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Some Genetic and Environmental Aspects of Blood Proteins in Dairy Cattle.

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SOME GENETIC AND ENVIRONMENTAL ASPECTS OF BLOOD PROTEINS IN DAIRY CATTLE

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ABSTRACT

Investigations were conducted on the environmental and genetic control of blood proteins in dairy cattle. In the environmental aspect of the study, two experiments were conducted. In the first experiment, 28 heifers were subjected to 65°F for 48 hours and then exposed for 8 hours at 95°F. In the second experiment, 10 Holstein heifers were exposed to 65°F for 4 weeks (treatment 1), simulated Louisiana summer condition (temperature cycling diurnally from 65°F to 95°F) for 8 weeks (treatment 2) and at 65°F for 4 weeks (treatment 3). The measure of responses were hemoglobin, hematocrit, RBC, total serum protein and its electrophoretic components.

There were no significant differences between the mean values of the blood constituents (except hemoglobin $P < 0.05$) at 65°F and 95°F in short term study. In the second experiment, hematocrit value declined ($P < 0.05$) in treatment 2 while total serum protein concentration increased ($P < 0.01$). There was no significant difference between treatment 1 and 3. No significant differences were observed in relative concentrations of α, β and γ-globulins. Mean body weight gain per day declined ($P < 0.01$) in treatment 2. Treatment 3 had a higher rate of gain ($P < 0.05$) than treatment 1.

In the genetic phase, fractions of starch gel electropherogram were identified. Transferrin and hemoglobin gene frequency were obtained on 350 adult females of four pure breeds (Holstein, Jersey, Brown Swiss and a red Sindhi bull) and cross breeds from Louisiana herds.

No activity of haptoglobin and glucuronidase could be localized on gel electropherogram. Lactic dehydrogenase was distributed among four bands. Three fractions tested positive for cholinesterase. Leucine aminopeptidase and ceruloplasmin activity were localized in single bands. None of these showed differences in mobility, in four breeds examined. Transferrin and aromatic esterases showed differences in migration rates between individuals within breed. The transferrin Tf-E gene was absent from pure breeds (except Sindhi bull) and Brown Swiss X Jersey. Holstein crosses had lower ($P < 0.05$) Tf-E gene than Sindhi crosses.

No statistically significant differences were observed in milk and milk fat production of different Tf genotypes in Holsteins. There were no mean differences in fertility of different Tf genotypes.

Bovine hemoglobin Hb^B gene was completely absent from Holstein herds. Bov. Hb^B were present in Brown Swiss and Jersey. The frequency of Hb-B gene was lower ($P < 0.01$) in Jersey and Brown Swiss crosses than Sindhi crosses.

I. GENERAL INTRODUCTION

A major problem confronting the dairy cattle breeder is the selection of animals which will be productive and economical (36). Investigators in this field have searched for characteristics present in young animals that would be an index of their future performance. Many experimenters have taken the approach of experimental physiology (18, 61). Those with genetic inclination have examined the distribution and heredity of blood types (28, 43, 51, 55, 56, 64). Although both approaches have met with limited success, each has focused attention upon blood. The physiological studies have shown that the concentration of blood proteins vary with physiological and pathological states. The blood type studies have shown that the structure of blood proteins is under genetic control.

A number of studies have suggested that blood proteins vary in cattle subjected to climatic stress. Initial studies of Dale et al. (32) and of Blincoe and Brody (19) indicated that the plasma protein levels did not change in cattle exposed to hot, humid environments in a climatic chamber. Recent investigators (17, 54), using animals of European and of Indian lineage, have reported a decrease in total plasma proteins. Bhosrekar and Sadhu (17) found that the opposite effect, a rise in total plasma protein, occurred if Indian cattle were exposed to a hot dry environment for short periods. Lundgren (59)

indicated that a number of fractions of the total protein were altered by heat stress.

The discovery of hereditary variant forms of a number of blood proteins has stimulated efforts to discover relationships between blood protein genotypes and climatic tolerance or other traits of economic importance (6, 7, 8, 9, 10). Three plasma proteins (transferrins, alkaline phosphatases and "thread proteins") and hemoglobin exist in variant forms in cattle blood (5, 13, 39, 72). Extensive work on the hemoglobins has shown that such variants usually differ from each other structurally by a single amino acid residue (50). Such differences are assumed to arise as a point mutation on a structural gene which alters the nucleotide sequence of the nucleic acid triplet code (74, 75). Variant forms of these proteins are usually inherited as multiple alleles at the same locus (13, 72).

The retention of a mutation in a population in high frequency is dependent upon a balance of selective processes. The occurrence together in a population of two or more different proteins in such proportion that the rarest form cannot be retained by recurrent mutation is called polymorphism (1). Transient polymorphism involves only temporary diversity and follows a change in the environment. When selective agencies favor diversity and oppose uniformity, balanced polymorphism results. Such is the case for the transferrins and hemoglobins of cattle. Attempts have been made to relate the balanced polymorphism of cattle hemoglobin and transferrin genes to the action of specific selective agents. Much of this research was stimulated by the discovery linking the high frequency of the gene for human hemoglobin S with survival of

man in areas in which malaria is endemic. Bangham and Blumberg (14) found that Bov.Hb^B gene might offer similar selective advantage to cattle exposed to trypanosomiasis. Although very controversial, Ashton's work, suggesting correlation between transferrin Tf - E gene and cold tolerance (7) and between other transferrin genes and milk productivity (8), fertility (9), and embryonic mortality (10), has stimulated much research on cattle blood.

Information on the effect of controlled hot climatic conditions on blood proteins of cattle is limited. In addition, more data are needed on the interrelationships between the blood proteins and such economically important traits as milk production and fertility, both from a hereditary and environmental standpoint. The objectives of the studies reported herein were as follows:

1. To determine the effect of a high temperature, high humid environment on blood proteins.

2. To study hemoglobin and transferrin polymorphism in Louisiana dairy herds and to relate these to milk and fat production and fertility of the cattle.

II. THE EFFECT OF HOT, HUMID ENVIRONMENT ON BLOOD SERUM PROTEIN AND HEMATOLOGICAL ATTRIBUTES

In an effort to develop a more satisfactory index of adaptability of cattle to hot, humid climatic conditions, physiologists have used various criteria, including body temperatures, respiration rates, cardiac function, vaporization rate and blood composition. More recently, attention has been directed toward blood serum proteins. This interest, no doubt, has been prompted by the important role that serum proteins play in metabolic processes (2, 38, 44, 46). However, as yet, data on the changes in the serum proteins and electrophoretic components in cattle under controlled heat stress conditions are limited (54, 59). It has been reported that the protein components are under both genetic and environmental control (34, 52, 72). However, the impact of environmental factors such as heat stress on these components has not been defined clearly. Therefore, the objectives of the investigations reported in this chapter were as follows:

1. To determine the effect of controlled climatic stress on total serum protein concentration and electrophoretic components of serum protein in dairy cattle.

2. To measure changes in the hematological attributes in the heifers.

3. To determine the growth response of the heifers and to relate this response to changes in blood components.

Material and Methods

Two experiments were conducted using controlled environments in a psychrometric chamber. The first experiment was of short term duration and involved exposure of Holstein heifers to a constant hot temperature. The second experiment involved continuous exposure of Holstein heifers to cyclic hot climatic conditions in a psychrometric chamber.

Twenty-eight Holstein heifers ranging in age from nine to twenty-four months were selected from the Louisiana State University herd for the first experiment. Ten Holstein heifers between twelve and fifteen months of age were selected for the second experiment. These heifers were daughters of eight different sires. Their body weights at the beginning of the experiment ranged from 576 lb to 658 lb.

Animals were fed a ration providing 120% of Morrison's standard for growing heifers (62). The concentrate feed consisted of a pelleted grain and mineral mixture containing 18% crude protein. One-half of the concentrate was fed daily at 0700 hours along with silage; the other half of the concentrate and the alfalfa hay were fed at 1600 hours. The rate of feeding of the grain and alfalfa hay was six pounds of each per heifer daily. Corn silage was fed ad libitum. Animals had free access to salt and water.

