RESEARCH BULLETIN 859 APRIL 1964

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
ELMER R. KIEHL, *Director*

# Seasonal Variation in Levels of Some Chemical and Hematological Components in the Blood of Hereford Cows

C. E. STUFFLEBEAM, L. L. WILSON, D. T. MAYER, B. N. DAY,
J. E. COMFORT, AND J. F. LASLEY

(Publication Authorized April 22, 1964)

COLUMBIA, MISSOURI

## SUMMARY

Chemical and hematological determinations were made on the blood of 10 Hereford cows monthly for 13 months. Comparisons were made on a monthly and seasonal basis. In general, results agree quite well with other reports in the literature, especially those of Brody and some of his successors who worked with dairy cattle in a climatic laboratory at the University of Missouri.

Values for glutathione, glucuronic acid, serum phosphorus, serum protein, neutrophils and possibly true glucose, are apparently reduced under conditions of cold atmospheric temperatures (below 20°F.). Serum cholesterol levels and percent lymphocytes were generally lower in the summer than in winter. Hemoglobin, hematocrit and eosinophil numbers seem to be increased both by low and high temperatures. No important seasonal differences were observed in levels of blood glucose, blood creatinine, and serum calcium or in numbers of erythrocytes and total leukocytes.





This bulletin is a report on Department of Animal Husbandry research project 198, Cattle Improvement

# Seasonal Variation in Levels of Some Chemical and Hematological Components in the Blood of Hereford Cows

C. E. Stufflebeam, L. L. Wilson, D. T. Mayer, B. N. Day, J. E. Comfort, and J. F. Lasley

## INTRODUCTION

Inherited differences in the physiological make-up of cattle as they affect growth have been the object of many investigations in recent years. Studies have been and are being made to determine the genetic relationship between certain metabolic precursors and their end products in the blood and the rate and efficiency of growth in beef cattle. Since it is necessary to collect blood samples at various times throughout the year, the question arises as to what effect uncontrolled seasonal environment has upon the levels of certain chemical and hematological components. The purpose of this study was to determine if there were any normal seasonal trends in the levels of some of these components.

Many of the blood components determined in this study were also studied by Brody (1949), Blincoe and Brody (1951), and Kamal et al. (1959) under conditions of controlled temperature and humidity in a climatic laboratory at the Missouri Station.

## MATERIALS AND METHODS

### Animals Used

Blood samples were collected monthly from 10 Hereford cows, two and three years of age, beginning in August, 1962, and continuing through August, 1963. The cattle were maintained on grass pasture during the grazing season and on mixed hay in the winter. Adequate protein and mineral were supplied throughout the year.

### Design of Experiment

Comparisons were made among the four seasons. The three coldest months (December, January, and February) were designated the winter season. The other three seasons were divided logically among the

other nine months of the year. The data for August, 1962, were not used in the comparison of season means. However, these data were used in preparing the graphs in Figures 1, 2, and 3. Autumn data were not available for serum calcium and blood glucuronic acid. Serum cholesterol determinations were not made before November, 1962. For these three components, comparisons on a seasonal basis were made among winter, spring, and summer means only.

The data on total serum protein were collected during only eight months (November through June). In Table 1, these data are presented in four groups of two months each. Though data were not collected for the full four seasons, comparisons can be made between the two coldest months (January and February) and the two warmest months (May and June). These two groups of monthly means were designated winter and summer, respectively. November and December were called autumn while March and April were designated spring.

Erythrocyte counts were not made in October and November, 1962. The counts for August and September, 1962, are presented in Table 1 to represent the autumn season.

Duncan's New Multiple Range Test (Steel and Torrie, 1960) was used to test the significance of differences between all possible combinations of season means. The results of these tests are presented in Table 2.

## Whole Blood Chemistry

For all the analyses on whole blood, filtrates were prepared according to Haden's modification of the Folin and Wu method (Hawk et al., 1954). To obtain a sufficient amount of tungstic acid filtrate, five ml. of blood was added to 40 ml. of one-twelfth normal sulfuric acid in 25 x 200 mm. culture tubes. The tubes were stoppered and inverted once and allowed to set for about five minutes. Five ml. of 10 percent sodium tungstate was then added to each tube and the contents were mixed by shaking. The contents of each tube were filtered through a single layer of Whatman number 40 filter paper. A yield of about 25 ml. of filtrate was obtained from each sample.

Total reducing substances (Folin and Wu, 1920) will hereafter refer to the values obtained from the Folin-Wu method of glucose determination.

Two ml. of tungstic acid filtrate was placed in a Folin-Wu sugar tube graduated at 12.5 and 25 ml. To each tube two ml. of alkaline copper tartrate solution (Fisher reagent number So-A-324) was added. The tubes were immersed in boiling water for eight minutes and cooled in tap water for about 10 minutes. Two ml. of phosphomolybdic acid solution (Fisher reagent So-A-114) was added and diluted to the 12.5 ml. mark with distilled water. The tubes were covered, inverted, shaken vigorously and allowed to set for 10 minutes. The optical density of each sample was determined with a Fisher Electrophoto-

meter and a blue, number 425 filter and converted to units of mg. percent.

True glucose was determined according to the method reported by Saifer and Gerstenfeld (1958). The prepared glucose oxidase reagent used was a Dade product obtained from Scientific Products. Each vial of reagent contained a complete enzyme system consisting of the dried coupled enzymes, glucose oxidase and peroxidase, plus the chromogen o - dianisidine. The contents of a single vial were ready for use when reconstituted in 100 ml. of distilled water. The reagent was prepared shortly before use and set in a 37°C. water bath long enough for the temperature of the reagent to rise to that level.

Two ml. aliquots of tungstic acid filtrates were placed in 20 x 150 mm. culture tubes and set in a 37°C. water bath. Eight ml. of the pre-warmed glucose oxidase reagent was added to each tube and mixed with the contents. The reaction was allowed to proceed for exactly 30 minutes; then one-half ml. of one normal hydrochloric acid was added to each tube and mixed. The tubes were removed from the water bath and allowed to set for 10 minutes. The optical density was determined using a blue no. 425 filter and converting to units of mg. percent.

