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TEMPORAL CHANGES OF SERUM PROTEINS IN THE CALF AFTER COLOSTRUM FEEDING

By
GHAZI SARKIS

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A THESIS

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AMERICAN UNIVERSITY OF BEIRUT

In partial fulfillment of
the requirements for the
degree of

MASTER OF SCIENCE IN
AGRICULTURE

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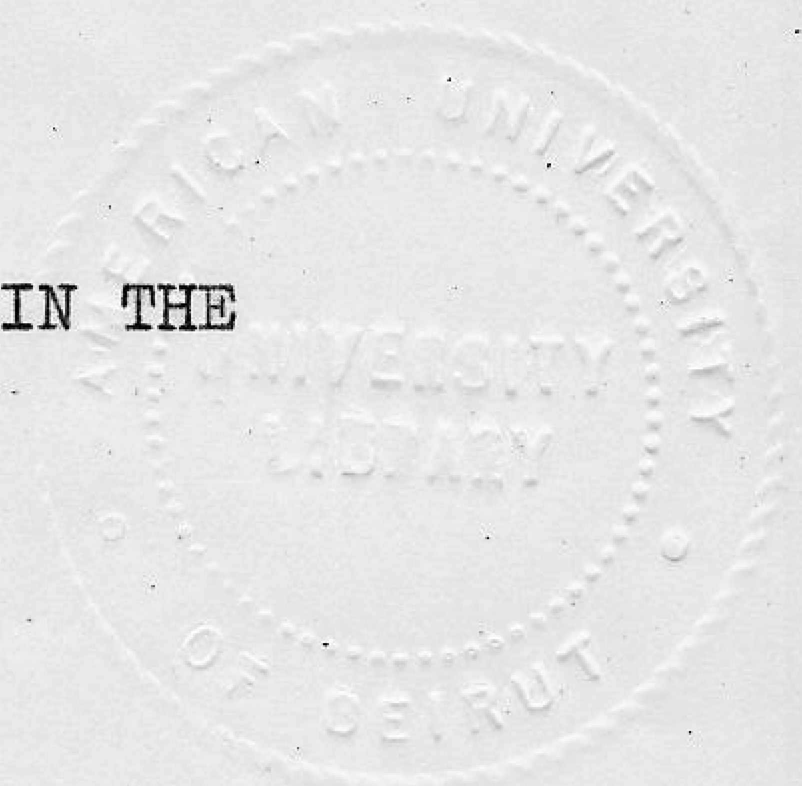

TEMPORAL CHANGES OF SERUM PROTEINS IN THE
CALF AFTER COLOSTRUM FEEDING

By

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NEWBORN CALF SERUM PROTEINS

SARKIS

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AN ABSTRACT OF THE THESIS OF

Ghazi Sarkis for M.S. in Animal Nutrition.

Title: Temporal changes of serum proteins in the calf after colostrum feeding.

Changes occurring in the serum proteins of Holstein-Friesian newborn calves were studied during a 4-hour period following colostrum feeding.

Seven newborn calves were removed from their respective dams immediately after birth in order to prevent suckling, and were given 800 g from a pool of colostrum collected from 8 cows of the AREC dairy herd. Blood samples were drawn from the jugular vein prior to and one hour, 2 hours and 4 hours after colostrum feeding. The serum was separated and analyzed by the biuret reaction for total protein, by paper electrophoresis for quantitative variations in individual serum protein fractions and by immunoelectrophoresis for comparison of protein fractions of adult and newborn calf sera.

Results obtained indicated the occurrence of a variation among newborn calves of the Holstein-Friesian breed in the rate of absorption of colostral immune globulins. The levels of immune globulins in the serum varied between 0.51 and 1.45 g/100 ml 4 hours after colostrum intake. Immunoelectrophoretic analysis indicated that during the first few hours of life absorption of immune fractions is selective, and is restricted to the fast moving beta 2 and gamma 1 immune globulins. Gamma 1 M, although present in colostrum, could not be detected in the newborn calf serum 4 hours post colostrum feeding.

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I. INTRODUCTION

In contrast to human infants who acquire parental immunity transplacentally during fetal life, newborn farm animals acquire it postnatally through the ingestion of colostrum. In addition to transmitting immunity, first milking colostrum is a highly nutritious food, rich in protein (14.0-17.6 g/100 g), in fats (6.7 g/100 g), and in minerals and vitamins.

The importance of various fractions of colostrum (crude fatty fraction, clarified fatty fraction, non-fatty fraction) for the newborn calf has been studied (Aschaffenburg et al., 1949). The non-fatty fraction of colostrum was found to promote best gains in calves and prevent scouring. As little as 80 ml of this non-fatty fraction of colostrum have been found to give adequate protection against scouring and other diseases for the newborn calf. However, calves deprived completely of this aqueous phase of colostrum had a reduced rate of survival.

While only 1-1.5 percent of the milk proteins are immune globulins, 50-60 percent of the colostrum proteins belong to that cetegory (Smith, 1948). These globulins are transferred from the dam's plasma into colostrum during the last period of pregnancy. They are absorbed

from the intestinal lumen during the first 36 hours following birth, and seem to protect the newborn against infectious agents prevalent in his environment.

Calves deprived of colostrum have reduced chances of survival because of higher incidence of infections, particularly colibacillosis. However, the same infections cause mortality in some colostrum-fed calves. It is not yet known whether this apparent inconsistency is due to variations in the protective properties of colostrum itself, to changes in the microbial serotypes involved, or to differences among calves in the absorptive capacity of their intestinal epithelium.

Recent studies on colostrum proteins showed that the immune globulins are normal blood proteins which are secreted into the milk from the blood stream without degradation and resynthesis (Larson, 1958). However biophysical and immunological studies indicate selective transport of proteins within the mammary gland from maternal plasma to colostrum (Murphy *et al*., 1964). The udder has been found to have selective preference for the electrophoretically fastest immune globulins thus resulting in a higher concentration of these fractions in colostrum. Pierce and Feinstein (1965) reported no selective absorption for immunoglobulins from the intestine of the newborn calf.

There is limited information about changes in the

serum proteins of the calf during the first few hours after suckling colostrum. Furthermore, the theory of selective absorption of protein fractions from the intestine is still debated.

This investigation was initiated to determine whether individual variations exist in the rate of absorption of immune globulins among newborn calves of the Holstein-Friesian breed, and whether the absorption of colostral immune globulins from the gut is an indiscriminate or selective process.

II. REVIEW OF LITERATURE

Transmission of Antibodies via Colostrum

Early in the twentieth century the importance of colostrum as a source of antibodies to the newborn animal was demonstrated (Famulener, 1912). Immunizing pregnant goats against sheep red blood cells and comparing the relative amount of specific hemolysine found in the colostrum and the serum of the kid respectively, Famulener (1912) established that the kid's serum contained no sheep red blood cell hemolysine until after the first colostrum meal. Furthermore, he postulated that the antibodies appearing in the newborn's serum after suckling are derived from the dam and that colostrum plays a greater role in the transmission of passive immunity than transplacental transfer.

Little and Orcutt (1921) studied the transmission of B. abortus specific agglutinins from dam to calf via colostrum, and found a high concentration of agglutinins in the blood and colostrum of infected dams, but none in the blood of the newborn calf which had not received colostrum.

Higher concentrations of antibodies in the cow's colostrum than in the cow's blood were also reported to

occur at the time of parturition or immediately thereafter. These findings supported Famulener's findings (1912) which prescribed a minor role for the placenta in intra-uterine transfer of antibodies for congenital immunity.

Smith and Little (1922), Aschaffenburg *et al.* (1949b), and Fey *et al.* (1963) observed that calves deprived of colostrum had reduced chances of survival. It was concluded that colostrum-deprived newborn calves lacked some factor whose absence permitted the invasion of the body by microorganisms. Howe (1921a), fractionating serum proteins with increasing concentrations of anhydrous sodium sulfate, established that in addition to euglobulin the blood serum contained pseudoglobulin I and pseudoglobulin II fractions which precipitated completely at 17.5 and 21.5 percent sodium sulfate concentrations, respectively. The author (Howe, 1921b) further demonstrated that, prior to colostrum ingestion, the plasma of the newborn calf contained no euglobulin and pseudoglobulin I fractions. These proteins however, appeared in the calf's serum following the feeding of colostrum. Aschaffenburg *et al.* (1949a) confirmed the above findings of Howe (1921b).

