York.

35. Jeter, W.S., R. Kibler, and C.A.L. Stephens. 1977. Oral administration of dialyzable transfer factor to tuberculin to human volunteers. J. Reticuloendothel. Soc. 22:46.

36. Jeter, W.S., R.E. Reed, T.C. Soli, and J.D. Cramer. 1977. Transfer factor to Coccidioides Immitis in cattle. From: "Coccidioidomycosis: Current clinical and diagnostic status" pp. 359-363. (Ajillo, A. Ed.) Symposia Specialists, Miami, Fl.

37. Jeter, W.S., M.M. Tremaine, and P.M. Seebolm. 1954. Passive transfer of delayed hypersensitivity to 2,4-dinitrocholobenzene in guinea pigs with leukocyte extracts. Proc. Soc. Exp. Biol. 86:251.

38. Kirkpatrick, C.H., A. Khan, and N.O. Hill. 1979. Meeting Report: Third International Symposium on Transfer Factor. Cell. Immunol. 46:184.

39. Klaus, G.G., A. Bennett, and E.W. Jones. 1969. A quantitative study of the transfer of colostral immunoglobulins to the newborn calf. Immunology. 16:293.

40. Klesius, P.H. 1981. Modulation of cell-mediated responses with dialyzable leukocyte extract containing transfer factor. Adv. Exp. Med. Biol. 137:293.

41. Klesius, P.H., and H.H. Fudenberg. 1977. Bovine transfer factor: In vivo transfer of cell-mediated immunity to cattle with alcohol precipitates. Clin. Immunol. Immunopath. 8:238.

42. Klesius, P.H., T.T. Kramer, D. Burger, and A. Malley. 1975. Passive transfer of coccidian oocyst antigen and diptheria toxoid hypersensitivity in calves across species barriers. Transplant. Proc. 7:449.

43. Klesius, P.H., and F. Kristensen. 1977. Bovine transfer factor: effect on bovine and rabbit coccidiosis. Clin. Immuno. Immunopath. 7:240.

44. Kmetz, M., H.W. Dunne, and R.D. Schultz. 1970. Leukocytes as carriers in the transmission of bovine leukemia: invasion of the digestive tract of the newborn by ingested, cultured, leukocytes. Am. J. Vet. Res. 31:637.

45. LaMotte, C.B., and R.J. Eberhart. 1976. Blood leukocytes, neutrophil phagocytosis, and plasma corticosteroids in colostrum-fed and colostrum-deprived calves. Am. J. Vet. Res. 37:1189.

46. Landsteiner, K., and M.W. Chase. 1942. Experiments on transfer of cutaneous sensitivity to simple compounds. Proc. Soc. Exp. Bio. Med. 49:688.

47. Lascelles, A.K., B.W. Gurner, and R.R.A. Coombs. 1969. Some properties of human colostral cells. Aust. J. exp. Biol. Med. Sci. 47:349.

48. Lawrence, H.S. 1954. Transfer of generalized cutaneous hypersensitivity of the delayed tuberculin type in man by means of the constituents of disrupted leukocytes. J. Clin. Invest. 33:951.

49. Lawrence, H.S. 1955. The transfer in humans of delayed skin sensitivity to streptococcal M substance and to tuberculin with disrupted leukocytes. J. Clin. Invest. 34:219.

50. Lawrence, H.S. 1959. In "Cellular and Humoral Aspects of the Hypersensitivity States," (H.S. Lawrence, Ed.) pp. 279-318, Hoeber-Harper, New York.

51. Lawrence, H.S., and A.M. Pappenheimer, Jr. 1956. Transfer of delayed hypersensitivity to diphtheria toxin in man. J. exp. Med. 104:321.

52. Lawton, J.W.M., K.F. Shortridge, R.L.C. Wong, and M.H. Ng. 1979. Interferon synthesis by human colostral leukocytes. Arch. Dis. Child. 54:127.

53. Lee, C.S., F.B.P. Wooding, and P. Kemp. 1980. Identification, properties, and differential counts of cell populations using electron microscopy of dry cow secretions, colostrum, and milk from normal cows. J. Dairy Res. 47:39.

54. Lesourd, B., R. Moulias, M.R. Marescot, M. Thiomet, J.F. Parson, J.R. Pillet, and J. Hainout. 1979. Guinea pigs as experiment model for testing activity and specificity of human and bovine transfer factor. In "Immune Regulators in Transfer Factor" p. 55. (Ed. by Kahn, Kirkpatrick, and Hill), Academic Press, New York.

55. Little, R.B., and M.L. Orcutt. 1922. The transmission of agglutinins of *Bacillus abortus* from cow to calf in the colostrum. J. exp. Med. 35:161.

56. Lombardo, P.S., D.A. Todhunter, R.W. Scholz, and R.J. Eberhart. 1979. Effect of colostrum ingestion on indices of neutrophil phagocytosis and metabolism in newborn calves. Am. J. Vet. Res. 40:362.

57. Mach, J.P., and J.J. Pahud. 1971. Secretory IgA, a major immunoglobulin in most bovine external secretions. J. Immunol. 106:552.

58. McAlpine, J.G., and L.F. Rettger. 1925. Serological studies on bovine infectious abortion. J. Immunol. 10:881.

59. McDiarmid, A. 1946. The transference of agglutinins for Brucella abortus from cow to calf and their persistance in the calf's blood. Vet. Rec. 58:146.