Saccharoids: These were determined by difference between the values obtained by the Folin-Wu method and those for true glucose. The units are mg. percent, which represents the amount of non-glucose reducing substances measured by the Folin-Wu method in glucose units or equivalents.

Glutathione (Stevenson et al., 1960) determinations were made within 30 minutes after the preparation of the filtrates had begun. The stability of glutathione in the filtrates began to decrease about an hour after preparation. The reagents used were reagent grade acetone, phosphate buffer pH 8.4 (Harleco reagent 4040) and recrystalized bis p-nitrophenyldisulfide (Eastman Organic Chemicals T 1855). The latter is hereafter referred to as PHPD. Recrystallization of the PNPD was accomplished by adding some of the crystals to glacial acetic acid and boiling the substance under a hood for 15 minutes. upon cooling, the acetic acid was decanted. The wet PNPD crystals were placed on a watch glass and air dried for 12 hours. Further drying was attained in an oven at 105 C. for another 12 hours. The working PNPD reagent was prepared by placing 30.8 mg. of the dry crystals in a 100 ml. volumetric flask and diluting to the mark with reagent grade acetone. The prepared reagent was refrigerated and prepared fresh about every two months.

Two ml. aliquots of freshly prepared tungstic acid filtrates were placed in micro cuvettes (one cm. in diameter with a volume of about six ml.). One-half ml. of phosphate buffer and two ml. of acetone were added to each cuvette. One-half ml. of PNPD reagent was then added and the contents were mixed by inversion. As soon as the bubbles had disappeared (about 30 seconds), the optical density was determined

using the blue no. 425 filter. The color produced was unstable so it was necessary that the readings be made immediately. Values for glutathione were expressed in units of mg. per 100 ml. of red blood cells. These values were obtained by dividing whole blood glatathione by the hematocrit value and multiplying by 100.

Uric Acid (Caraway 1955): Three ml. aliquots of tungstic acid filtrates were placed in micro cuvettes. One ml. of 10 percent sodium carbonate was added and mixed with the filtrate. Uric acid reagent (Aloe no. VS-3790) was diluted to 10 percent and one ml. of this was added to each cuvette and mixed with the contents. After it had set for 10 minutes, the optical density of the mixture was determined using a red no. 650 filter and converting to units of mg. percent.

Glucuronic Acid Miettinen et al., 1957): A solution of 0.2 percent naphthoresorcinol was prepared and allowed to set 24 hours in a 37°C. water bath. Immediately before use, equal volumes of filtered naphthoresorcinol solution and concentrated hydrochloric acid were combined. Four ml. of the freshly prepared reagent was added to two-ml. aliquots of tungstic acid filtrates in 20 x 150 mm. culture tubes. The tubes were placed in a water bath at 98°C. for 30 minutes and cooled in running tap water for about 10 minutes. Ten ml. of butyl acetate was added to each tube and the contents were shaken vigorously until the precipitate was dissolved (15 to 20 seconds was usually sufficient). The colored top layer was poured into dry cuvettes and allowed to set 10 minutes. Sometimes a fluorescence was seen but this did not interfere with the optical density. The green no. 525 filter was used in determining the optical densities which were then converted to units of mg. percent.

Creatine and Creatinine (Hawk et al., 1954): Alkaline picrate reagent was prepared just prior to its use by adding one volume of 10 percent sodium hydroxide to five volumes of saturated picric acid.

Three ml. aliquots of tungstic acid filtrates were placed in culture tubes for the creatine determination. The tubes were covered with aluminum foil and placed in an autoclave for 30 minutes at 250°F. This procedure converted the creatine to creatinine. Three ml. quantities of filtrate were also placed in micro cuvettes for the creatinine determination.

Two ml. of alkaline picrate reagent was added to each tube and cuvette, and mixed with the contents by inversion. The mixture was allowed to set for 10 minutes, then optical densities were determined, using the no. 525 filter and converting readings to mg. percent. (NOTE: After filtrates are removed from the autoclave, they must be cooled to room temperature before adding the alkaline picrate solution. Hot filtrates mixed with the picrate solution produced much darker colors.)

Serum Chemistry

Total Cholesterol (Ferro and Ham, 1960): A color development mixture (CDM) was prepared by adding one volume of concentrated sulfuric acid to a stock solution of acetic acid-anhydride made by mixing three volumes of acetic anhydride with two volumes of glacial acetic acid. The CDM was prepared at least one hour before it was to be used and allowed to cool down to at least 10°C. in a refrigerator. Cooling of the CDM was necessary to prevent charring of the serum. The CDM is reported to be stable for one day.

Ten ml. of cold CDM was added to 0.2 ml. of serum in a culture tube and mixed with it. After the mixture had been allowed to set for ten minutes at room temperature, optical densities were determined using the red no. 650 filter and converting results to mg. percent.

Total serum protein was determined using a procedure similar to the one reported by Reinhold (1953). Fifty ml. of 16 percent sodium hydroxide was placed in a liter flask containing about 500 ml. of distilled water. Nine gm. of sodium potassium tartrate (Rochelle salt), three gm. of finely powdered copper sulfate crystals ($CuSo_4 - 5H_20$), and five gm. of potassium iodine were added to the flask and thoroughly mixed. After the crystals had been dissolved, water was added to the one liter mark and mixed. The reagent was stable.

Five ml. of the biuret reagent above was added to 0.1 ml. of serum in micro cuvettes and mixed with it. After 30 minutes the optical densities were determined, using the green no. 525 filter and converting results to units of percent.