Orcutt and Howe (1922) demonstrated the role of euglobulin and pseudoglobulin I in the transfer of passive immunity. These authors thought that the appearance of *B. abortus* agglutinins in the blood stream of the young calf after colostrum intake ought to be correlated with the appearance of euglobulin and pseudoglobulin I fractions.

Experimenting on 2 calves born to dams with a definite _B. abortus_ infection, they showed that the removal of the above-mentioned proteins from colostrum, caused the simultaneous disappearance of _B. abortus_ antibodies. It was concluded that immune bodies or antibodies are carried by, or constitute an integral part of the euglobulin and pseudoglobulin I molecules. These findings were later confirmed by several workers (Howe, 1924; Mason _et al._, 1930; Hansen and Phillips, 1947; Graves, 1963; Coggins, 1964).

Jameson _et al._ (1942) reported that the euglobulin and pseudoglobulin I fractions of the calf serum behaved similarly to the gamma globulins of adult bovine serum when studied electrophoretically. Though absent from the calf blood at birth, the gamma globulin fraction increased markedly 18 hours after feeding, and reached a maximum concentration 3 days after birth. Simultaneously, a decrease in the alpha globulin and albumin fractions was noted during suckling.

In order to verify the chemical nature of immune globulins of colostrum and those appearing in the blood of the calf after suckling, Smith (1946b, 1948) fractionated colostrum and isolated pseudoglobulin I and euglobulin fractions, both of which have immune activity. Moreover, the author separated electrophoretically the calf's serum immune proteins into gamma and T fractions. After comparing

the immune proteins from the 2 different sources, it was concluded that the fractions were not identical. The results obtained implied that the immune globulins of colostrum and those in calf serum have inherent basic differences, or that they are identical, but while being absorbed, the colostral immune proteins undergo chemical or physical changes affecting their chemical and electrical properties.

The contentions suggested by Smith (1948) were later disproved through the work of Blakemore and Garner (1956), and Larson and Kendall (1957), who demonstrated with the use of tracers that the immune globulins of colostrum are normal blood proteins which migrate intact into milk. Pierce (1955) found no significant difference between calf and cow sera proteins in respect to electrophoretic mobility. Using electrophoretic and ultracentrifugation methods, Johnson and Pierce (1959) presented evidence of passive absorption of immune lactoglobulins of colostrum by the newborn calf, and concluded that colostrum immune proteins and the newborn calf immune serum proteins, post-suckling, are identical. Subsequent work by Murphy _et al_. (1964), and Pierce and Feinstein (1965) revealed some differences in the concentrations of the serum protein fractions of the dam and the calf. Murphy _et al_. (1964) reported that the newborn serum, post-feeding, does not contain all the protein fractions identified in the mature

bovine serum. Moreover, it was reported that complete discrimination in the udder against the secretion of slow moving 7_s gamma globulins in favor of a fast moving component occurs. These findings were later confirmed by Pierce and Feinstein (1965) who reported that the gamma globulins found in colostrum are solely of the fast moving 7_s class. Furthermore, the authors reported that among the protein fractions of gamma mobility, only the fast moving 7_s gamma globulin is found in the serum of the newborn calf post-feeding colostrum.

Period during Which Absorption Takes Place

Comline et al. (1951) observed rapid absorption of globulins when introduced into the duodenum of the newborn calf. The presence of these fractions was detected in the serum within 60-120 minutes following introduction.

Little and Orcutt (1921) reported that antibodies are absorbed from the digestive tract fairly rapidly, appear in the circulating blood 1-3 hours post-feeding, and reach a maximum concentration in 5 hours. Mason et al. (1930) detected tetanus antitoxins in the blood of lambs within 30 minutes from the time they were infused in their gut. Balfour and Comline (1962) using radiosotopes found that radio-activity appeared in the lymph 80-120 minutes post-feeding colostrum, and reached a maximum in about 200

minutes.

Famulener (1912) noted that there was no absorption of globulins in calves older than 3 days. Hansen and Phillips (1947) confirmed this early observation and reported no measurable increase in the gamma globulin fraction of the newborn calf when colostrum was fed 24 hours past-calving. Moreover, when colostrum was withheld from newborn calves, 8 weeks were required for the protein fractions to reach their normal levels. Smith and Erwin (1959) noted that while immune proteins were absorbed by 6 and 18 hour old calves, they did not appear in the sera of older calves. Kaeckenbeeck *et al.* (1961) and Kaeckenbeeck and Shoenaers (1964) established that absorption of colostral antibodies by the newborn remained at its maximum for only 12 hours, then fell rapidly, and completely stopped 36 hours after birth.

Smith and Erwin (1959) introduced colostrum directly into the duodenum after ligating it 3 inches posterior to the pyloric sphincter and posterior to the point where colostrum was introduced. Their results indicated that gastric juice had no role in inhibiting immune protein absorption in calves 20 hours old. Balfour and Comline (1962) reported that a colostrum whey-protein-fraction of probably low molecular weight plays a major role in absorption of globulins. When labelled globulins devoid of this fraction were administered in a solution of Na, K,

Mg and Ca chlorides, very little absorption was detected. However, when inorganic phosphate and glucose-6-phosphate were added, the globulins were absorbed rapidly. The question, as to why immune globulin absorption by the intestinal lumen of the newborn calf is only effective during the first 36 hours of the calf's life, remains to be answered.

Breed and Individual Calf Variations in Globulin Absorption

The increase in the newborn's serum globulin content following colostrum intake entails increase in the total serum protein due mainly to the immune fraction transfer. Orcutt and Howe (1922) studied changes in the B. abortus agglutinin titre of the blood of calves after suckling. Individual variations were observed, since in one calf the immune protein level was 0.83 g/100 ml of serum, 3 hours and 50 minutes after feeding, while in another it was 1.98 g/100 ml, 4 hours post-feeding. Variations were also reported in respect to total protein content since, prior to colostrum feeding, one calf had 3.39 g/100 ml while another had a value of 4.39. Following colostrum ingestion the difference in total protein content between the 2 calves was maintained. From the data presented above it is evident that variations occur in the rate of absorption of immune colostrum globulins among individual newborn calves.

Marked variations were observed by Sarwar _et al._ (1964) between colostral antibodies of cows in the same herd and those in different herds. Variations in resistance to disease have also been noted to exist among breeds and herds. Aschaffenburg _et al._ (1949a) reported that in colostrum-deprived calves, Shorthorns were less resistant to infection than Ayrshires or Guernseys. Even when colostrum was fed and the performance of these breeds recorded, there was some indication that newborns of the Shorthorn breed were slightly more susceptible to scours than Ayrshires, and on the whole had a poorer performance. This poor performance could not be attributed to a difference in quality of colostrum, since both breeds were given colostrum from the same pool. It seems more likely that the Shorthorn breed has inherent characteristics that render it more prone to scours and possibly to other diseases than the Ayrshire.

III. MATERIALS AND METHODS

Colostrum

First-milking colostrum was collected from 8 parturient cows belonging to the dairy herd at the Agricultural Research and Education Center (AREC) of the American University of Beirut. Each cow's yield of colostrum was stored in a glass container at $-14^{o}C$. When enough colostrum was collected, all samples were thawed, pooled, and the pool divided in 800 g amounts which were stored in individual plastic containers in the freezer until used.

Experimental Animals

Two female and 5 male calves born to Holstein-Friesian cows at AREC were included in this study. Immediately following calving, the calves were separated from their dams, in order to prevent suckling, and were housed in separate pens. Within 60 minutes from birth each experimental calf was fed 800 g of the pooled colostrum from a sterilized bottle with a nipple.

Blood Serum Samples

Blood samples (8 ml) were collected from the jugular

vein of each calf prior to feeding colostrum and at 1, 2 and 4 hours after feeding. Blood was collected in 10 ml sterilized screw-capped tubes, wetted with sterile physiological saline solution immediately prior to use. The blood samples were held for 3 hours in a water bath at 37°C to hasten clot retraction and serum separation. The serum was separated from the clot and centrifuged for 20 minutes at 2000 revolutions per minute, in a clinical centrifuge, to remove all cells. After centrifugation the serum was carefully transferred into small size screw-capped tubes and immediately placed in the freezer. The serum was kept frozen until used for the various tests.