As mentioned previously, the first experiment involved short term exposure of heifers to the different climatic conditions in the psychrometric chamber. The schedule of treatments are given in Table 1. The ten animals in the second experiment were exposed to the schedule of treatments in the psychrometric chamber as shown in Table 2. The schedule of the hot cyclic temperatures (second phase) are shown in Table 3.

Table 1. Schedule of treatments in experiment 1

Phase	Treatment	Duration
1.	Continuous exposure to 65°F (DBT) and 55°F (WBT)	48 hours
2.	Short duration exposure to 95°F (DBT) and 85°F (WBT)	8 hours

Table 2. Design used in experiment 2

Phase	Treatment	Duration
First	Continuous exposure to 65°F (DBT) and 55°F (WBT)	Dec. 16, 1963 to Jan. 12, 1964
Second	Continuous exposure to cyclic hot condition	Jan. 13, 1964 to Mar. 10, 1964
Third	Continuous exposure to 65°F (DBT) and 55°F (WBT)	Mar. 11, 1964 to Apr. 8, 1964

DBT = Dry bulb temperature
WBT = Wet bulb temperature

Table 3. Schedule of treatments during second phase of experiment 2

Period	Hours	Dry bulb temp. (°F)	Wet bulb temp. (°F)
I	0600 - 1000	85	75
II	1000 - 1600	95	85
III	1600 - 2200	85	75
IV	2200 - 0600	75	70

Artificial light was provided from 0600 to 1900 hours.

In experiment 1, blood samples were collected at the end of the first and second phases. In experiment 2, blood samples were collected every seventh day at 1300 hours.

Total red blood cell count was made in a Coulter electronic counter as described by Brecher (23). The cyan-met hemoglobin method as described by Benjamin (15) was followed for the estimation of hemoglobin. Hematocrit value was recorded by a microhematocrit graphic method as described by Wintrobe (80).

Total serum protein was determined by the method described by Weichselbaum (78). Electrophoretic fractionation of serum protein was conducted in a Beckman Spinco model R electrophoretic cell. Samples of 0.01 ml were applied on the paper strips. Electrophoresis was conducted in a veronal buffer of pH 8.6 and ionic strength 0.075. A constant current of 2.5 mA was applied for 24 hours. Subsequent treatments of the paper were done as described by Block et al.(20). The strips were then stained with bromphenol blue and scanned in a Spinco Analytrol model densitometer.

Body weights during the second experiment were recorded every seventh day at 0700 before feeding. No body weight data were obtained in the first experiment.

All statistical analyses were done according to the methods described by Snedecor (76). All of the data in the first experiment were used for statistical analysis. In the second experiment data were grouped in two-week periods. Statistical analysis were performed on the data from the third, fourth, eleventh, twelfth, fifteenth, and sixteenth weeks. It was assumed that animals show an initial period of adjustment

when switched from one phase to another. In order to provide allowance for this compensatory period, statistical analysis was done on the last two weeks of each treatment, i.e., week 3 and 4, 11 and 12, and 15 and 16 (test periods).

The statistical model of analysis of variance for the two experiments is presented in Tables 4 and 5.

Table 4. Model for short term heat exposure

Source of variance	Degrees of freedom	Sum of squares	Mean square	Expected mean square
Total	55			
Among animals (A)	27			
Between treatments (T)	1			$\sigma^2 + 27\ \sigma^2_T$
Animal x treatment	27			σ^2

Table 5. Model for long term heat exposure (cyclic)

Source of variance	Degrees of freedom	Sum of squares	Mean square	Expected mean square
Total	59			
Among animals (A)	9			
Between treatments (T)	2			$\sigma^2 + 2\ \sigma^2_{AT} + 20\ \sigma^2_T$
Animals x treatment	18			$\sigma^2 + 2\ \sigma^2_{AT}$
Within animal x treatment	30			σ^2

Results and Discussions

Experiment 1.

The effects of heat stress on the blood constituents of the heifers are presented in Table 6. There was no significant difference between the mean values of the blood constituents at 65°F and 95°F except for hemoglobin ($P < 0.05$). These results are in contrast to the findings of Bhosrekar and Sadhu (17) who reported a decrease in hematocrit and RBC values in Hariana cattle. These workers also reported an increase in serum protein concentration under dry hot conditions and a decrease in total serum proteins in hot humid conditions.

The alterations in hematological attributes and serum components could arise in two ways: a) effect on the rates of anabolism or catabolism of the proteins and production of blood constituents; b) hemodilution or hemoconcentration. The first possibility is precluded in this experiment since there is always a pronounced lag in the biosynthesis of plasma proteins (2). It is, therefore, not possible that short term heat could directly influence the metabolism of proteins. Probably the duration of heat stress was too short to even produce a hemodilution effect. Therefore, the results as shown in Table 6 are in agreement with these possibilities. Consequently, it appears that the use of short term exposures of cattle to heat stress to formulate heat tolerance adaptability indices is highly questionable. This contention is supported by other workers (18, 61).

Table 6. Effect of short term climatic stress on the blood constituents

Constituents	Temperature	
	65°F	95°F
Hemoglobin (g/100 ml)	11.86[a]	12.41[a]
Hematocrit value (%)	29.9	30.00
RBC count (millions/cu mm)	781.3	775.70
Total serum protein (g/100 ml)	6.79	6.77
Serum albumin (%)	42.64	42.33
α-globulin (%)	13.65	13.40
β-globulin (%)	14.03	14.31
γ-globulin (%)	29.66	29.94
Albumin (g/100 ml)	2.89	2.86
α-globulin (g/100 ml)	0.92	0.91
β-globulin (g/100 ml)	0.95	0.97
γ-globulin (g/100 ml)	2.02	2.03

[a]Mean values.

Experiment 2.

The average values for RBC, hemoglobin and hematocrit value for the different treatments are presented in Figure 1. The mean values for the various blood constituents and body weight during test portions of each of the periods are given in Table 7. There was a significant difference ($P < 0.01$) in hematocrit values, the value in the cyclic phase being significantly different from those for treatment 1 and 3. There was no significant difference between treatments 1 and 3. Hematocrit value showed a decline in the last two weeks of the cyclic hot phase as will be seen in Figure 1. This is in accord with the findings of some workers (40, 53, 79). However, other workers have reported (25, 69) no change in hematocrit value either in controlled climatic heat stress or under natural summer conditions.

No significant differences were obtained for hemoglobin and RBC values due to treatments. This is in contrast to the findings of many authors (40, 41, 60). Some authors (25, 69) have reported no seasonal trend for hemoglobin and RBC.

Data for total serum proteins and its electrophoretic components are presented in Figure 2 and Table 7. There was a significant difference ($P < 0.01$) in total serum protein content, the value being higher in the cyclic phase than in the cool phases. However, there was no significant difference between treatments 1 and 3 (the two cool phases). These results are contrary to the findings of some workers (54, 67). Appleman and Delouch (3) have reported a progressive increase in the plasma protein concentration in goats at increasing temperatures from 20°C to 40°C. Bhosrekar and Sadhu (17) have reported an increase in