Calcium (Ferro and Ham, 1960) determinations required the use of 50 percent isopropyl alcohol, five percent aqueus solution of tetrasodium ethylenediaminetetraacetate (EDTA), and sodium chloranilate. Sodium chloranilate was prepared as follows: seven ml. of 10 percent sodium hydroxide was placed in a 250 ml. volumetric flask containing about 125 ml. of distilled water; to this 2.5 ml. of chloranilic acid (Eastman Organic Chemicals 4539) and enough distilled water were added to bring the volume to the 250 ml. mark. The contents were then thoroughly mixed/filtered. A calcium standard (1.0 ml. = 0.1 mg.) was prepared by dissolving 0.3594 gm. of dry calcium chloride in distilled water and diluting to one liter.

One ml. of sodium chloranilate was added to one ml. of serum in a glass centrifuge tube and mixed well. The tubes were allowed to set for 30 minutes, then centrifuged for 10 minutes. The supernatant liquid was decanted and the tubes were allowed to drain for two or three minutes on absorbent paper. About five ml. of 50 percent isopropyl alcohol was introduced into each tube in a fine stream from a polyethylene wash bottle. The precipitate was broken up and resuspended. The tubes were again centrifuged, decanted, and drained as before. Five ml. of five percent EDTA was added and the precipitate

dissolved by shaking. Para film was used to cover the tubes while they were being shaken. Optical densities were determined using the green no. 525 filter and converting results to mg. percent. The pink color was stable and could be read immediately or after several hours.

Phosphorus (Fiske and Subbarow, 1925). A molybdic acid solution was prepared by adding 42 ml. of concentrated sulfuric acid to 150 ml. of distilled water in a 500 ml. volumetric flask; after the solution cooled, 100 ml. of solution containing 12.5 gm. of ammonium molybdate in distilled water was added. Distilled water was then added to the mark and the contents of the flask were thoroughly mixed.

Amino naphtholsulfonic acid (ANSA) was prepared by adding 2.5 ml. of 20 percent sodium sulfite to a 100 ml. volumetric flask containing 0.25 gm. of ANSA crystals and diluting to the mark with 15 percent sodium bisulfite. This reagent was prepared monthly and stored in a brown bottle in a refrigerator.

A stock standard solution was prepared by dissolving 0.3509 gm. of dry monopotassium phosphate in 500 ml. of distilled water in a liter volumetric flask and diluting to the mark with distilled water.

One ml. aliquots of 10 percent serum filtrates were added to two ml. of water in micro cuvettes. The filtrates were prepared by adding four ml. of water and five ml. of 10 percent trichloroacetic acid (TCA) to a tube containing one ml. of serum; the tubes were shaken and the contents filtered through Whatman no. 40 filter paper. One ml. of molybdic acid solution and one-half ml. of ANSA were added to each cuvette and mixed with the contents. The cuvettes were protected from direct light and allowed to set for 10 minutes. Optical densities were determined using the red no. 650 filter.

## Hematology

Hematocrit: Non-heparinized capillary tubes 1.5 mm. in diameter and 75 mm. long were filled to about three-fourths full with well mixed whole blood. One end of each tube was sealed over a gas flame. Tubes were placed in a hematocrit centrifuge and spun for 10 minutes at 7000 r.p.m. The height of the packed column of cells was expressed as a percent of the total height of cells plus plasma.

Red cell count: Well mixed whole blood was drawn to the 0.5 mark in a standard red cell diluting pipette and diluted to the 101 mark with 0.9 percent sodium chloride. The pipette was shaken for several minutes by hand and the first few drops were discarded. The red cell counting chamber on a standard hemacytometer was flooded and about three minutes were allowed for the cells to settle. The cells in 80 small squares were counted and four ciphers were added. The resulting figure was an estimate of the number of circulating erythrocytes per cmm. of whole blood.

Hemoglobin was determined by adding 0.02 ml. of well mixed whole blood to five ml. of hemoglobin diluent (Ortho Aculute) in a cuvette. The substance was mixed and, after 10 minutes, the optical density was determined using a green no. 525 filter. Hemoglobin was expressed in terms of gm. percent of whole blood and in gm. percent of the packed cells. The latter is usually referred to as mean corpuscular hemoglobin concentration and is determined by dividing the hemoglobin value by hematocrit and multiplying by 100.

Total white cell count: Well mixed whole blood was diluted 1:19 in standard white cell diluting pipettes with a diluting fluid prepared by mixing 2.0 ml. of glacial acetic acid and 1.0 ml. of Gentian violet with 100 ml. of distilled water. Counts were made on a standard hemacytometer with counting chambers 0.1 mm. deep. An estimate of the number of white cells per cmm. of whole blood was determined by multiplying the number of cells counted in 4 large squares by 50.

Lymphocytes and Neutrophils: Differential counts were made from dry blood smears stained with modified Wright's stain. This stain was prepared as follows: 3.0 gm. of powdered Wright's stain, 0.3 gm. of Giemsa's stain and 30 ml. of glycerine were ground together to a paste in a mortar. A buffer solution of 1.6 gm. of monobasic potassium phosphate and 3.2 gm. of dibasic sodium phosphate was dissolved in 970 ml. of methyl alcohol. The buffer solution was mixed with the stain-glycerine paste and shaken for 20 minutes, allowed to set for 24 hours, and then filtered. The dry smears were flooded with the modified stain for 15 seconds, diluted with water for 15 seconds and washed with distilled water and allowed to dry. One hundred cells (lymphocytes, neutrophils and eosinophils) were counted on each smear to determine the percentage of each kind of cell. On the basis of the number of total white cells and the percentages of each kind present, the absolute or total numbers of lymphocytes and neutrophils per cmm. were calculated.