Colostrum whey was obtained by precipitating the casein of a 500 ml pooled colostrum sample with commercial rennet at 39°C. After 45 minutes in the water bath, the whey was separated by centrifugation for 20 minutes, at 2000 revolutions per minute, in an International Centrifuge (I.E.C.).

Protein determinations. Protein in serum and colostrum whey was determined using the biuret method of Wolfson et al. (1948).

The biuret reagent consisted of 1.5 g copper sulfate and 6.0 g sodium potassium tartrate dissolved in 500 ml of distilled water. Three hundred ml of a carbonate-free 10 percent sodium hydroxide solution were added, and the

volume made up to one liter with distilled water. The reagent was stored in a polyethylene bottle in the refrigerator.

Sodium sulfate solution (27.2%) was prepared by dissolving 272 g of anhydrous sodium sulfate in 500 ml of warm distilled water. The volume was made up to one liter, and the solution stored in a polyethylene bottle in the incubator at 37°C.

All samples were analyzed for total protein in duplicates in the following manner: Two-tenth of a ml of the sample was pipetted into a large test tube containing 4 ml of the sodium sulfate solution. The contents of the tube were mixed thoroughly. One ml of this mixture was pipetted and added to a test tube containing 2.5 ml of the biuret reagent. After mixing by inversion 3 times, the tubes were placed for 10 minutes in an incubator at 37°C, after which the optical density was determined at 540 mμ in a Beckman model B spectrophotometer. Protein concentrations corresponding to optical densities were read directly from a standard curve.

The standard curve for the biuret protein determination was prepared as follows: Bovine serum albumin, (Sigma Chemical Company, St. Louis 18, Missouri, U.S.A.) was poured in a small beaker and placed under vacuum in a dessicator containing anhydrous calcium chloride. After 48 hours, 0.3688 g of albumin was weighed, dissolved in

distilled water, and made to a final volume of 5 ml. The resulting solution had a protein concentration of 7.376 g/100 ml. Aliquots of 0.40, 0.50, 0.80 and 1 ml of the albumin standard solution were transferred to test tubes, and the volumes adjusted to 1 ml with distilled water. The protein concentrations thus obtained were as follows: 2.950, 3.688, 5.900 and 7.376 g/100 ml. The standard solutions were developed in a similar manner to the unknown, and a standard curve was drawn by plotting optical density values against protein concentrations on regular graph paper.

Electrophoretic procedure. All electrophoretic separations were conducted in an LKB 6800 A apparatus (LKB-Produkter AB, Stockholm, Sweden).

Paper electrophoresis was done according to a procedure described by Aronsson and Gronwall (1958). The high resolution buffer described by the same authors was used and consisted of 60.5 g of trishydroxymethyl-amino-methane (TRIS), 6.0 g of ethylene diaminetetraacetic acid (EDTA), and 4.6 g of boric acid. These ingredients were dissolved in distilled water and the final volume made to 1.0 liter. The resulting solution had a pH of 8.9.

The Amido black stain was prepared by dissolving 1.7 g of Amido Black 10B (E. Merk AG. Darmastadt, Germany) in 240 ml of a solvent obtained by mixing 940 ml of methanol, 210 ml of glacial acetic acid and 940 ml of distilled water. The same solvent was used for rinsing the

stained strips.

The electrode chambers were filled with 675 ml of the buffer solution and hydrostatic equilibrium between the 2 chambers was obtained by means of a glass siphon. Four "Selecta" type paper strips 40 x 410 mm, number 2043 (Carl Schleicher and Schull, Dassel, Western Germany) were used in each run. They were soaked in the buffer, left to drain in a vertical position, placed in the electrophoresis cassette and allowed to equilibrate with the buffer solution for half an hour. Eight microliters, measured with a micropipette, were placed on the applicator and applied to the paper strips through the slot nearest to the cathode. The current was adjusted to 0.25 m A/mm of paper width (4 m A, total amperage), and maintained for 16 hours. Following this period, the electricity was turned off and the strips transferred immediately to an oven preheated to 120°C where they were kept for 30 minutes to dry. Each run's strips were stained twice in 80 ml of Amido Black solution. They were then washed 7 consecutive times with the rinsing solution, dried in a preheated oven for 15 minutes and scanned in a Spinco model R Analytrol (Beckman Instruments, Inc., Fullerton, California), using filter number 600.

<u>Immunoelectrophoresis</u> was done according to the procedure described by Scheidegger (1955).

A barbital buffer solution slightly modified from

the one described by Crowle (1961), and having a pH 8.6 was prepared. It consists of 7.43 g of sodium barbiturate dissolved in 500 ml of distilled water. To this solution 3.3 ml of a 10 percent stock solution of sodium azide were added and the volume made up to one liter with distilled water. This solution was expanded with an equal volume of distilled water prior to use in the immunoelectrophoretic analysis.

A one percent agar gel was prepared by adding 50 ml of the above buffer solution to a 100 ml beaker. To a similar beaker one gram of Ionagar No. 2 (Oxoid. The oxoid division of OXO Ltd., London, E.C. 4) and 50 ml of distilled water were added. The contents of both beakers were heated in a pressure cooker until the agar was properly dissolved, then mixed, divided in 10 ml portions and filled into sterile screw-capped tubes.

Rabbit antiserum to bovine whole serum was raised locally in rabbits. It was tested for suitability against a commercial imported standard antiserum (Hyland Laboratories, Los Angeles, California) and was found to be superior.

A thiazine red staining solution was prepared by dissolving one gram of thiazine red in one liter of a one percent acetic acid solution, then adding 20 ml of trichloracetic acid.

A 70 percent ethanol solution containing one percent acetic acid was used to differentiate the stained slides.

Precoated slides were placed in series of 3 in the special plastic slide racks, and 10 ml of melted agar media was poured over each series. The quantity was found sufficient to cover the slides and fill both ends of the plastic holder. When the agar had properly set, a one mm trough width with 4.1 mm distance between trough and well pattern was obtained using gel punch type 6808 A (LKB-Produkter, Stockholm, Sweden). After filling each of the apparatus chambers with 675 ml of the expanded buffer solution, the serum samples were filled in the antigen wells and electrophoretically separated at a current adjusted to 0.25 mA per set of 3 slides. After 120 minutes of operation, the antiserum trenches were cut out and filled with rabbit antibovine serum. When immuno-precipitation was complete 36 hours later, the slides were removed and soaked for 48 hours in one percent sodium chloride solution. They were then transferred to a distilled water bath and kept there for 10 minutes. At the end of this period they were removed and dried at 37°C in an incubator. Staining was performed by dipping the slides for one hour in the thiazine red solution after which they were differentiated in the rinsing solution until the background became clear.

IV. RESULTS AND DISCUSSION

Total Serum Protein

The study of blood serum protein changes in the young calf, early after colostrum feeding, is of interest mainly because it indicates the rate at which immune globulins are being absorbed from the intestinal tract of the newborn.

Results for total protein in the serum samples of the experimental animals are presented in Table 1. The figures indicate that there is an increase in the total serum protein of the newborn calf during the first 4 hours following colostrum feeding. It was found that prior to colostrum intake the 7 Holstein-Friesian newborn calves had an average of 4.20 g/100 ml of protein in their blood serum, the range being 3.56-4.69 g/100 ml. Most of the increase took place at the fourth hour after colostrum feeding when the serum protein level (of the newborn) reached an average of 5.13 g/100 ml, showing a mean rise of 0.93 g/100 ml. Analysis of variance done according to Snedecor (1956, pp. 237-250) and presented in Table 2 showed that while the increase of the fourth hour was significant (at the 5 percent level) it was not so at the first hour (mean increase 0.10 g/100 ml), nor at the

Table 1. Total serum proteins in g/100 ml prior to and following colostrum intake.