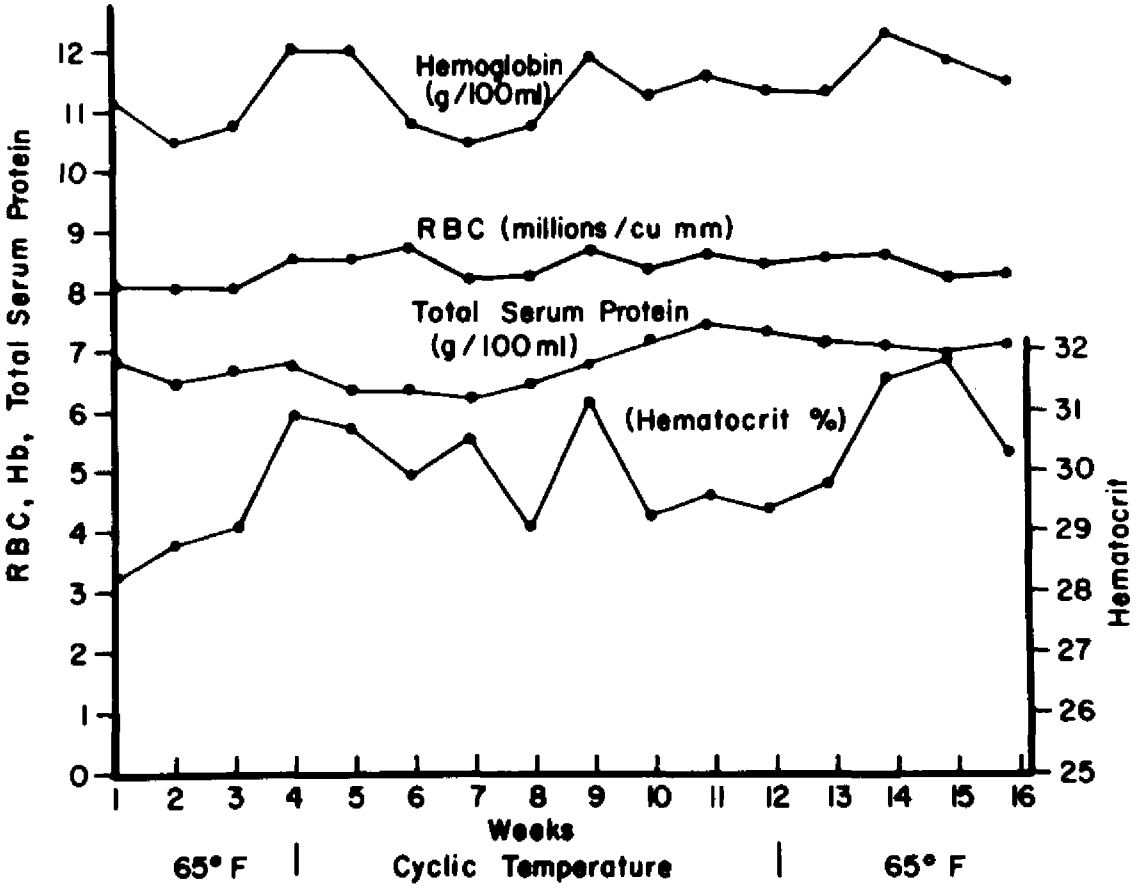

Figure 1. Effect of long term heat stress (cyclic on hematological attributes and total serum protein concentration.

Table 7. Effect of climatic heat stress on the blood constituents and body weight

Constituents	Treatments 1	2	3
Hemoglobin (g/100 ml)	11.60	11.55	11.69
Hematocrit value (%)	30.60	29.50	31.20
RBC Count (millions/cu mm)	840.19	864.92	828.73
Total serum protein (g/100 ml)	6.77	7.44	7.09
Serum albumin (%)	44.13	42.66	42.23
Serum α-globulin (%)	12.80	14.05	13.69
Serum β-globulin (%)	12.88	12.80	13.36
Serum γ-globulin (%)	30.18	30.48	30.70
Albumin (g/100 ml)	2.98	3.15	2.96
α-globulin (g/100 ml)	0.86	1.04	0.97
β-globulin (g/100 ml)	0.86	0.95	0.95
γ-globulin (g/100 ml)	2.05	2.29	2.20
Body weight gain/day (lbs)	1.62	0.56	3.52

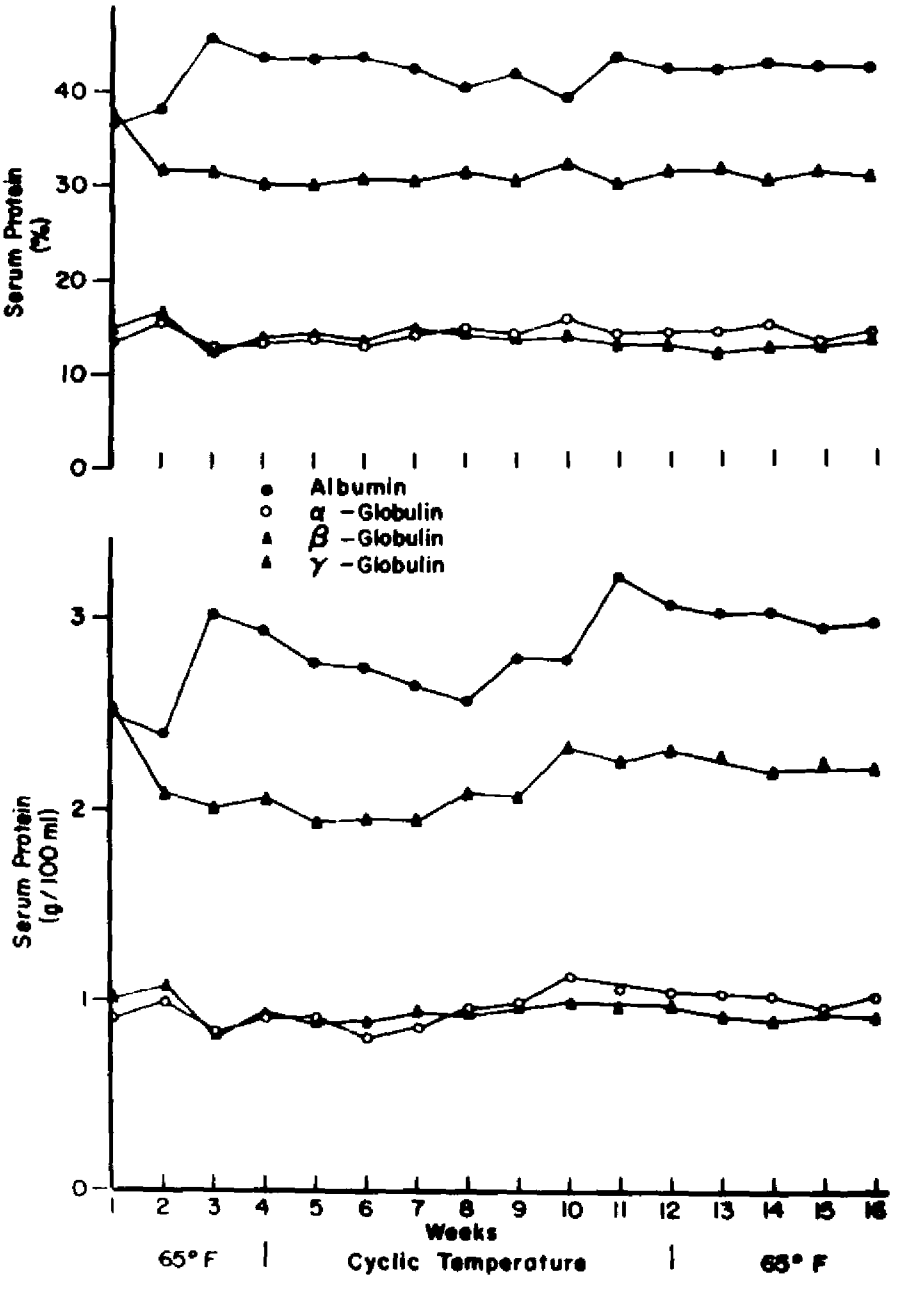

Figure 2. Effect of long term heat stress on serum protein fractions.

plasma protein in dry, hot temperature and a decrease in hot, humid temperature for Hariana cattle. They attribute this difference to hemodilution and the nature of the interstitial fluid permeating into blood.

Plasma protein concentration could be changed by an alteration in rates of protein synthesis and catabolism during heat stress. Nutritional status is another factor which determines the concentration of serum proteins. Randel (67) found a decrease in serum protein concentration during heat stress of Holstein calves. However, when the value was corrected for nitrogen intake there was no change in serum protein concentration with treatments. Such was not the case in this experiment. The animals showed an increase in serum protein concentration during the cyclic hot phase.

Conn and associates (30) developed the concept that heat acclimatization was associated with an increase in the activity of the pituitary-adrenal system. Precisely how climatic stress influences the pituitary-adrenal system to act on protein metabolism in cattle is not known. Kamal (54) has indicated that a decline in protein concentration due to heat stress may result from increased protein catabolism. However, more positive evidence is needed to understand the direct influence of heat stress on metabolism of serum proteins in cattle.

The relative value of albumin, α-globulin, β-globulin, and γ-globulin did not show any significant difference between treatments. The absolute concentration of α-globulin, β-globulin and γ-globulin showed significant differences, the values being higher in cyclic phase than in the cool phases. These differences are due to the fact that total serum protein concentration is involved in the calculation of these values.