Eosinophils: Because of the relatively few numbers present in the blood, eosinophils were counted by the more accurate direct method of Randolph (1949). The staining solution was prepared by mixing three parts of 0.1 percent phloxine in propylene glycol and two parts of 0.1 percent Methylene Blue in propylene glycol with five parts of distilled water just prior to use. If a drop of the fresh stain is put on filter paper, the methylene blue remains in the center of the spot and the phloxine moves to the edges of the spot forming a red ring. After four to six hours this differentiation does not occur and fresh stain must be prepared. Well mixed whole blood was diluted 1:9 in standard white cell pipettes and shaken for seven to 10 minutes. The first three or four drops in the pipette were discarded and the counting chamber of a Fuchs-Rosenthal Ultra Plane hemacytometer was flooded. The chambers of this particular hemacytometer were 0.2 mm. deep. Because of the viscosity of the staining solution, the cells

were allowed to settle for about five or 10 minutes after the chambers were flooded. The number of cells per cmm. of whole blood was determined by multiplying the number of cells counted in eight large squares by 6.25.

## RESULTS AND DISCUSSION

The first graph in Figure 1 shows the atmospheric temperatures in terms of the overnight lows on the mornings when blood samples were collected and high temperature of the previous day. Generally, temperatures decreased steadily from August through February. Temperatures began to rise sharply after February until about June when they reached a plateau.

### Glucose and Saccharoids

Total reducing substances (actually, substances determined by the Folin-Wu method of glucose determination) and true glucose levels were quite erratic except for the high values in February, the coldest month. For several weeks previous to that time, the temperature had been near 0 °F. and below. Values for total reducing substances reached a low in April. The mean value for the spring season was low enough to be significant at the one percent level according to Duncan's New Multiple Range Test. A similar low was noted in the levels of saccharoids. Except for the high values in February and March, no important seasonal trend was noticed for true glucose.

These results are similar to those of Brody (1949) who observed no particular response in glucose (Folin-Wu) levels between 50° and 100° F. Blincoe and Brody (1951) observed a general increase in glucose levels in dairy cows with decreasing temperatures to near 5° F. These two studies were made in a climatic laboratory under controlled conditions.

### Hematology Associated with the Red Blood Cell

In general, it appears that hemoglobin and hematocrit values are increased under conditions of either high or low temperatures. In the present study, values were highest in the summer months ($P < .01$). Lowest values were observed in November and April with relatively high values in the cold months. Patterson et al. (1960) also observed higher hemoglobin levels in summer than in winter in dairy cows. However, on eight Japanese subjects, Yoshimura (1958) reported lower hematocrit values in the summer. He further reported that these variations were explained by changes in the water content of the serum.

Munday and Blane (1960) found that hematocrit levels in rats decreased steadily for 48 hours in a cold environment. Reducing the rectal temperature of hamsters to 14° C. resulted in an increase in hematocrit (Beaton, 1956). After cooling to 25 ° or warming to 42.5 °C., Spurr and Barlow (1959) reported that menatocrit levels increased. Blincoe and Brody (1951) reported no definite relationship between ambient temperatures in the range from 40 to 105 ° F. in a climatic laboratory and hemoglobin and hematocrit levels or erythrocyte numbers in dairy cows.

In the present study, no definite seasonal trend could be seen in erythrocyte numbers. However, the highest numbers of cells were counted in the two coldest months and in June.

The bottom row of graphs in Figure 3 shows seasonal variation in hemoglobin per 100 ml. of erythrocytes, hemoglobin in a single erythrocyte, and erythrocyte size. These three components are referred to in the literature as mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV), respectively. MCHC is calculated by multiplying the hemoglobin-hematocrit ratio by 100. Standard deviations are not presented since the data for the graph were calculated from monthly means and not from individual values. No consistent seasonal trend is apparent; but there does appear to be a general decrease with increasing age after October. Ten times the hemoglobin-erythrocyte ratio produced the data for MCH. Erythrocyte size (MCV) was determined as 10 times the hematocrit-erythrocyte ratio. These data were also calculated from monthly means so standard deviations were not calculated. Corpuscular hemoglobin as well as corpuscle size tended to increase with advancing age of the cows. Corpuscle size appears to be greater in summer than in winter.

## Glutathione and Glucuronic Acid

Glutathione is presented as mg. per 100 ml. of red cells because preliminary work had shown no trace of glutathione in the plasma. Also, due to variations in hematocrit, expression in these terms should be more accurate. Except for the relatively high values in January, glutathione levels follow a definite seasonal trend with lower levels associated with colder temperatures. Kamal et al. (1959) measured blood glutathione in dairy calves during growth from six to 12 months of age in controlled temperatures of 50° and 80° F. Mean values were 60 percent higher in the warmer environment. Patterson et al. (1960) also reported higher values for blood glutathione and glutathione-hemoglobin ratios in summer in Holstein and Jersey cows. A slight increase in blood glutathione after reducing the rectal temperature of rats to 14° C. was reported by Beaton (1961).

Except for the extreme drop in glucuronic acid levels in August, 1963, this blood component followed a definite seasonal trend. The lowest value occurred in the coldest month and levels increased