Calf No.	Prior	1 hr	2 hr	4 hr	Total increase
153	4.30	4.18	4.18	4.80	0.50
155	3.56	4.30	4.18	5.42	1.86
306	4.18	4.30	4.68	5.42	1.24
308	3.94	4.18	4.42	5.04	1.10
310	4.69	4.56	4.92	5.04	0.35
312	4.30	4.30	4.30	5.04	0.74
313	4.42	4.30	4.42	5.16	0.74
Average	4.20 ± 0.12*	4.30 ± 0.05	4.44 ± 0.10	5.13 ± 0.08	0.93 ± 0.48

* Standard error.

second hour (mean increase 0.24 g/100 ml). One calf only had a serum protein content less than 5 g/100 ml 4 hours after colostrum intake, the other 6 had values ranging between 5.04-5.42 g/100 ml. The analysis of variance (Table 2) showed no significant difference in the blood serum protein between the individual calves. The values in Table 1 fall within the range reported by Howe (1921b), and Orcutt and Howe (1922). Unfortunately, these authors have used one and 2 calves respectively, of unspecified breeds, and no valid comparison between breeds or individuals can be drawn from their data.

Table 3 shows the total protein values of a mature cow serum used for reference, and of the whey of the pooled colostrum fed to the experimental calves. The values, 7.14 g/100 ml for the cow serum and 14.75 g/100 ml for the whey, are close to those reported by Smith (1948).

Paper Electrophoresis

Figure 1 shows the analytrol recording of the electrophoretic pattern obtained with the colostrum whey. The high peak represents the immune globulins, (83.45 percent of the total whey proteins), in which both the gamma and the beta fractions merge. The alpha globulins were virtually non existant whereas the albumin represented 11.87 percent of the total proteins.

Table 2. Analysis of variance of total serum protein in g/100 ml serum.

Source of variation	D.F.	M.S.	F
Individuals	6	0.11	1.4
Time of colostrum feeding	3	1.11	13.9*
Error	18	0.08	

* $P < .05$

Table 3. Results of the biuret and paper electrophoresis tests on cow serum and colostrum whey in g/100 ml.

	Total protein	Globulins Gamma	Beta	Alpha	Albumin
Cow serum	7.14	2.05	1.19	1.07	2.83
Colostrum whey	14.75	- 12.31 -		0.49	1.94

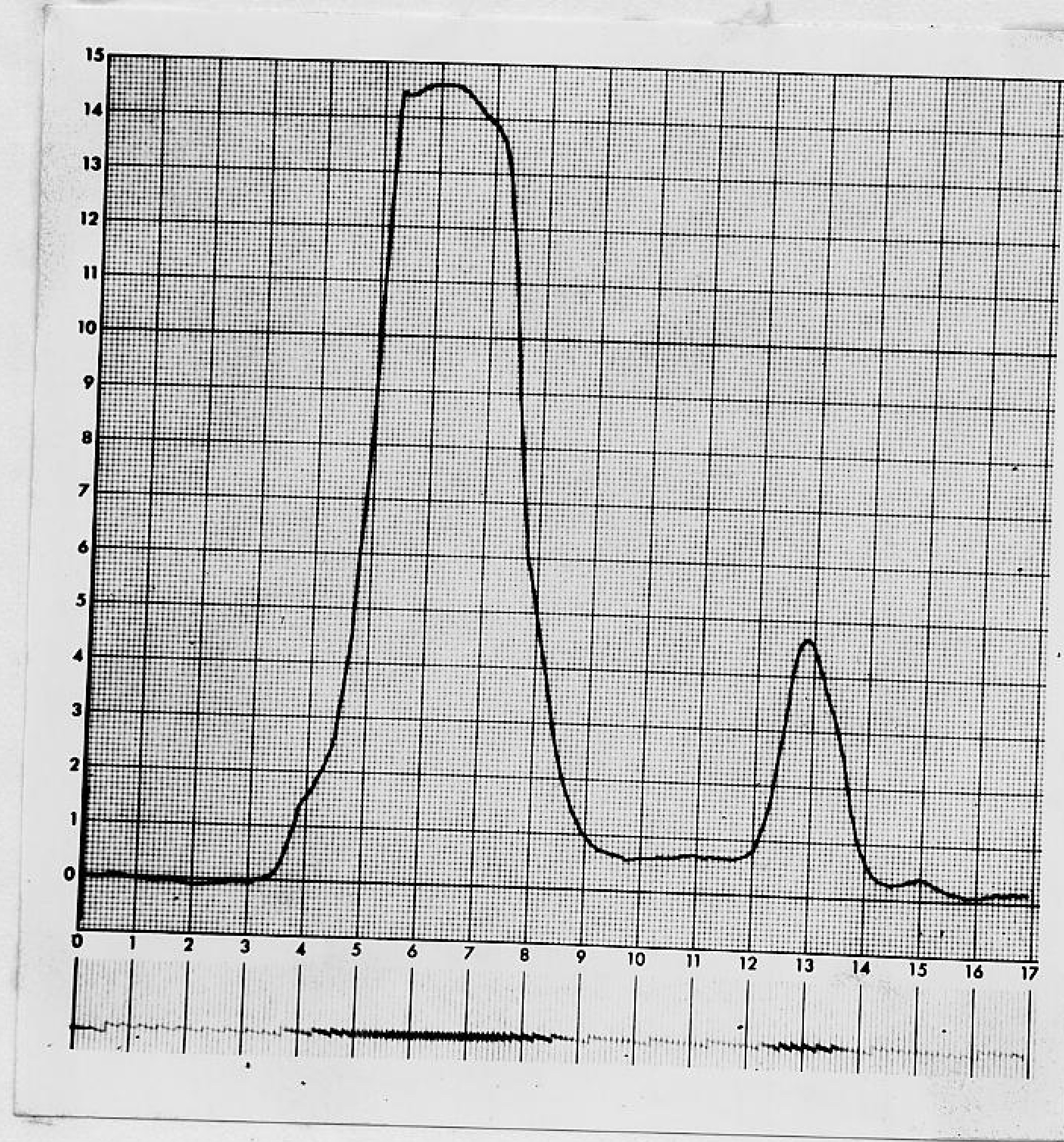

Figure 1. Distribution of colostrum whey proteins in paper electrophoresis.

Figure 2 shows the electrophoretic profile of a mature cow serum in mid lactation. Figures 3, 4, 5, 6, show the patterns obtained with the serum of a typical experimental calf (calf No. 308) prior to, and at one, 2 and 4 hours post colostrum feeding. In Figure 2, 7 peaks corresponding to 7 electrophoretically distinct proteins can be seen. Serum of the newborn calf right after delivery (Figure 3) showed only 3 distinct peaks corresponding to the alpha, beta, and albumin fractions. There was however a minor peak corresponding to the gamma globulins. At one, and 2 hours after colostrum feeding (Figures 4 and 5), no significant changes in the curve occurred apart from a slight increase in the area corresponding to the gamma fraction. However, at the fourth hour (Figure 6), the latter area increased appreciably giving a distinct 4 peak pattern and indicating that, sometime between the second and fourth hour, a surge of gamma globulin has occurred. The serum of the newborn, even at the fourth hour post colostrum feeding, is more homogenous and is not as well differentiated as the serum of a mature cow, since no distinct peaks for the alpha 1, alpha 2, beta 1 and beta 2 fractions can be observed.

From the analytrol graphic charts the areas under the curve corresponding to different fractions were calculated and the relative amount of each fraction computed.