These data reveal that no disturbance or alteration takes place in the biochemical mechanism of the synthesis and catabolism of serum protein fractions due to hot environment. This further confirms the importance of examining both relative and absolute values for determining the nature of changes in the plasma proteins.

The long term exposure to heat stress allows compensation for thermally induced changes. The animal may restore the biological displacement that has occurred at the beginning of the thermal exposure. To what extent the animals can shift the comfort zone is to be determined precisely. In this connection, Berman et al. (16) have reported recently that lactating Holstein cows may adapt to hot summer conditions by decreasing their metabolic rates and by adapting themselves to higher than normal body temperatures.

The intensity of heat stress used in this experiment may not have been sufficient to evoke metabolic changes in plasma protein components. However, the cyclic hot environment employed in these studies were simulated Louisiana summer climate. Under these conditions certain physiological responses such as changes in rectal temperature, respiration rate and sweating rate are quite evident (12, 40, 61). Thus, it would appear that one or more of these responses would be of more value in assessing adaptation of cattle to hot climatic conditions than changes in plasma proteins.

The mean body weight gain per day is presented in Table 7. Figure 3 shows the body weight changes during the different treatment periods. It will be noted in Figure 3 that there was a considerable decline in body weight gains during the cyclic, hot phase (treatment 2). The mean

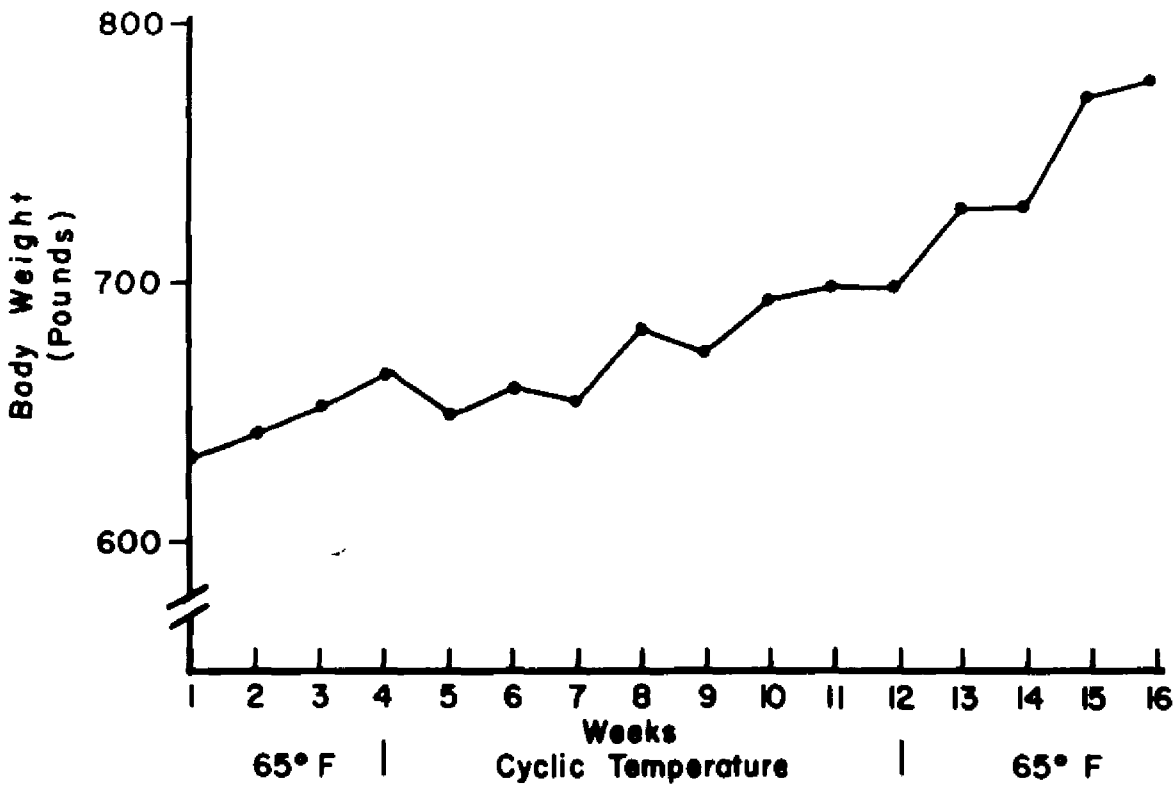

Figure 3. Effect of long term heat stress on body weight of Holstein heifers.

body weight gain showed a steady decline in the first three weeks and thereafter it showed a period of adjustment. This decline in body weight may have been due to reduction in food intake. Unfortunately, no data were obtained on dry matter consumption. It will be observed in Figure 3 that the rate of gain during the third phase was considerably greater than during the cyclic hot phase. However, when the growth data presented in Figure 3 are considered for all the phases, the responses appear to have been linear. Statistical analysis of the test period portions of these data revealed that differences in rates of daily gain in body weight were significantly greater ($P < 0.01$) during periods 1 and 3 (cool phases) than during period 2 (cyclic hot phase). These findings are in accord with those of many authors (12, 40, 53). It appears that a period of three or four weeks is required for Holstein heifers to adjust to hot climatic conditions.

The rates of gain during the two cool phases were significantly ($P < 0.05$) different. Perhaps these responses can be explained on the basis of compensation that the heifers made for the low rates of gain during the cyclic hot phase.

III. GENETIC STUDIES OF TRANSFERRIN AND HEMOGLOBIN

For many years dairy scientists have attempted to find blood characteristics which are associated with economic traits and could be used to select animals for improvement in these traits (18, 24, 37, 61, 66). During the first half of this century, routine blood chemistry and hematology were extensively examined (18, 37, 61). With the discovery of serological variants, interest shifted to correlation of blood groups with productive traits (28, 43, 51, 55, 56, 64). Following the development of paper electrophoretic methods for separating proteins, Cabannes and Serain (29) found two hemoglobin types in cattle red blood cells. Almost simultaneous to his discovery of high resolution electrophoresis in starch gel, Smithies (72) reported the discovery of five beta-globulin types in cattle blood plasma. Later Giblett et al. (42) showed that these variants were transferrins, iron binding proteins. Subsequent work of Ashton (8) indicated that cattle transferrin type might be associated with certain characteristics of economic importance.

The principal objective of the present study was to test Ashton's findings, which some investigators question (21, 26, 27, 33, 48, 68). An additional objective was to identify other protein fractions of starch gel electropherograms and to determine gene frequencies of hemoglobins and transferrins of herds of cattle in a subtropical region.

Material and Methods

Blood was collected from 350 adult females from the dairy herds of Louisiana State University and the Iberia Livestock Experiment Station (Table 8). Collections were made from only two males, a Red Sindhi bull (Bos indicus) and one of its half breed progeny. Blood was taken aseptically from the jugular vein directly into the sampling tubes. Tubes containing citrate were used to obtain blood for hemoglobin studies; tubes without anticoagulants were used to obtain blood for serum studies. After centrifuging the blood, red cells were separated from plasma by centrifugation and were washed three times with physiological saline. Serum was decanted into storage tubes after clot retraction. Washed cells and serum were stored at - 20°C until analyzed.