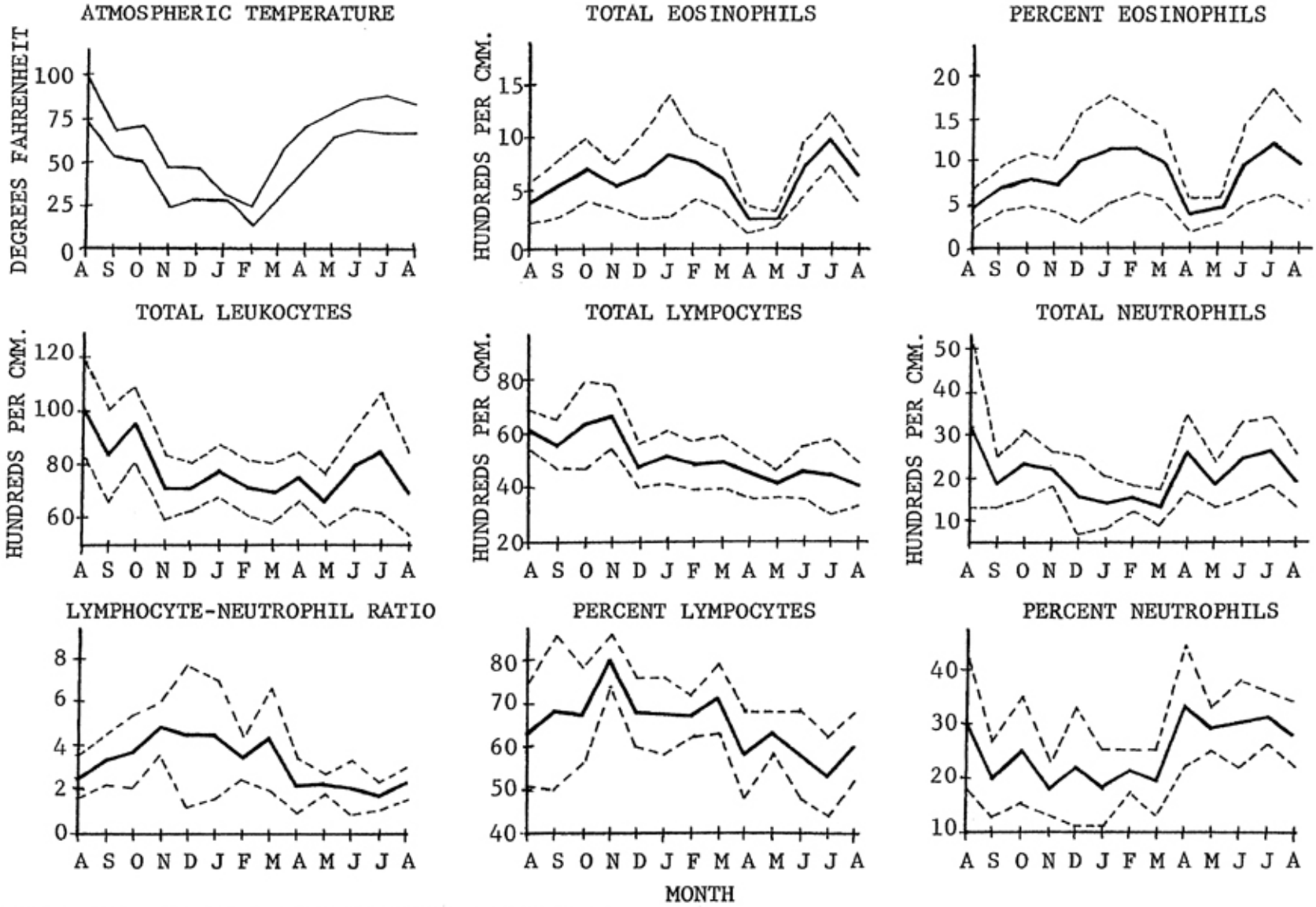


*Fig. 1—Seasonal variation in atmospheric temperature and in totals and percentages of various kinds of leukocytes in beef cows. Heavy lines represent monthly means; dotted lines represent one standard deviation on either side of the mean.*