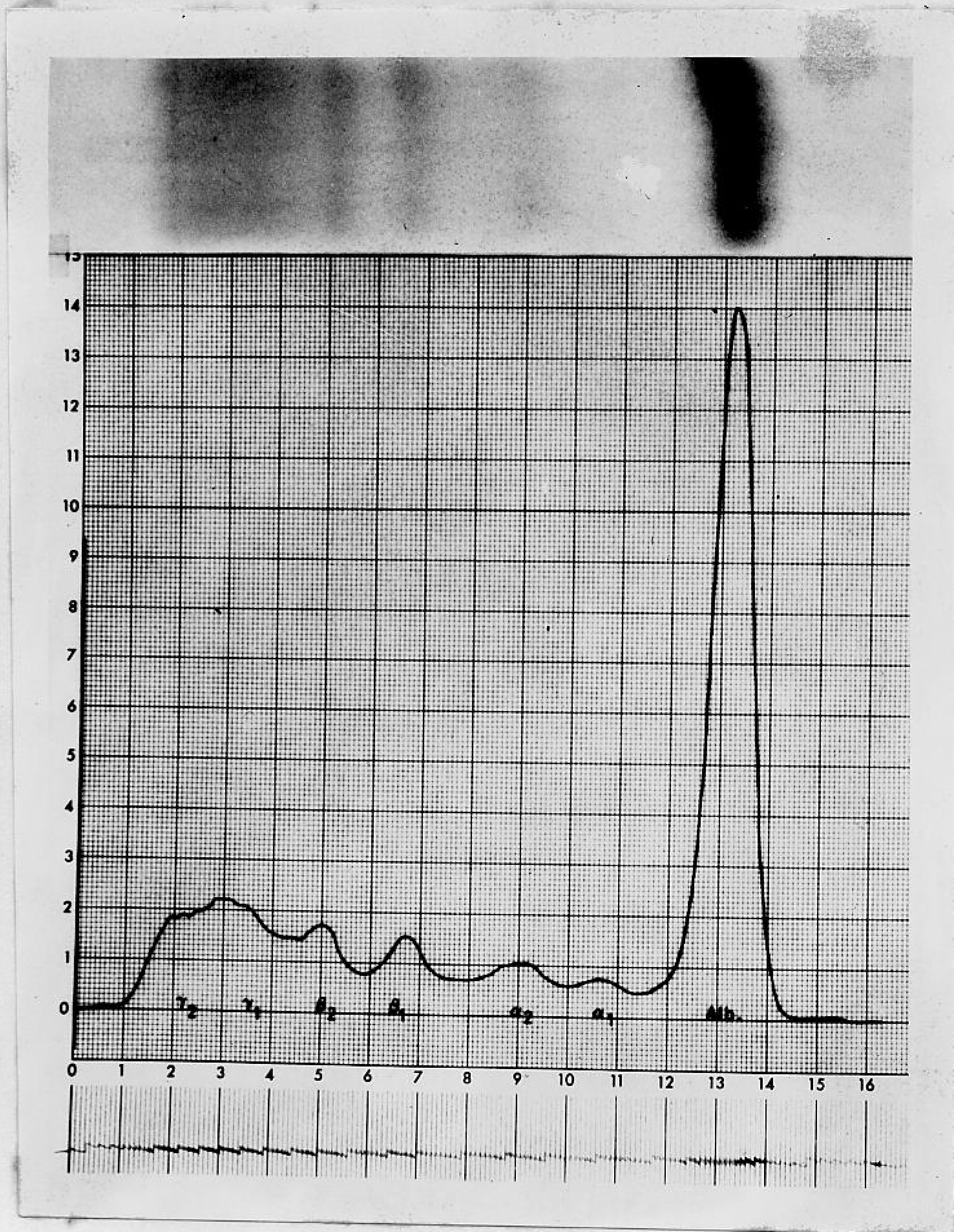

Figure 2. Paper electrophoresis pattern of mature cow serum and its analytrol profile.

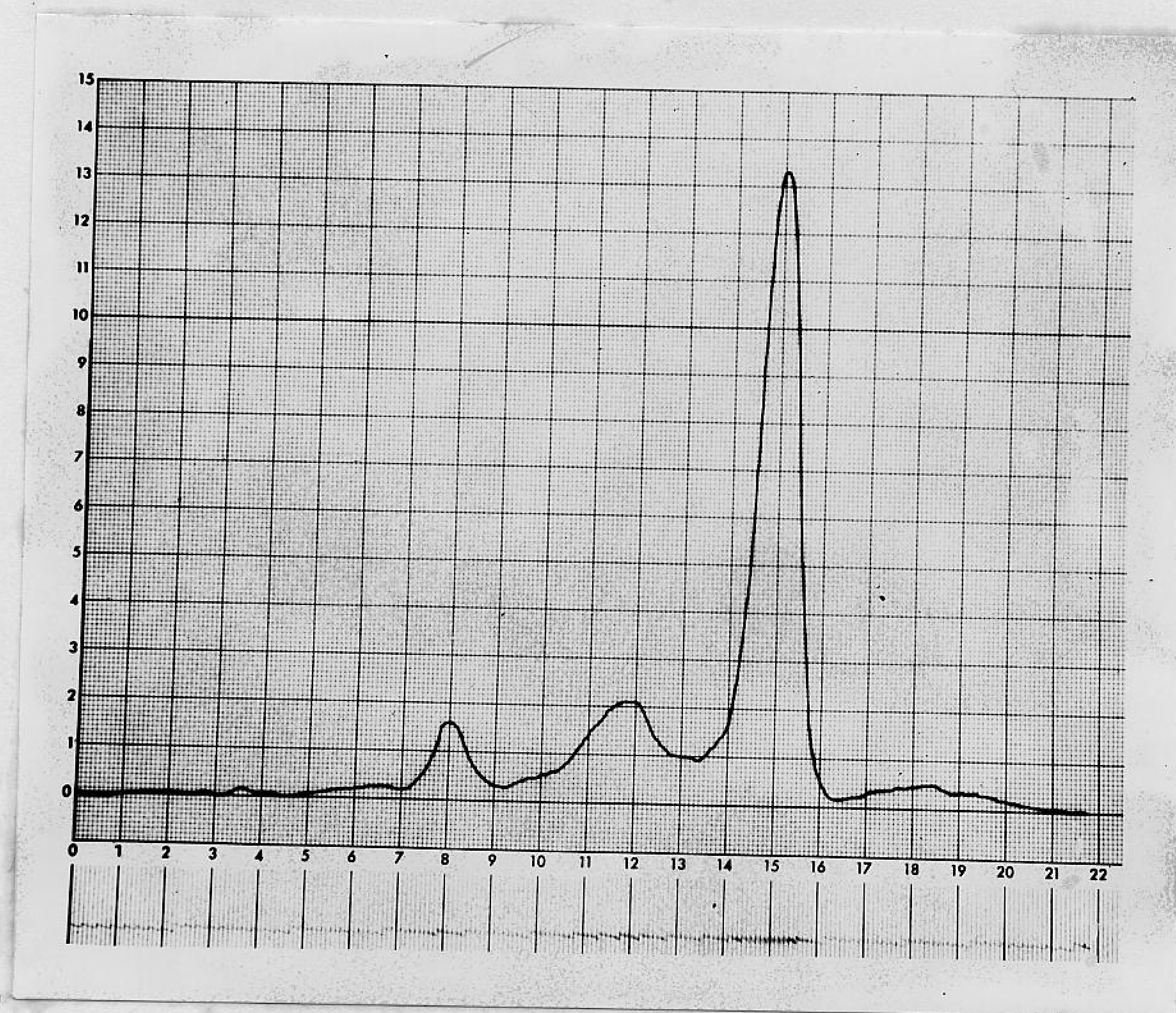

Figure 3. Paper electrophoresis of calf (No. 308) serum before colostrum feeding.