Hemoglobins and plasma proteins were analyzed by means of vertical starch gel electrophoresis in 0.01 M sodium borate buffer, pH 8.6 (73). To expedite the survey of hemoglobin and transferrin types, hemolyzates were analyzed on the same gel slab. Serum was pipetted into the slots near the upper end of the gel. Hemolysates were applied to pieces of Whatman 3 mm filter paper, and the wetted papers were inserted into slots in the gel close to its lower end, 15 cm from the serum samples. To permit transferrin localization, 2 to 4 μc of Fe^{59} were mixed with each sample before electrophoresis. Electrophoresis was carried out in a cold room (3 to 5°C) for 22 hours at 170 volts. After electrophoresis, gels were sliced horizontally. One half of each gel was stained with the dye amidoschwartz to localize all major protein fractions. The other half gel was treated to localize specific proteins. Transferrins were identified by radioautograph (42). Enzymes were localized by methods

Table 8. Distribution of transferrin and hemoglobin types in Louisiana dairy herds

Breed composition	Transferrin types						Hemoglobin type			
	DE	AE	DD	AD	AA	Total	AA	AB	BB	Total
Louisiana State University Herd										
Holstein (H)	0	0	56	64	18	138	137	0	0	137
Jersey (J)	0	0	12	12	9	33	18	16	1	35
Brown Swiss (B)	0	0	6	6	1	13	10	4	0	14
Iberia Livestock Dairy Herd										
Holstein	0	0	13	17	9	39	37	0	0	37
1/2 H [1/] 1/2 J	0	1	3	4	3	11	9	2	0	11
1/2 B 1/2 H	1	0	0	8	2	11	11	0	0	11
1/2 B 1/2 J	0	0	2	2	0	4	3	0	0	3
3/4 H 1/4 S [2/]	2	1	2	0	0	5	3	2	0	5
1/2 H 1/4 S 1/4 J	0	0	3	3	2	8	4	4	0	8
1/2 H 1/4 B 1/4 J	0	1	1	0	0	2	1	0	0	1
3/4 H 1/4 B	0	0	0	3	1	4	5	0	0	5
3/4 H 1/4 J	1	0	0	1	0	2	2	0	0	2
1/2 B 1/4 S 1/4 J	1	2	1	0	2	6	2	3	0	5
1/2 B 1/4 H 1/4 J	0	2	3	4	0	9	7	2	0	9
1/2 B 1/8 S 3/8 J	0	1	1	1	0	3	3	0	0	3
1/2 H 1/8 S 3/8 J	0	1	1	1	0	3	2	1	0	3
1/2 H 1/4 B 1/8 S 1/8 J	1	4	1	2	0	8	6	3	0	9
7/8 H 1/8 S	0	0	0	1	0	1	1	0	0	1
1/2 B 1/4 H 1/8 S 1/8 J	5	3	4	5	1	18	12	6	0	18
1/2 B 3/8 H 1/8 S	0	0	1	2	1	4	3	1	0	4
1/2 B 1/4 H 1/16 S 3/16 J	0	0	0	1	0	1	0	0	1	1
3/4 B 1/8 S 1/8 J	0	0	1	0	0	1	1	0	0	1
9/16 H 1/4 B 1/8 S 1/16 J	0	1	5	1	1	8	4	4	0	8

1/ First letter represents breed of sire of the animal.

2/ Red Sindhi.

slightly modified from those outlined by Lawrence et al. (57). Trihydroxy methyl aminomethane-maleic acid buffer (45) was used except in the haptoglobin method.

The records on milk and milk fat production and on number of services per conception of cattle used in this study were related to their transferrin types. For milk and milk fat production, data were available from October, 1961, through September, 1963. Data from the Iberia herd were uncorrected for age at calving, but those for the purebred cattle of the Louisiana State University herd were adjusted to a 2 x -305 day-M.E. basis. Production figures during different seasons were used in statistical analyses involving the Louisiana State University Holstein herd. Pooled data for the entire year were used in tests involving other animals of both herds, due to the limited number of observations.

Gene frequencies of transferrins (4) and hemoglobins (13, 71) were calculated on the basis of inheritance as autosomal alleles at the same loci (58). All other statistical analyses were performed according to Snedecor (76).

Results

A diagram of starch gel electropherogram of plasma proteins of cattle is presented in Figures 4 and 6. Serum components are numbered for descriptive purpose in order of decreasing anodal migration. Activities of some of the proteins in the different fractions are identified. Haptoglobin activity is absent.

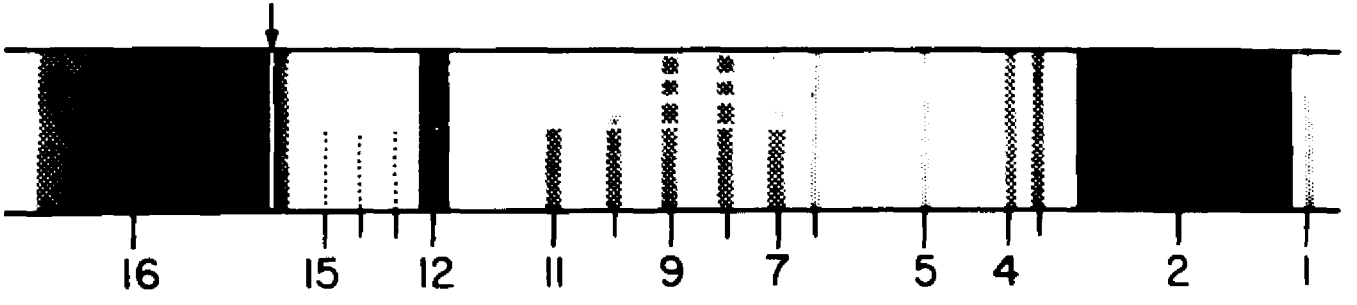

Figure 4. Diagram of starch gel electropherogram of serum proteins of cattle in borate buffer pH 8.6. Relative concentration of protein is proportional to the density of the vertical bars; broken bars indicate intensity may vary. Arrow indicates point of sample application; anode is to the right. Properties of proteins migrating in the various bands have been identified as follows:

Prealbumin = band 1. Absent in some sera.
Albumin = band 2.
Lactic dehydrogenase = present in band 1, band 2 (2 fractions), band 4.
Leucine aminopeptidase - catalyzes hydrolysis L-leucyl-B-naphthylamide found in band 4.
Ceruloplasmin = catalyzes oxidation of p-phenylene diamine found in band 6.
Cholinesterase = catalyzes hydrolysis of 6-bromo-2-naphthyl-carbonaphthoxy choline iodide found in bands 7, 8, and 9.
Aromatic esterases = catalyze hydrolysis of a-naphthyl-acetate found in bands 6, 7, 13, 14 and 15.
Transferrins = iron binding proteins found in bands 7, 8, 9, 10 and 11.
-globulin = band 16.

Migration rates of a number of plasma enzymes were identical in different individuals and in different breeds. Lactic dehydrogenase activity was distributed among four fractions. Weak leucine aminopeptidase activity was found in fraction 4 (Figures 4 and 6). Band 6 catalyzed the oxidation of p-phenylene diamine, a characteristic of mammalian ceruloplasmin. Three fractions tested positive for cholinesterases. Since localization of these enzymes was carried out on only 10 animals, the possibility of occurrence of intraspecific variation of these enzymes in cattle is not ruled out.

Transferrins and aromatic esterases varied in migration rates in different individuals and in their distribution among breeds. In the 350 sera tested five different components were found that exhibited aromatic esterase activity. Three of these were present in each serum. All sera contained the two weak esterases of fast migration rate in bands 6 and 7 and a third very active esterase. In about 97% of the animals, this very active esterase migrated in band 15; in 14 animals it occurred in band 14. The very active esterase migrated in band 13 only in Sindhi bull. Neither the calf progeny of the Sindhi bull nor 20 cattle containing one-fourth or less of Sindhi heritage, exhibited the band 13 esterase.

Five electrophoretically distinct iron-binding proteins were found in sera of these cattle. Three to five components were found in individual sera; these were distributed in five different patterns (Figures 5 and 6) as described by Smithies (72). Distribution of transferrin types in the purebreds and crossbreds is given in Table 8; gene frequencies are listed in Table 9. Transferrin types $Tf^{D/E}$ and $Tf^{A/E}$ were not present in purebred Holsteins, Brown Swiss, and Jersey nor in Brown Swiss x Jersey. The gene frequencies of transferrins of the Louisiana State