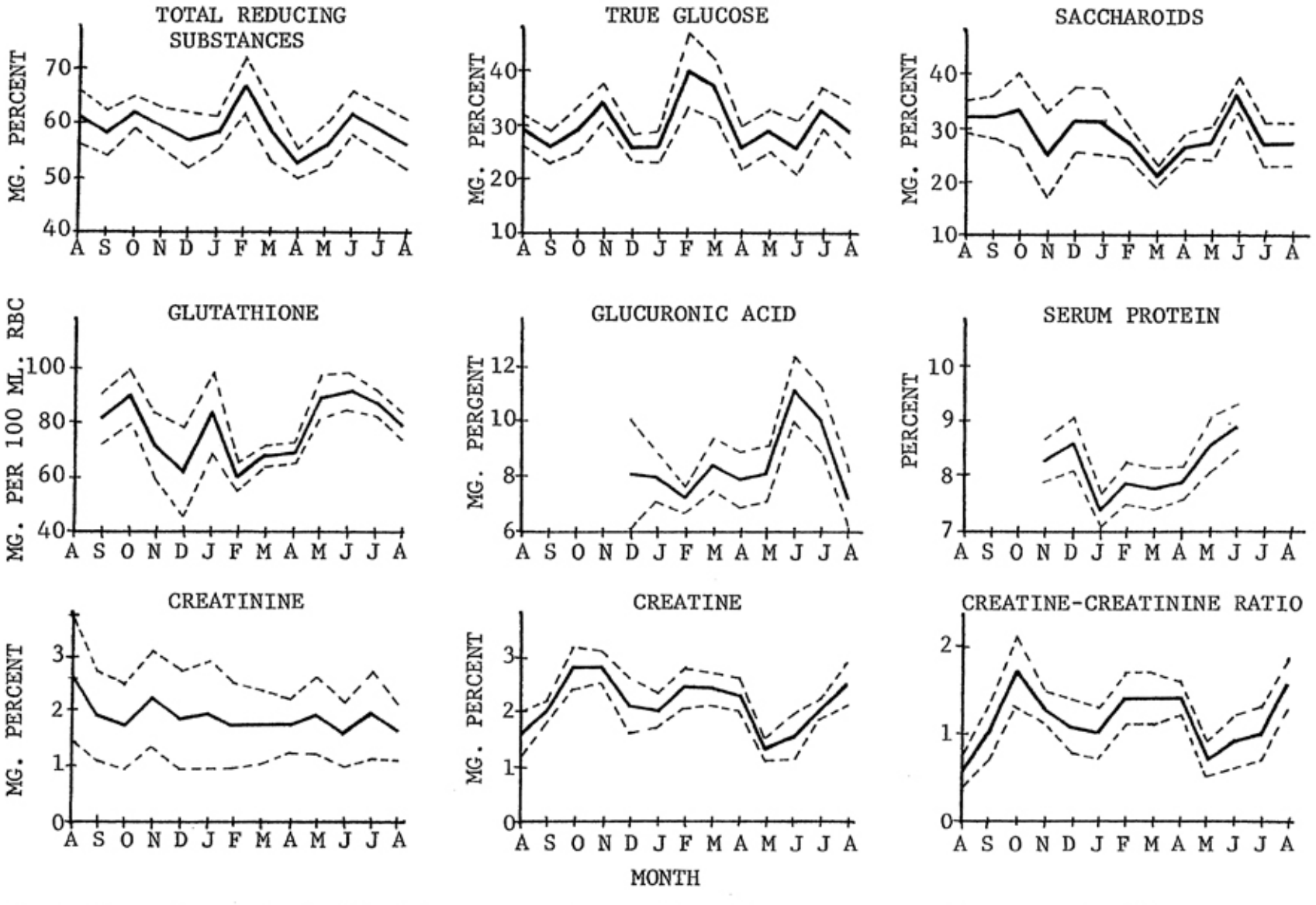


*Fig. 2—Seasonal variation in blood glucose, non-glucose reducing substances. and total serum protein in beef cows. Heavy lines represent monthly means; dotted lines represent one standard deviation on either side of the mean.*

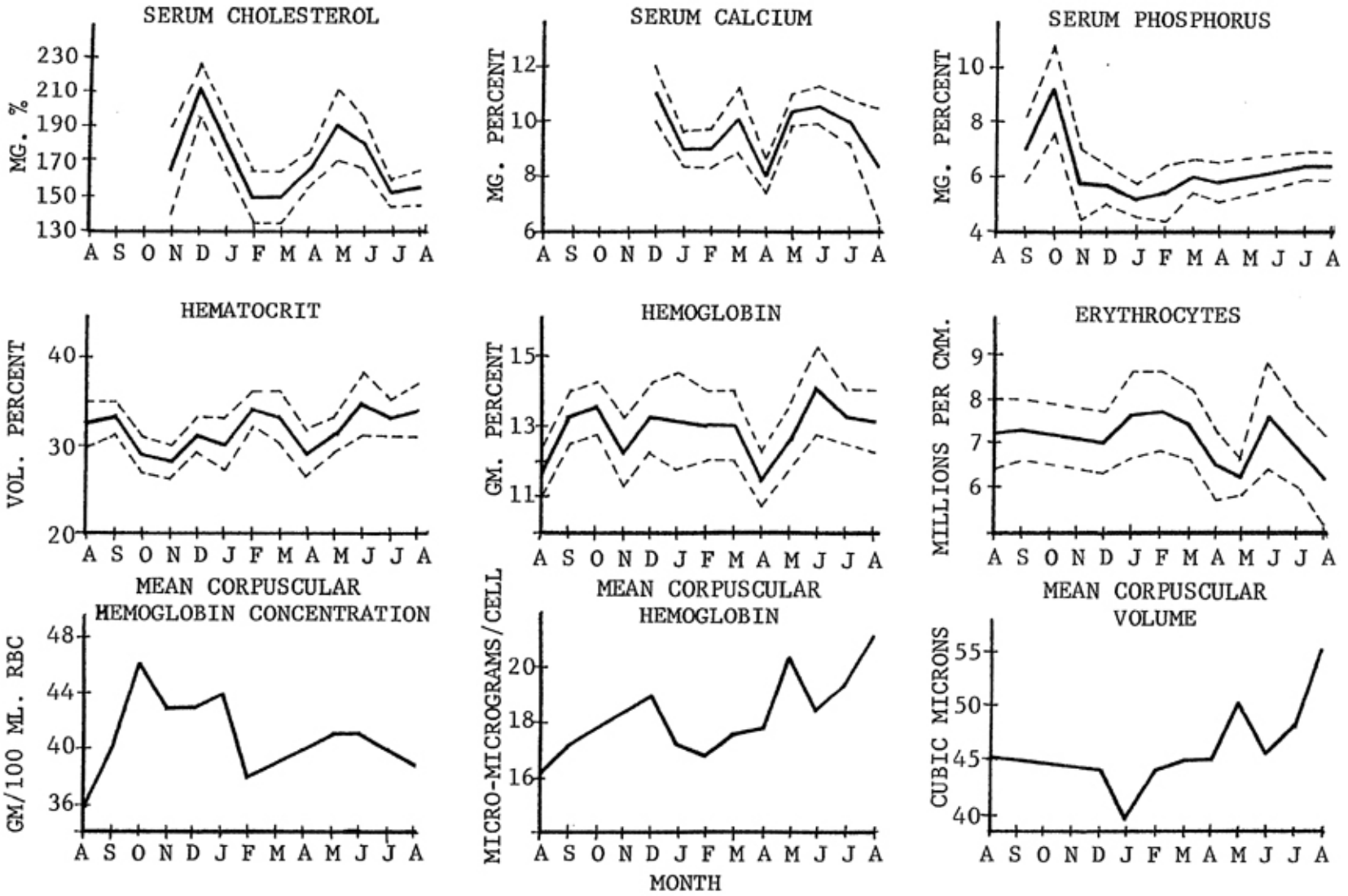


*Fig. 3—Seasonal variation in serum total cholesterol, calcium, and inorganic phosphorus and in ceratin hematogical measurements in beef cows. Heavy lines represent monthly means; dotted lines represent one standard deviation on either side of the mean.*