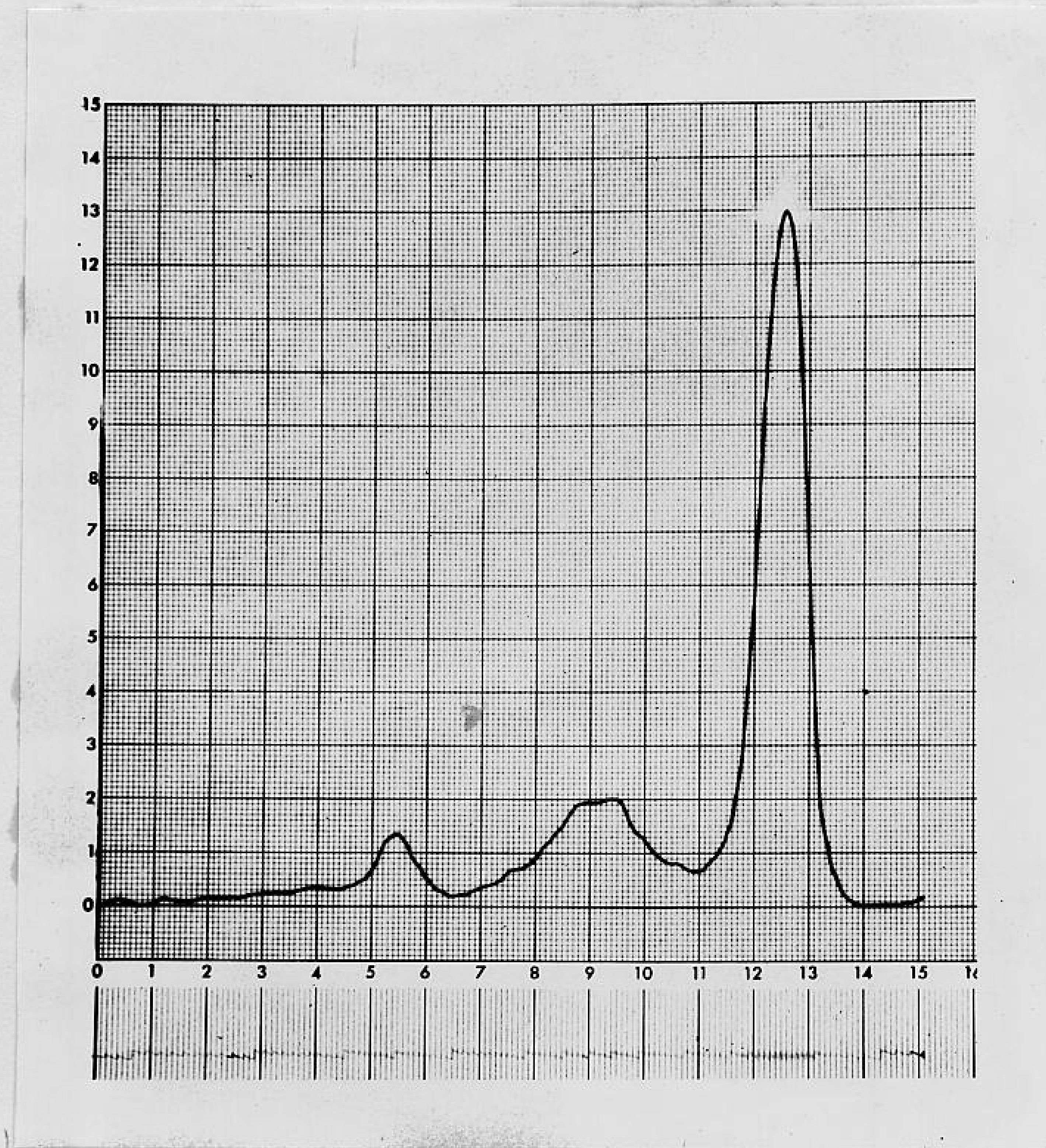

Figure 4. Paper electrophoresis of calf (No. 308) serum one hour after colostrum feeding.

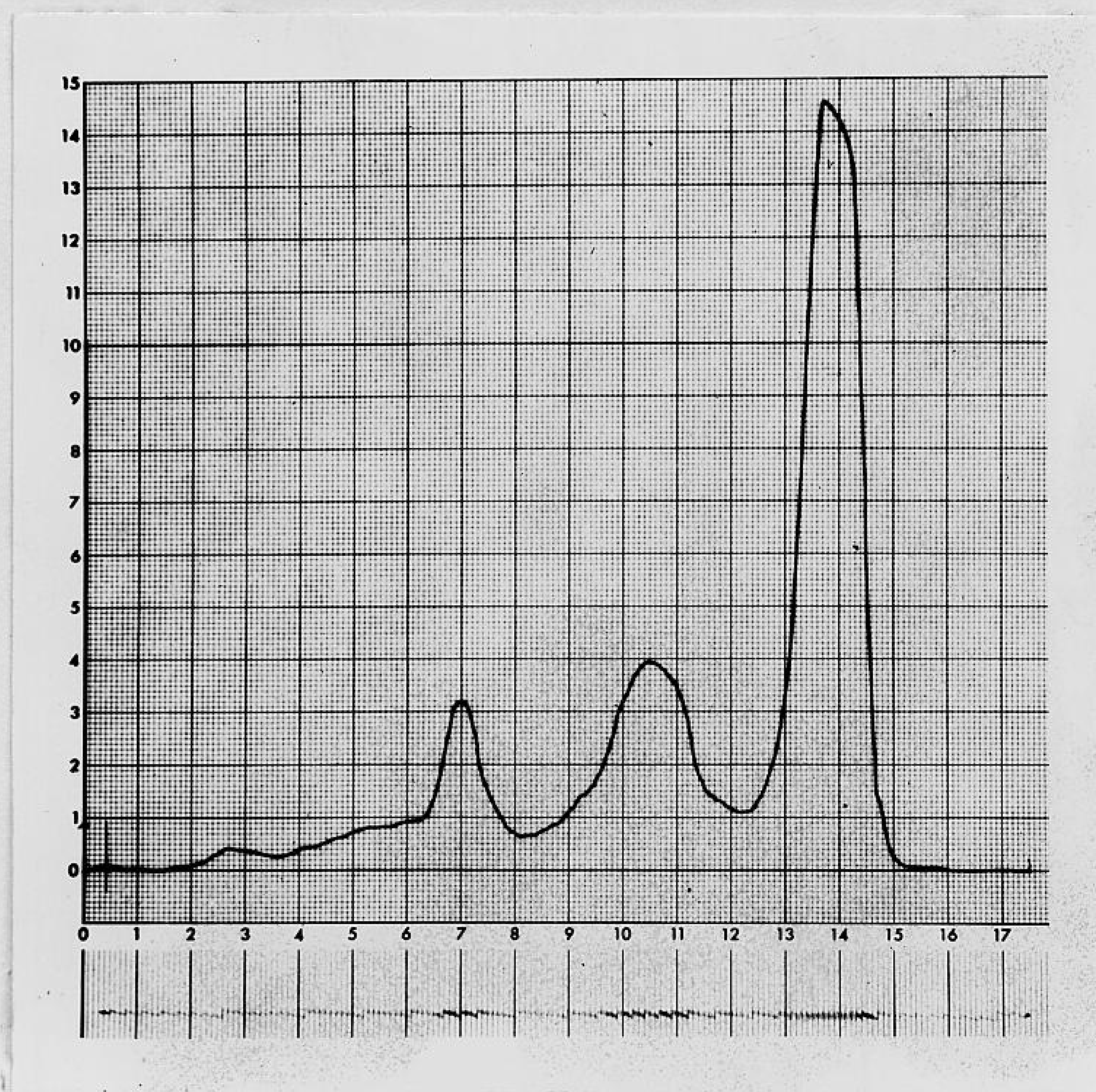

Figure 5. Paper electrophoresis of calf (No. 308) serum 2 hours after colostrum feeding.