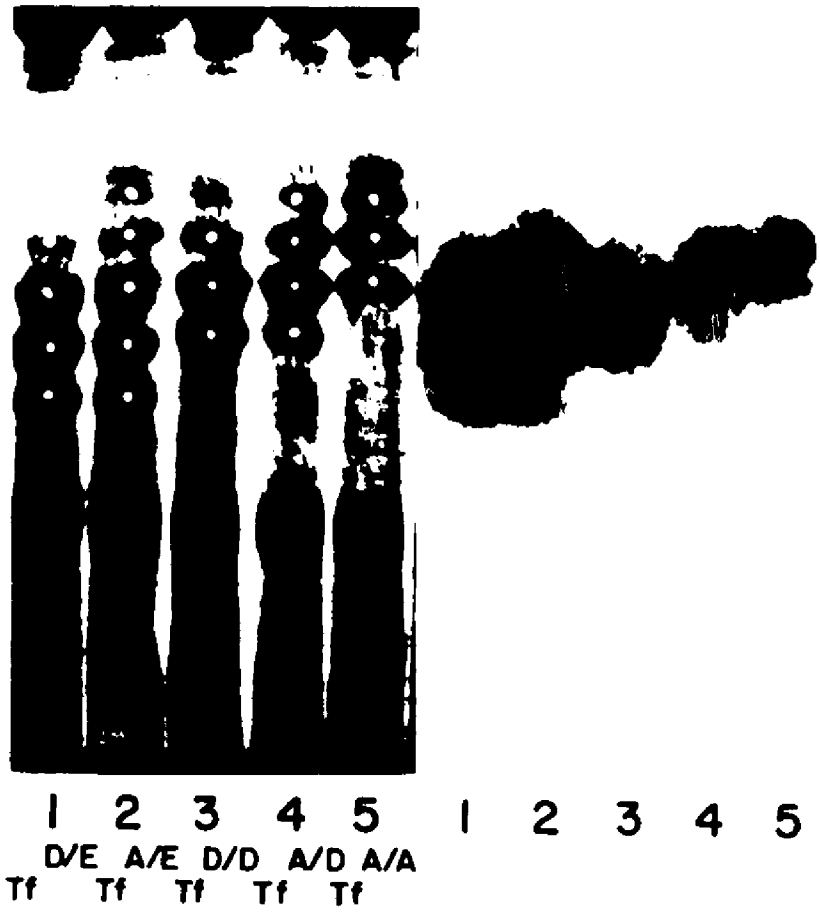

Figure 5. Transferrin variants in cattle. The picture on the left is of starch gel pattern of serum proteins. Dots identify fractions with iron-binding activity. These were localized from the radio-autograph on the right. Letters below the photographs indicate transferrin genotype. Anode is toward the top of the picture; sample slots toward the bottom.

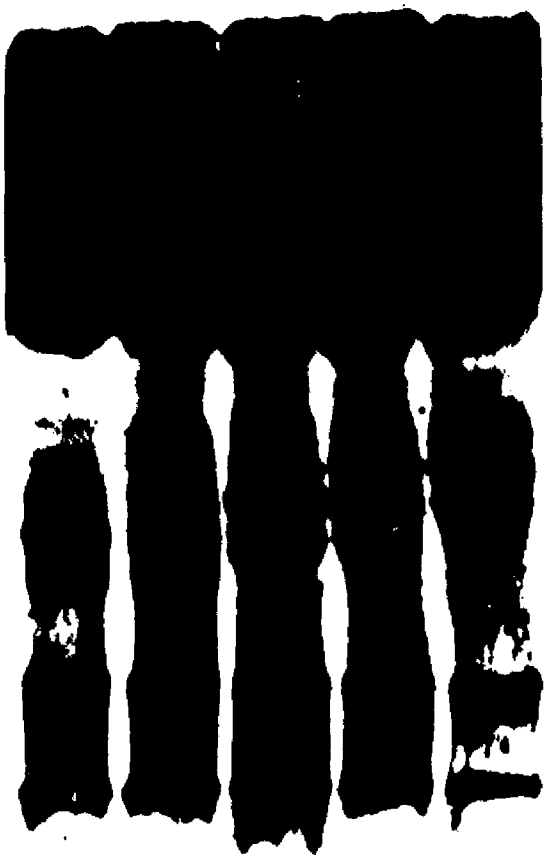

Figure 6. Starch gel electropherogram of plasma proteins of cattle. Numbers to the left of the bands refer to those numbers used to describe the fraction in the text and in Figure 4. Anode is to the top of the photograph.

Table 9. Gene frequencies of Transferrin and hemoglobin genes in Louisiana State University and Iberia Livestock Experimental Station dairy herds

Breed group	Transferrin gene	Gene frequency	Hemoglobin	Gene Frequency
		Louisiana State University Herd		
Holstein	Tf - A	0.362 ± 0.029	Bov. Hb ^{A}	1.00
	Tf - D	0.638 ± 0.029		
Jersey	Tf - A	0.455 ± 0.061	Bov. Hb^{A}	0.743 ± 0.052 1/
	Tf - D	0.545 ± 0.061	Bov. Hb^{B}	0.257 ± 0.052
Brown Swiss	Tf - A	0.308 ± 0.090	Bov. Hb^{A}	0.857 ± 0.066
	Tf - D	0.692 ± 0.090	Bov. Hb^{B}	0.143 ± 0.066
		Iberia Livestock Dairy Herd		
Holstein	Tf - A	0.449 ± 0.056	Bov. Hb^{A}	1.00
	Tf - D	0.551 ± 0.056		
European Crosses	Tf - A	0.442 ± 0.053	Bov. Hb^{A}	0.952 ± 0.023
	Tf - D	0.489 ± 0.054	Bov. Hb^{B}	0.048 ± 0.023
	Tf - E	0.069 ± 0.027		
Sindhi Crosses	Tf - A	0.333 ± 0.041	Bov. Hb^{A}	0.811 ± 0.034
	Tf - D	0.500 ± 0.043	Bov. Hb^{A}	0.189 ± 0.034
	Tf - E	0.167 ± 0.032		

1/ Standard error.

University Holstein herd did not differ significantly from those of the Iberia Holstein herd (Appendix Table 1). Likewise, there were no statistically significant differences in transferrin frequencies between herds of different purebreds. Although transferrin types $Tf^{D/E}$ and $Tf^{A/E}$ were present in the crossbred cattle of Holstein as well as Sindhi lineage, the Tf - E gene was more frequent in Sindhi crosses than in European crosses ($P<0.05$) (Appendix Table 1).

There were no statistically significant differences in milk production or fat production between animals of different Tf genotypes in the Louisiana State University Holstein herd (Appendix Tables 2 - 3). Calculations based on pooled data on Holsteins of both herds indicated a lack of relationship between transferrin genotype and the number of services required per conception (Appendix Tables 4 - 6). Milk and fertility data on other breeds were not extensive enough to obtain a reliable estimate of possible relationship with transferrin types (Appendix Tables 4 - 6).

The two electrophoretically distinct hemoglobins described by Bangham (13) were found in sera of these cattle (Figure 7). These occurred in different individuals in three patterns, including one or both proteins. Distribution of hemoglobin types (Figure 7) in the purebreds and crossbreds is given in Table 8; gene frequencies are listed in Table 9. Holstein blood contains only Bov. Hb^{A}. The frequency of the gene for Bov. Hb^{B} is greater in cattle of Sindhi lineage than in European crosses ($P<0.01$) (Appendix Table 1). The purebred Sindhi bull was type Bov. Hb^{BB}.

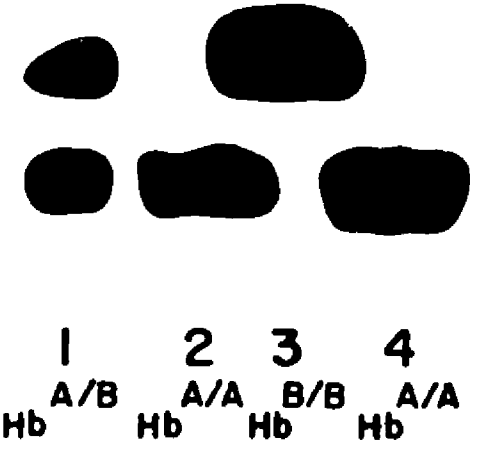

Figure 7. Hemoglobin variants in cattle. Letters below photograph indicate the hemoglobin genotype. Anode is toward the top of the photograph; sample slots toward the bottom.

Discussion

Proteins of six different activities were localized on gel electropherogram of cattle sera. Haptoglobins, present in plasma of many vertebrates, were absent in the four breeds examined. Glucuronidase and alkaline phosphatase were not found. Gahne (39), in his report on alkaline phosphatase variants in cattle, did localize phosphatase on gel electropherograms; however, he found many plasma samples which gave negative results. An enzyme which catalyzed the hydrolysis of leucine beta-naphthylamide occurred as a single band of weak activity. This enzyme attained high activity in certain reptilian forms (35) and during pregnancy, in female anthropoids (65). Leucine naphthylamidase, ceruloplasmin and lactic dehydrogenase migration rates did not vary among the different breeds. Vessel and Bearne (77) observed one band with the activity of lactic dehydrogenase in red cell hemolysates of a steer. Five different bands, three in each sample, had esterase activity. Of the three fractions catalyzing the hydrolysis of choline esters, one also utilized an aromatic ester as substrate. One aromatic esterase, that of the fastest mobility, occurred in all serum samples. The esterase of the slowest mobility occurred in three electrophoretic variants. One variant was found only in the Red Sindhi bull. As yet no evidence is available linking the variation to either genetic or physiological control. Augustinsson (11) previously resolved cattle plasma esterase by electrophoresis on cellulose column into aromatic and cholinesterase fractions.