TABLE 1 - MEANS AND STANDARD DEVIATIONS OF VARIOUS BLOOD COMPONENTS IN TEN HEREFORD COWS AT VARIOUS SEASONS OF THE YEAR

| Blood Component (Units in Mg. % except where noted) | AUTUMN Means and Standard Deviations | | WINTER Means and Standard Deviations | | SPRING Means and Standard Deviations | | SUMMER Means and Standard Deviations | | Overall Means |
|---|---|---|---|---|---|---|---|---|---|
| Total Reducing Substances | 59.9 | 3.6 | 60.7 | 4.2 | 55.5 | 4.0 | 59.2 | 4.2 | 58.8 |
| True Glucose | 29.8 | 3.3 | 30.7 | 4.0 | 30.5 | 4.9 | 29.3 | 4.4 | 30.1 |
| Saccharoids | 30.1 | 6.3 | 30.0 | 4.8 | 25.0 | 3.7 | 29.9 | 3.8 | 28.8 |
| Glutathione (Mg. % of RBC) | 80.2 | 10.3 | 68.4 | 11.8 | 75.1 | 5.4 | 85.6 | 5.7 | 77.3 |
| Glucuronic Acid[a] | | | 7.7 | 1.1 | 8.1 | 1.0 | 9.5 | 1.1 | 8.4 |
| Creatinine | 1.91 | .84 | 1.83 | .91 | 1.75 | .64 | 1.69 | .65 | 1.80 |
| Creatine | 2.51 | .28 | 2.17 | .70 | 2.01 | .28 | 1.98 | .34 | 2.17 |
| Creatine-Creatinine Ratio | 1.33 | .31 | 1.20 | .31 | 1.16 | .25 | 1.19 | .25 | 1.22 |
| Serum Cholesterol[a] | | | 179 | 15 | 170 | 16 | 162 | 12 | 170 |
| Serum Protein (%) | 8.44 | .46 | 7.88 | .32 | 7.85 | .34 | 8.74 | .44 | 8.23 |
| Serum Calcium[a] | | | 9.66 | 1.2 | 9.54 | 1.2 | 9.67 | 1.8 | 9.62 |
| Serum Phosphorus | 7.3 | 1.4 | 5.5 | .8 | 5.9 | .6 | 6.4 | .5 | 6.3 |
| Hematocrit (Vol. %) | 30.2 | 2.0 | 31.6 | 2.5 | 31.1 | 2.5 | 33.9 | 2.7 | 31.7 |
| Hemoglobin (Gm.%) | 13.0 | .8 | 13.1 | 1.0 | 12.3 | .9 | 13.5 | 1.0 | 13.0 |
| Erythrocytes (Millions/cmm.) | 7.3 | .7 | 7.4 | .9 | 6.7 | .7 | 6.9 | 1.0 | 6.9 |
| Total Leukocytes (100's/cmm.) | 76.8 | 14.6 | 72.8 | 10.1 | 69.9 | 10.3 | 76.8 | 18.2 | 74.1 |
| Total Lymphocytes (100's/cmm.) | 61.6 | 12.9 | 49.2 | 8.9 | 44.8 | 8.6 | 43.3 | 10.5 | 49.7 |
| Total Neutrophils (100's/cmm.) | 18.9 | 7.2 | 15.1 | 6.5 | 18.8 | 7.8 | 23.2 | 8.2 | 19.0 |
| Total Eosinophils (100's/cmm.) | 6.4 | 2.5 | 8.0 | 3.8 | 4.3 | 2.4 | 8.3 | 2.8 | 6.75 |
| Percent Lymphocytes | 71.7 | 13.5 | 67.5 | 7.4 | 64.2 | 9.5 | 56.8 | 9.1 | 65.0 |
| Percent Neutrophils | 21.2 | 7.9 | 20.5 | 7.8 | 26.6 | 9.3 | 29.6 | 6.3 | 24.5 |
| Percent Eosinophils | 7.0 | 2.8 | 11.0 | 5.9 | 6.2 | 3.9 | 10.7 | 5.3 | 8.7 |
| Lymphocyte-Neutrophil Ratio | 4.04 | 1.66 | 4.11 | 2.56 | 2.94 | 1.83 | 2.10 | .91 | 3.14 |

[a]Data not available for the autumn season.

TABLE 2 - LEVEL OF SIGNIFICANCE AS MEASURED BY DUNCAN'S NEW MULTIPLE RANGE TEST BETWEEN SEASONAL MEANS OF VARIOUS BLOOD COMPONENTS OF TEN HEREFORD COWS[a]

| Blood Component (Units in Mg. % except where noted) | Autumn vs Winter | Autumn vs Spring | Autumn vs Summer | Winter vs Spring | Winter vs Summer | Spring vs Summer |
|---|---|---|---|---|---|---|
| Total Reducing Substances | | ** | | ** | | ** |
| True Glucose | | ** | | ** | | ** |
| Saccharoids | ** | | | * | ** | ** |
| Glutathione (Mg. % of RBC) | *** | *** | *** | | ** | ** |
| Glucuronic Acid | * | ** | ** | | | |
| Creatinine | | | | | | |
| Creatine | *** | *** | *** | | ** | |
| Creatine-Creatine Ratio | * | * | | | ** | ** |
| Serum Cholesterol | *** | *** | *** | | | |
| Serum Protein (%) | ** | ** | | | ** | |
| Serum Calcium | ** | ** | ** | | | ** |
| Serum Phosphorus | ** | * | ** | ** | | ** |
| Hematocrit (Vol. %) | | * | | | ** | |
| Hemoglobin (Gm. %) | | | | | | |
| Erythrocytes (Millions/cmm.) | | | | | | |
| Total Leukocytes (100's/cmm.) | ** | ** | ** | | * | |
| Total Lymphocytes (100's/cmm.) | | | * | | ** | * |
| Total Neutrophils (100's/cmm.) | * | ** | * | ** | | ** |
| Total Eosinophils (100's/cmm.) | | ** | ** | | ** | ** |
| Percent Lymphocytes | | ** | ** | ** | ** | |
| Percent Neutrophils | ** | | ** | ** | | ** |
| Percent Eosinophils | | * | ** | * | ** | |
| Lymphocyte-Neutrophil Ratio | | | | | | |

* P .05 ** P .01 *** No data available.

[a]A blank space indicates that the difference between means is not great enough to be significant at the five per cent level.

through the spring months to a high in June. The differences between the mean value for summer and either winter or spring were great enough to be significant at the one percent level according to Duncan's test.