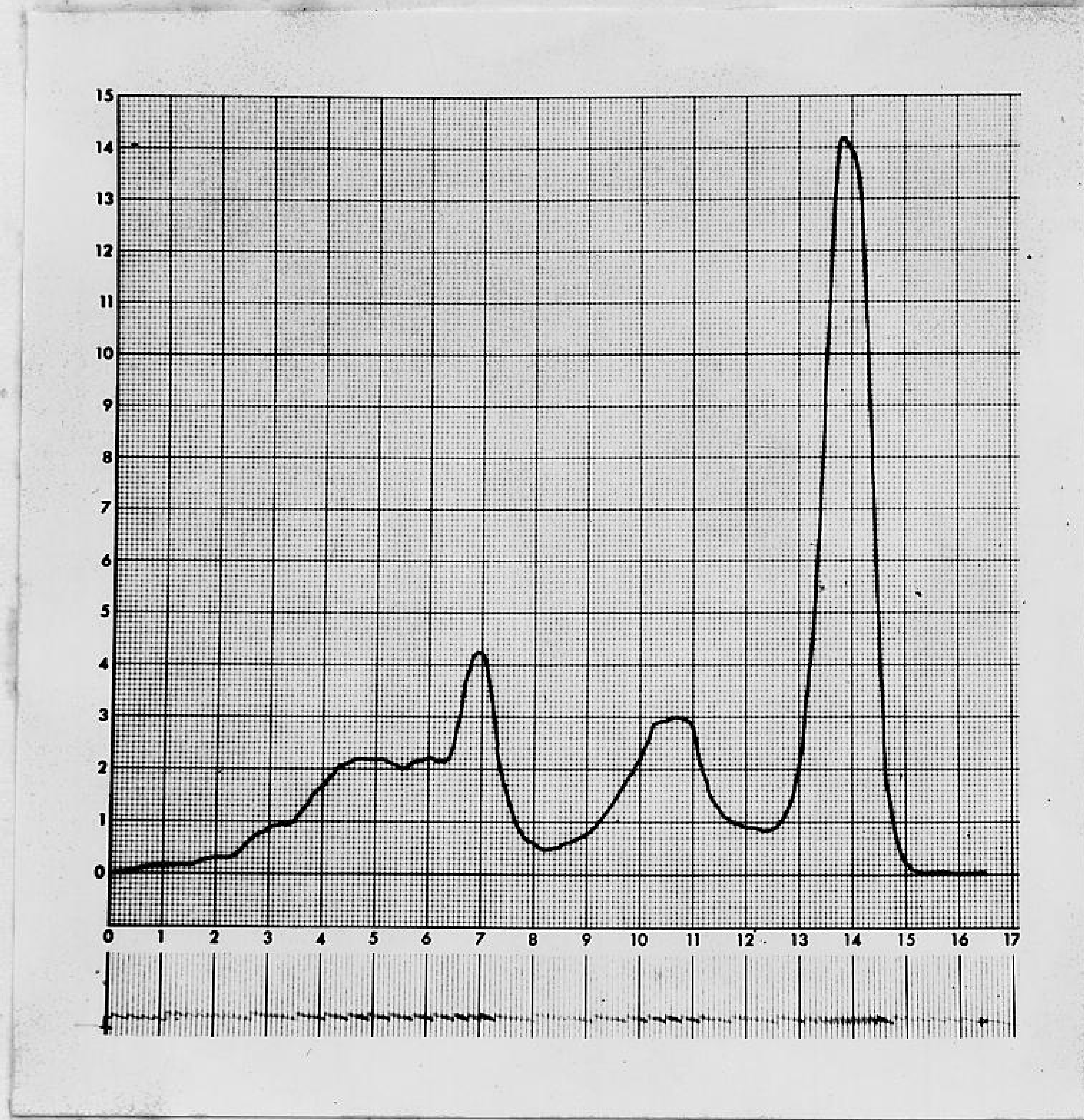

Figure 6. Paper electrophoresis of calf (No. 308) serum 4 hours after colostrum feeding.

The results are presented in Table 4. Data in this table shows that during the 4 hour period of the experiment the mean serum gamma globulin percentile value rose by a significant 11 points. There was a drop in the relative amounts of alpha globulins and albumin whereas the beta fraction value remained unchanged.

Changes in actual protein concentration of the various fractions, expressed in g/100 ml, do not follow however the same pattern, since the total amount of protein in the calf's serum is changing during the time interval studied. Table 5 shows the concentration values for the different fractions. The data in Tables 3 and 5 indicate higher concentrations in the cow serum than in the calf serum for all protein fractions with the exception of the alpha fraction. It seems that as the calf matures and its serum composition approaches that of the normal adult, there is a decrease in the alpha content and a gradual increase in the gamma, beta, and albumin content. Thus, our results for the first 4 hours that follow the feeding of colostrum do not agree with the findings of Jameson *et al.* (1942) who stated that the concentrations of the alpha and albumin fractions decrease during the nursing period.

Table 4. Percentile distribution of proteins among paper electrophoresis fractions.

	Calf number							
	153	155	306	308	310	312	313	Average
Gamma								
Prior	9.91	15.89	11.25	9.13	7.45	11.18	4.82	9.95±1.31*
1 hr	6.43	22.15	12.00	10.98	11.74	13.97	6.42	11.95±1.90
2 hr	5.58	18.97	16.49	11.97	11.50	12.71	9.94	12.45±1.65
4 hr	20.74	26.81	24.34	25.32	10.50	29.10	9.83	20.95±2.95
Beta								
Prior	9.01	16.82	10.62	10.32	13.04	12.50	7.57	11.41±1.15
1 hr	9.29	16.82	11.50	10.16	15.65	8.94	7.75	11.44±1.32
2 hr	8.18	16.67	11.58	11.40	10.50	8.29	10.26	10.98±1.08
4 hr	8.36	16.67	10.55	13.95	9.50	8.96	8.55	10.93±1.20
Alpha								
Prior	33.33	34.58	37.50	26.98	35.40	34.21	39.67	34.52±1.50
1 hr	36.07	31.01	37.00	27.64	20.00	37.99	40.91	32.95±2.74
2 hr	34.57	28.74	35.44	29.63	29.50	28.73	32.69	31.33±1.08
4 hr	26.09	23.91	27.27	22.48	29.50	28.36	34.19	27.40±1.46
Albumin								
Prior	47.75	32.71	40.62	53.57	44.10	42.11	47.94	44.11±2.51
1 hr	48.21	32.91	39.50	51.22	52.60	39.11	44.92	44.07±2.73
2 hr	51.67	35.63	36.49	47.01	48.50	50.28	47.12	45.24±2.46
4 hr	44.82	32.61	37.83	38.24	50.50	33.58	47.44	40.72±2.63

* Standard Error.

Table 5. Amounts of protein per electrophoretic fraction in g/100 ml.

	Calf number							
	153	155	306	308	310	312	313	Average
Gamma								
Prior	0.43	0.57	0.47	0.36	0.35	0.48	0.21	0.41 ± 0.04*
1 hr	0.27	0.95	0.52	0.46	0.54	0.60	0.28	0.52 ± 0.09
2 hr	0.23	0.79	0.77	0.53	0.57	0.55	0.44	0.55 ± 0.07
4 hr	1.00	1.45	1.32	1.28	0.53	1.47	0.51	1.08 ± 0.16
Beta								
Prior	0.39	0.60	0.44	0.41	0.61	0.54	0.33	0.47 ± 0.04
1 hr	0.39	0.72	0.50	0.42	0.71	0.38	0.33	0.49 ± 0.06
2 hr	0.34	0.70	0.54	0.50	0.52	0.36	0.45	0.49 ± 0.05
4 hr	0.40	0.90	0.57	0.70	0.48	0.45	0.44	0.56 ± 0.07
Alpha								
Prior	1.43	1.23	1.57	1.06	1.66	1.47	1.75	1.45 ± 0.09
1 hr	1.51	1.33	1.59	1.16	0.91	1.63	1.76	1.41 ± 0.11
2 hr	1.45	1.20	1.66	1.31	1.45	1.24	1.44	1.39 ± 0.05
4 hr	1.25	1.30	1.48	1.33	1.49	1.43	1.76	1.43 ± 0.05
Albumin								
Prior	2.05	1.16	1.70	2.11	2.07	1.81	2.12	1.86 ± 0.13
1 hr	2.02	1.42	1.70	2.14	2.40	1.68	1.93	1.90 ± 0.12
2 hr	2.16	1.49	1.71	2.08	2.39	2.16	2.08	2.01 ± 0.12
4 hr	2.15	1.77	2.05	1.93	2.55	1.69	2.45	2.08 ± 0.12

* Standard error.

Our results in Table 5 indicate also that contrary to the belief that the newborn calf serum is devoid or contains only minute amounts of slow moving gamma globulins prior to colostrum intake (Jameson _et al_., 1942; Hansen and Phillips, 1947; Graves, 1963), it contains rather a good deal of it, 0.41 g/100 ml. This is approximately 40 percent of its concentration 4 hours post feeding, and represents 20 percent of the concentration of gamma globulins found in the mature cow serum. Among the 7 calves studied, calves Nos. 310 and 313 showed considerably lower values in their gamma globulin fractions ($P < .01$). The analysis of variance for the gamma globulins of the individual calves is presented in Table 6.

Table 6. Analysis of variance of gamma globulins in g/100 ml serum.

Source of variation	D.F.	M.S.	F
Individuals	6	0.17	4.3**
Time of colostrum feeding	3	0.63	15.8**
Error	18	0.04	

** $P < .01$.

The results obtained in this study support the findings of Howe (1921b), and Orcutt and Howe (1922), who found a euglobulin and pseudoglobulin I concentration, in the blood of colostrum deprived newborns, ranging from 0.027-0.090 g of nitrogen per 100 ml of

serum, which corresponds to 0.169-0.56 g of gamma globulins per 100 ml. The present results are further in agreement with the findings of Hansen and Phillips (1949), who reported that even before the ingestion of colostrum the serum of the newborn calf gives decided evidence for the presence of proteins immunologically similar to cow colostrum pseudoglobulin.