Of the six patterns of transferrins reported in populations of cattle of European breeds, five were found in the animals of the Louisiana

herds. Transferrin Tf-A and Tf-D gene frequencies in the Holstein herds were similar to frequencies reported for other North American and European Holstein herds (4, 48, 72). Frequencies of Tf-A and Tf-D genes in the Jersey herd were similar to those found in a group of Jersey bulls from New York (48). Large variations were observed in frequencies of Tf-A and Tf-D genes in three Jersey herds from England; however, in all cases, the Tf-A gene was more common in North American population. The Louisiana Brown Swiss animals had the lowest frequency of Tf-A and highest frequency of Tf-D of the purebred Louisiana herds. The Tf-B and Tf-F transferrins found by Ashton in Sindhi and Sindhi crosses (7) were not present in Louisiana cattle.

The Tf-E gene was absent from the Holstein, Jersey and Brown Swiss cattle of Louisiana herds. Although present in crossbreds, the frequency of Tf-E gene was lower in European crosses than in Sindhi crosses. Ashton (7), on the basis of gene distribution among cattle of British Isles, suggested that Tf-E gene was less frequent in breeds with high adaptability coefficients. Braend et al.(21), on the basis of a study of transferrins of Icelandic cattle, did not support Ashton's contention. Ashton (7), in a later study, reported very high frequencies of Tf-E in Zebu breeds. Thus, even though the lower frequency of Tf-E genes found in the Louisiana herd would support Ashton's hypothesis, the evidence as a whole indicates that Tf-E gene does not have selective significance for hot climatic tolerance.

Ashton (8) obtained a positive correlation between $Tf^{D/D}$ genotype in bulls and the average milk production of their daughters. Rausch et al. (68) found that cows of Tf-E genotypes were poorest milk producers.

Although Datta and Stone (33) observed that cows of $Tf^{D/D}$ genotype produced slightly higher milk yields than cows of $Tf^{A/A}$ and $Tf^{D/D}$ genotype, their data did not show the presence of a statistically significant relationship between the transferrin types and milk or milk fat productivity. Results of Brummerstedt-Hensen et al. (27) working with the Danish cattle were similar to the findings of the present study indicating a lack of relationship between transferrin variants and milk or milk fat productivity.

Ashton (9) also found higher fertility rates in matings between cattle of homozygous transferrin types. Rates were highest for crosses in which both mates were of the same genotype. Hickman and Dunn (48) obtained data which agreed with the relation between transferrin type and fertility; however, their results indicated that unlike matings had the reproductive advantage. Other workers have failed to confirm a relationship between transferrins and fertility (27, 33). Likewise, results of this study based on pooled data on Holsteins relative to number of services per conception did not indicate a correlation with transferrin type.

Comparison of proteins of different organisms can shed light upon their ancestry (13). Evidence from this study on the distribution of hemoglobin variants confirms the high incidence of hemoglobin B gene among cattle of Zebu ancestry, and the moderate occurrence of Bov. Hb^{B} gene in Jersey and Brown Swiss breeds. Bangham (13) suggested that the presence of B gene in Jersey cattle might indicate Zebu ancestry. However, the Tf-E gene, which is absent in Jersey, is of high frequency in Zebu cattle. Further, Bov. Hb^{B} has not been found in Holsteins, yet

Holstein herds exhibit Tf-E frequency as high as 8.7% (68). Perhaps the Bov. Hb^B of Zebu and European breeds arose as independent mutant of identical or of different primary structure. The fact that two proteins have equal electrophoretic migration does not prove that they are of identical primary structure (50).

IV. GENERAL DISCUSSION

The investigations were centered around the environmental and genetic control of blood proteins in dairy cattle. Exposure of Holstein heifers to short term heat stress did not result in any change in either serum protein components or hematological attributes. When animals were exposed to long term heat stress, there was a decrease in hematocrit and an increase in serum protein. The results suggested, however, that there was no disturbance in either the synthesis or catabolism of the serum proteins. The heat stress imposed in a climatic chamber was not sufficient to elicit a response that could be useful as an index of adaptability.

Even though physiological differences in adaptability have been observed, these are difficult to use in selecting individuals with high climatic adaptability. Environmental chamber studies are costly and time consuming. Discovery of characters, genetically determined and linked to adaptability and productive efficiency would be of great economic importance. Such characters are often present from birth, and are usually easily determined. Although it was not possible to show any economic importance of biochemical polymorphism in these studies, the molecular approach to problems of selection may have great potential. Such studies are making important contributions to our knowledge of natural selection. A comparison of homologous proteins from different populations of a single species or of different species will contribute

estimates of minimum structural features of a protein that are essential for its biological function. Knowledge of structural differences, compatible with retention of function, should give clues concerning rates of mutation and their selectivity.

Hemoglobins offer excellent examples of how forces of natural selection affect a protein (50). All hemoglobins have, at least, one structural feature in common, the presence of the heme prosthetic group. Braunitzer (22) has shown that the sequence of amino acids on polypeptide chains adjacent to the heme group are almost identical in hemoglobins of widely divergent species. Most mutations about this "basic center" result in non-functional hemoglobins. Such mutants are selected against and remain in the population only as heterozygotes. Other sites along the hemoglobin polypeptide chains undergo mutation without affecting oxygen carriage. Certain mutant hemoglobins offer selective advantage to an animal in one environment and are selected against in another environment. For example, the hemoglobin of sheep living in low altitudes is apparently selected against in sheep living at high altitudes (49). Cattle Hb^A, probably, is selected against in areas of endemic trypanosomiasis (14). Hemoglobin S of man had selective advantage to Negroes when they lived in the malaria belt of Central Africa. When the Negro was transferred to North America, where malaria was absent, the frequency in the population of hemoglobin S gene decreased greatly within a few generations (63).

Evidence on a number of other proteins illustrate broader potential of research in this area. Rhodopsins of deep sea fish vary in their sensitivity to wave lengths of light from species to species.

These differences, related to protein structure, are associated with the quality of light in the environment (31). The number of residues of hydroxyproline in the collagens of fish is related to the temperature of the environment in which the fish lives (47). Such evidences suggest the manner in which the species variations in homologous proteins arose.

Many questions of future research were suggested by these studies. Is bovine Hb^B of Sindhi cattle structurally identical to hemoglobin B of European breeds? Although Sasakawa (70) has been studying the chemistry of cattle hemoglobin, neither he nor other investigators have determined the reason for the differences between cattle hemoglobin variants. Do any of cattle hemoglobins give selective advantage to animals living at high altitudes? Hill cattle of India range to altitudes of 14,000 feet. Will hemoglobins of the water buffalo (Bos bubalis), which is adapted well to the hot, humid lowlands of India, exhibit extensive differences in structure from hemoglobin of Yak, a ruminant adapted to the cold highlands of Tibet? Will other blood proteins such as esterases suggest selectivity? Will blood proteins give an evidence on interrelationship and on the origin of the various breeds of cattle?

V. GENERAL SUMMARY AND CONCLUSIONS

Investigations were conducted on the environmental and genetic control of blood proteins. In the environmental aspect of the study two experiments were conducted. The first, a short term heat stress exposure in a psychrometric chamber involved 28 heifers. Animals were subjected to 65°F for 48 hours and then exposed for 8 hours at 95°F. In the second experiment, 10 Holstein heifers were exposed to 65°F for 4 weeks (treatment 1), simulated summer conditions (a regimen of environmental temperatures cycling diurnally from 65°F to 95°F) for 8 weeks (treatment 2) and at 65°F for 4 weeks (treatment 3). The measure of responses were hemoglobin, hematocrit, RBC, total serum protein concentration, serum protein fractions and growth response.