60. McDonald, J.S., and A.J. Anderson. 1981. Total and differential somatic cell counts in secretions from noninfected bovine mammary glands: the peripartum period. Am. J. Vet. Res. 42:1366.

61. McGuire, T.C., N.E. Pfeiffer, J.M. Weikel, and R.C. Bartsch. 1976. Failure of colostral immunoglobulin transfer in calves dying from infectious disease. J. Am. Vet. Med. Assoc. 169:713.

62. Mejbaum, W. 1939. Uber die bestimmung kleiner pentosemengen, insbesondere in derivaten der adenylsaure. Z. physiol. Chem. 258:117.

63. Mohr, J.A. 1973. The possible induction and/or acquisition of cellular hypersensitivity associated with ingestion of colostrum. J. Ped. 82:1062.

64. Mohr, J.A., R. Leu, and W. Mabry. 1970. Colostral leukocytes. J. Sur. Oncol. 2:163.

65. Moody, E.G., G.H. Wise, D.B. Parrish, and F.W. Atkeson. 1951. Properties of the colostrum of the dairy cow. VI. Creaming and rate of flow. J. Dairy Sci. 34:106.

66. Ogra, S.S., D.I. Weintraub, and P.L. Ogra. 1977. Immunologic aspects of human colostrum and milk. III. Fate and absorption of cellular and soluble components in the gastrointestinal tract of the newborn. J. Immunol. 119:245.

67. Ogra, S.S., D.I. Weintraub, and P.L. Ogra. 1978. Immunologic aspects of human colostrum and milk: interaction with the intestinal immunity of the neonate. Adv. Exp. Med. Biol. 107:95.

68. Olson, G.B., and C.G. Drube. 1981. Passive transfer of delayed-type hypersensitivity with fractions from sera of sensitized animals. Clin. Immunol. Immunopath. 19:19.

69. Orcutt, M.L., and P.E. Howe. 1922. The relationship between the accumulation of globulins and the appearance of agglutinins in the blood of newborn calves. J. exp. Med. 36:291.

70. Parmely, M.J., A.E. Beer, and R.E. Billingham. 1976. In Vitro studies on the T-lymphocyte population of human milk. J. exp. Med. 144:358.

71. Patt, J.A. 1977. Factors affecting the duration of intestional permeability to macromolecules in newborn animals. Biol. Rev. 52:411.

72. Penhale, W.J., and G. Christie. 1969. Quantitative studies on bovine immunoglobulins: I. Adult plasma and colostrum levels. Res. Vet. Sci. 10:493.

73. Penhale, W.J., B.F. Logan, I.E. Selman, E.W. Fisher, and A.D. McEwan. 1973. Observation on the absorption of colostral immunoglobulins by the neonatal calf and their significance in coli-bacillosis. Ann. Rech. Vet. 4:223.

74. Pittard, W.B., and K. Bill. 1979. Immunoregulation by breast milk cells. Cell. Immunol. 42:437.

75. Porter, P. 1972. Immunoglobulins in bovine mammary secretions. Immunology. 23:225.

76. Rothman, U.S. 1966. The involvement of a globulin fraction from serum and lymphoid cells in the passive transfer of contact allergy. Acta. Soc. Med. Upsal. 71:109.

77. Sawyer, M., B.I. Osburn, H.D. Knight, and J.W. Kendrick. 1973. A quantitative serologic assay for diagnosing congenital infections in cattle. Am. J. Vet. Res. 34:1281.

78. Schlesinger, J.J., and H.D. Covelli. 1977. Evidence for transmission of lymphocyte responses to tuberculin by breast-feeding. Lancet. ii:529.

79. Schultz, R.D. 1973. Developmental aspects of the fetal bovine immune response: a review. Cornell Vet. 63:507.

80. Sigma Technical Bulletin. No. 210. 1978. Sigma Chemical Company, St. Louis, Mo.

81. Smith, C.W., and A.S. Goldman. 1968. The cells of human colostrum. I. In vitro studies of morphology and functions. Pediat. Res. 2:103.

82. Smith, J.W., and R.D. Schultz. 1977. Mitogen- and antigen-responsive milk lymphocytes. Cell. Immunol. 29:165.

83. Smith, T., and R.B. Little. 1922. The significance of colostrum to the new-born calf. J. exp. Med. 36:181.

84. Spitler, L.E. 1980. Delayed hypersensitivity skin testing. In "Manual of Clinical Immunology" American Society for Microbiology. Washington, D.C.

85. Stott, G.H., W.A. Fleenor, and W.C. Kleese. 1981. Colostral immunoglobulin concentration in two fractions of first milking postpartum and five additional milkings. J. Dairy Sci. 64:459.

86. Tsuji, S., S. Oshima, M. Oshiro, and T. Izumi. 1964. Studies on the "transfer factor" of tuberculin hypersensitivity in animals. I. Observation of successful passive transfer of tuberculin hypersensitivity with fractions of either disrupted alveolar macrophages or serum of sensitized and challenged rabbits. J. Immunol. 93:838.

87. Wilson, M.R., J.R. Duncan, F. Heistand, and P. Brown. 1972. The influence of preparturient intramammary vaccination on immunoglobulin levels in bovine mammary secretions. Immunology. 23:313.

88. Zinsser, H. 1924. Bacterial allergy and tissue reactions. Proc. Soc. Exp. Biol. Med. 41:159.

89. Zinsser, H., and J.H. Mueller. 1925. On the nature of bacterial allergies. J. exp. Med. 41:159.