Immunoelectrophoresis

Figure 7-1 shows the immunoelectrophoresis patterns (IEP) of mature cow serum and colostrum whey. The latter was found to lack the alpha 1 and alpha 2 fractions which are clearly present in cow serum. This finding is in agreement with our paper electrophoresis results and compares favourably with the findings of Murphy *et al.* (1964). On the other hand, the colostral whey developed arcs for the gamma 1 immunoglobulins (Ig G1), gamma 1 M immunoglobulins (Ig M), beta 1 and beta 2 globulins, but does not show the precipitin line corresponding to the gamma 2 immunoglobulin (Ig G2) fraction.

Figure 7-2, 3, 4, 5 shows the IEP of a typical experimental calf (calf No. 313) whose serum was tested at the different time intervals of the experiment. Prior to colostrum intake, the serum of the newborn calf shows well developed albumin and beta 1 arcs. The Ig G1 and

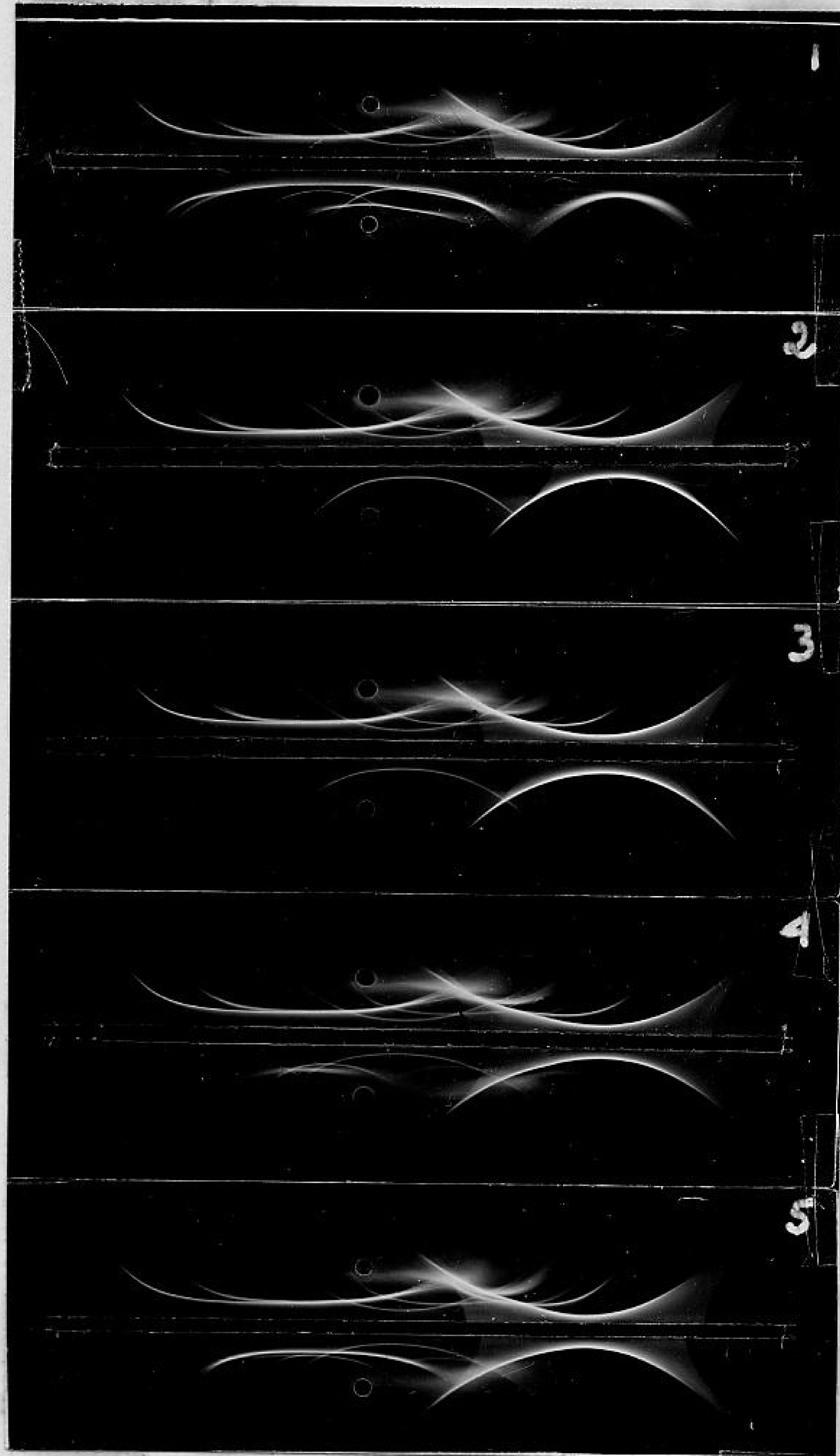

Figure 7. Immunoelectrophoretic analysis of colostrum whey and serum samples from calf No. 313 run in parallel with mature cow serum for reference. In all slides the upper antigen well was filled with cow serum. Slide 1, colostrum whey. Slides 2, 3, 4, 5, calf serum prior to colostrum feeding and one hour, 2 hours, 4 hours after colostrum feeding, respectively.

beta 2 arcs are completely absent. These results are in agreement with the paper electrophoresis results reported by Jameson _et al_. (1942), and Hansen and Phillips (1947). However, they do not agree with the findings of Howe (1921b), and Orcutt _et al_. (1922), and with our paper electrophoresis.

One hour after colostrum intake, a faint and fuzzy arc in the beta 2 area was observed in the IEP. This arc corresponds to the earliest protein population reaching the blood stream after colostrum feeding. In the 2-hour, post-feeding IEP, the same arc gained in intensity and extended toward the cathode. Its shape and location identify it clearly as Ig G1 intersecting with a thin, convex beta 2 arc, a feature which did not exist in the previous IEP. In the 4-hour, post-feeding slide the Ig G1 arc, compared to its homologue in the cow serum, reached its full size. However, no slow moving Ig G2 were detected in the newborn calf serum even 4 hours after colostrum had been fed.

Contrary to the findings of Johnson and Pierce (1959), and Pierce and Feinstein (1965), who reported that immune bodies are passively absorbed our findings indicate, at least for the first 4 hours post colostrum feeding, that immune bodies are selectively absorbed by the intestinal lumen in the newborn calf. The Ig M fraction, well present in the colostrum whey, did not

appear in the serum of the calf during the time interval studied.

The fact that paper electrophoresis revealed the presence of globulin of gamma mobility in the serum of the newborn calf prior to suckling, while immunoelectrophoresis failed to do so although it is by far the most sensitive, is probably due to an artifact inherent to the paper electrophoresis technique used. The prevalent conditions of analysis may have caused either marked trailing or altered the relative mobility of certain proteins.

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V. SUMMARY AND CONCLUSIONS

Changes in total serum protein, and in the serum immune globulins, alpha globulins, and albumin fractions of 7 Holstein-Friesian newborn calves were studied during a 4 hour period after colostrum feeding.

Data obtained indicated that no statistically significant increase (5 percent level of probability) in the newborn total serum protein occurred 2 hours after colostrum was fed. However, 4 hours after feeding, the mean total serum protein in the 7 experimental calves increased significantly by 0.93 g/100 ml of serum (5 percent level of probability). It was found that the differences in protein concentrations among newborn individuals of the Holstein-Friesian breed was not significant at the fourth hour after feeding colostrum. Apart from the alpha fraction, which seems to be unaltered, all other protein fractions in the newborn calf serum increased in concentration. However, the increase was most conspicuous in the gamma 1, beta 2 immune globulin fractions.

Individual calf variations in the gamma globulin fraction were found to be highly significant (P .01) during the 4 hour experimental period. A range of 0.51-1.47 g/100 ml was observed at the fourth hour, post

feeding colostrum.

Immunoelectrophoretic analysis indicated that the newborn calf serum is lacking in immune globulins. These globulins however, start to appear one hour after feeding colostrum (beta 2 first to be detected at this time). The Ig G1 started to appear at the second hour and developed at the fourth hour a distinct precipitin arc with rabbit antiserum.

Absorption of colostral proteins in the intestine of the newborn during the time interval studied was found to be selective. The Ig M fraction, detected in the colostrum whey, failed to appear in the newborn Holstein-Friesian calf serum 4 hours after feeding colostrum.

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