There were no significant differences between the mean values of the blood constituents (except in hemoglobin) at 65°F and 95°F in short term study. In the second experiment, there was a significantly lower ($P < 0.05$) value for the hematocrit in treatment 2 than in treatment 1 and 3. Total serum protein concentration and absolute values for α-globulin showed highly significant increases ($P < 0.01$). β-globulin and γ-globulin showed significant increases ($P < 0.05$) in treatment 2. There were no differences between treatments 1 and 3. Other constituents showed no change in any of the treatments. Mean body weight gain per day showed a highly significant decline ($P < 0.01$) in treatment 2. There was a significant difference ($P < 0.05$) between the mean values of treatments 1 and 3, the value being higher in the latter.

In the genetic phase of the research, transferrin and hemoglobin gene frequency was obtained on 350 adult females of four pure breeds (Holstein, Jersey, Brown Swiss and a Red Sindhi bull) and crossbreds from Louisiana State University and Iberia Livestock Experiment Station dairy herds. An attempt was made to relate the transferrin genotype to milk, milk fat and fertility. In addition, other protein fractions of starch gel electropherogram were identified. Proteins of six different activities were localized. Haptoglobin, alkaline phosphatase and glucuronidase were absent. Lactic dehydrogenase was distributed among four bands. Weak leucine aminopeptidase activity and ceruloplasmin activity were localized in single bands. Three fractions tested positive for cholinesterases. None of these showed differences in mobility, in the four breeds examined. Transferrin and aromatic esterases varied in migration rate between individuals within breed. Five different components were found that exhibited aromatic esterase activity. Those of slowest anodal migration showed three variants: 97% of animals had slowest, 14 moderate, and the Sindhi bull the fastest. Neither the half bred progeny of the Sindhi bull nor 20 animals with one-fourth or less of Sindhi heritage exhibited the characteristic band of Sindhi bull. The transferrin $Tf^{D/E}$ and $Tf^{A/E}$ were absent from purebreds and Brown Swiss x Jersey. Holstein crosses had significantly lower ($P < 0.05$) Tf-E genes than Sindhi crosses.

No statistically significant differences were found in milk and milk fat production of different Tf genotypes in the Louisiana State University Holstein data. There were no mean differences in fertility of

different Tf genotypes. Other data were inconclusive due to lack of sufficient records.

Two electrophoretically distinct hemoglobin homozygous forms were found. Bov. Hb^B gene was completely absent from Holstein herds. Bov. Hb^B gene was significantly lower ($P < 0.01$) in Jersey and Brown Swiss crosses than in Sindhi crosses.

From the results obtained, the following conclusions seem to be justified.

1. The effect of climatic stress of short duration is not sufficient enough to evoke responses in protein and hematological attributes.

2. The simulated Louisiana summer condition is not adequate to produce any disturbance in serum protein metabolism. Thus, there is no possibility of using serum protein changes in formulating an adaptability index.

3. At present, there does not appear to be any association between transferrin genotypes and economic characters. Hence the possibility of using transferrins as genetic markers for selection is precluded at present.

4. More extensive research is needed at the molecular level to relate molecular differences with economic characters.

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VII. APPENDIX

Appendix Table 1. Statistical analysis of gene frequency differences of transferrin and hemoglobin.

Breed	Location	Tf type	Gene frequency	Statistical analysis	"t" values	Significance
Holstein	L.S.U.	Tf^A	0.362 ± 0.029)	Difference within Breed of two herds	1.38	N.S.
Holstein	Iberia	Tf^A	0.449 ± 0.056)			
European Crosses	Iberia	Tf^E	0.069 ± 0.027)	Difference between Tf-E of European and Sindhi crosses	2.33	*
Sindhi Crosses		Tf^E	0.167 ± 0.032)			
			Hemoglobin			
European Crosses	Iberia	$Bov.Hb^B$	0.048 ± 0.023)	Difference between $Bov.Hb^B$ of European and Sindhi crosses	3.44	**
Sindhi Crosses	Iberia	$Bov.Hb^B$	0.189 ± 0.034)			

N.S. = Non Significant.
* = Significant at 5% level.
** = Significant at 1% level.

Appendix Table 2. Analysis of variance of milk production between transferrin genotypes in Louisiana State University Holstein herds

Component	D.F.	Sum of squares	Mean S.S.	F	
Total	14	2,605,895,644			
Between seasons	4	2,287,212,685	571,803,171		
Among genotypes	2	38,403,865	19,201,932	0.54	N.S.*
Residual	8	280,279,094	35,034,886		

* = Non Significant.

Appendix Table 3. Analysis of variance of fat production between transferrin genotypes in Louisiana State University Holstein herd

Component	D.F.	Sum of squares	Mean S.S.	F	
Total	14	4,077,811			
Between year-season	4	2,569,201	642,300		
Among genotypes	2	201,325	100,662	0.616	N.S.*
Residual	8	1,307,285	163,410		

* = Non Significant.

Appendix Table 4. Mean differences in milk production (pounds) between transferrin genotypes

Breed groups	Tf genotype				
	$Tf^{D/E}$	$Tf^{A/E}$	$Tf^{D/D}$	$Tf^{A/D}$	$Tf^{A/A}$
Holstein			13,735 (64)*	13,689 (91)	13,052 (22)
Jersey			8,276 (12)	8,500 (91)	7,871 (6)
Brown Swiss			14,038 (7)	11,472 (8)	14,264 (1)
Holstein crosses	11,915 (3)	11,127 (2)	10,412 (20)	9,841 (27)	11,379 (5)
Brown Swiss crosses	9,263 (6)	9,613 (2)	10,028 (15)	10,553 (19)	11,362 (5)
Any cross with Sindhi	10,292 (8)	10,983 (2)	10,773 (22)	10,274 (21)	11,761 (4)

*Figure in parenthesis represents number of observations.

Appendix Table 5. Mean differences in butter fat production between transferrin genotypes.

Breed groups	Tf Genotype				
	Tf D/E	Tf A/E	Tf D/D	Tf A/D	Tf A/A
Holstein			481 (64)*	479 (91)	475 (22)
Jersey			406 (12)	414 (17)	395 (6)
Brown Swiss			523 (7)	426 (8)	487 (1)
Holstein crosses	424 (3)	488 (2)	423 (20)	381 (27)	479 (5)
Brown Swiss crosses	410 (6)	344 (2)	388 (15)	394 (19)	424 (5)
Only crosses with Sindhi	419 (8)	442 (2)	430 (22)	392 (21)	434 (4)

*Figure in parenthesis represents number of observations.

Appendix Table 6. Mean differences in number of services per conception between transferrin genotypes.

Breed groups	Tf Genotypes				
	$Tf^{D/E}$	$Tf^{A/E}$	$Tf^{D/D}$	$Tf^{A/D}$	$Tf^{A/A}$
Holstein			2.88 (64)*	2.65 (91)	2.27 (22)
Jersey			3.25 (12)	3.00 (17)	1.50 (6)
Brown Swiss			3.14 (7)	4.13 (8)	5.00 (1)
Holstein crosses	1.33 (3)	1.50 (2)	1.50 (20)	1.70 (27)	2.20 (5)
Brown Swiss crosses	1.17 (6)	2.00 (2)	1.27 (15)	1.68 (19)	1.40 (5)
Any crosses with Sindhi	1.25 (8)	2.00 (2)	1.50 (22)	1.90 (21)	2.25 (4)

*Figures in parenthesis represent number of observations.

VIII. VITA

Ravindra Kumar Srivastava was born in Gorakhpur, U.P., India, on May 7, 1932. He received his undergraduate education from St. Andrews College, Gorakhpur.

He entered U.P. College of Veterinary Science and Animal Husbandry, Mathura, in 1949 and earned his B.V.Sc. and A.H. degree in 1953 from Agra University. From 1953 to 1957, he served as Instructor in the Department of Physiology at the U.P. College of Veterinary Science and Animal Husbandry, Mathura.

In September 1957, he was deputed by the State Government for higher studies. He earned his M. V. Sc. degree with major in Physiology in 1959 from Agra University.

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Major Field: Dairy Science

Title of Thesis: Some Genetic and Environmental Aspects of Blood Proteins in Dairy Cattle.

Approved:

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