## Creatine and Creatinine

No significant differences were observed between season means for blood creatinine. Creatine levels were highest in the autumn and lowest in the spring. No definite trend could be observed, although the autumn mean was high enough to constitute a real difference according to Duncan's test. There was an over-all decline in the levels of creatinine, creatine, and the creatine-creatinine ratio which corresponded to increases in age of the young cows. A marked difference can be seen in the variation associated with creatine and creatinine. Within-month variation in creatine was less than half that of creatinine (Figure 2).

Brody (1949) found that the creatinine levels increased two-fold in dairy cows subjected to increase in temperature from 80° to 100 °F. in a climatic laboratory. At that time, Brody and his associates had made no creatine determinations, but postulated that this increase in creatinine may have reflected an increase in creatine levels since creatinine was assumed to be derived from creatine. Later, Blincoe and Brody (1951) reported almost identical results from creatinine. Blood creatinine consistently rose with increasing temperatures and was associated with a decline in feed consumption. However, they found no corresponding rise in the creatine levels.

## Serum Cholesterol and Protein

When serum cholesterol values were summed by season, there seemed to be a definite decrease in summer as compared to winter and spring. Month to month variation seemed to establish no definite trend, however. Highest levels were observed in December, with lowest values in February and March. Levels then increased through May and fell again to another low in July.

Brody (1949) observed that cholesterol in dairy cows declined steadily with increasing ambient temperature. This decline was associated in part with a decline in milk production. The relationship between high cholesterol and low thyroid activity has led some workers to expect a reduction in thyroid activity and increased cholesterol levels in the summer. Data presented by Means and Andrews (1958), however, do not support this idea. Over 600 determinations were made on 157 ewes during a two-year period with no important differences between seasons. In another study by Fransen and Andrews (1958), no important seasonal effect was noticed. They did observe a decline following a severe cold period, however.

Total serum protein levels decreased in January and remained relatively constant through April. A sharp rise was noticed in May and

June. No protein determinations were made in the hottest months; however, it appears that warmer temperatures were associated with higher protein levels. Brody (1949) and Blincoe and Brody (1951) reported that increasing ambient temperatures did not affect the plasma protein levels of dairy cows.

In humans, Yoshimura (1958) reported protein decreases in summer and further stated that these decreases were explained by changes in the water content of the serum. In a much earlier report, Bazett et al. (1940) demonstrated that plasma protein levels in man increased with ambient temperature but soon returned to their initial value after continued exposure.

## Serum Calcium and Phosphorus

Phosphorus levels were highest in October, after which time there was a sharp decline to winter lows, followed by a gradual increase throughout the spring and summer months. This trend agrees in part with that observed by Marsh and Swingle (1960) who reported that in range cattle in Montana, phosphorus levels were higher in May, June, and July and dropped in late summer to autumn and winter lows. Brody (1949) also observed increases in serum phosphorus levels of dairy cattle with increases in ambient temperatures above 85° F. Cattle in 100 °C. temperatures had about 30 percent higher phosphorus levels than did the control animals. A general increase in phosphorus levels was noted with increases in temperatures from 70° to 100 °F. in dairy cattle by Blincoe and Brody (1951). They noticed no apparent change in phosphorus levels when temperatures were lowered from 50° to near 5 °F.

Monthly variations in serum calcium levels revealed no distinguishable seasonal trend. No differences were noted among the means for the three seasons. Brody (1949) as well as Blincoe and Brody (1951) observed no significant effect of ambient temperatures on blood calcium in dairy cows.

## Leukocytes

Month to month variations in numbers and percentages of the various kinds of leukocytes are presented graphically in Figure 1. In general, the number of total leukocytes appeared to be higher in the warmer months. A comparison of the season means produced no important differences. Total white cell count was highest in August, 1962, but the comparison among seasons does not include these data. Brody (1949) reported that the trend in leukocyte numbers in dairy cows under conditions of changing temperatures was uncertain.

Eosinophil numbers increased with decreasing atmospheric temperatures in autumn to monthly highs in January and February. The numbers decreased sharply with increasing temperatures to lows in April and May. Another sharp rise occurred in June and July. July values were

highest for the year. Eosinophils as percent of total leukocytes followed about the same trend. Blincoe and Brody (1951) reported a tendency for eosinophil numbers to decrease with increasing temperatures. The data from the present study would tend to support this. The period of sharpest increase in atmospheric temperatures (February through April) was associated with a sharp decline in eosinophil numbers.

Total numbers of lymphocytes decreased generally throughout the year except for increasing numbers in October and November, 1962. Expressed as the percent of total leukocytes, lymphocyte numbers tended to be lower in summer than in autumn and winter. Blincoe and Brody (1951) reported no decisive changes in lymphocytes in dairy cows with changes in ambient temperatures. Total neutrophil numbers as well as neutrophils as percent of total leukocytes were lowest in winter and highest in summer. The lymphocyte-neutrophil ratio was greatest in autumn and winter and lowest in summer.

## LITERATURE CITED

Bazett, H. C., J. W. Sunderman, J. Doupe and J. C. Scott. 1940. Climatic Effects on the Volume and Composition of Blood in Man. Am. J. Physiol. 129:69.

Beaton, J. R. 1956. Note on Blood Matabolite and Electrolyte Levels in the Hypothermic Hamster. Can. J. Biochem. Physiol. 39:1207.

Beaton, J. R. 1961. Further Observations on Metabolic Alterations in the Hypothermic rat. Can. J. Biochem. Physiol. 39:1.

Benedict, S. R. 1931. The Analysis of Whole Blood. II. The Determination of Sugars and of Saccharoids. J. Biol. Chem. 92:141.

Blincoe, C. and S. Brody. 1951. Environmental Physiology. XVII. The Influence of Temperature on Blood Composition of Cattle. Mo. Agr. Exp. Sta. Bul. 488.

Brody, S. 1949. Environmental Physiology. III. Ambient Temperatures, 50 ° to 100° F., On the Blood Composition of Jersey and Holstein Cows. Mo. Agr. Exp. Sta. Bul. 433.

Caraway, W. T. 1955. Determination of Uric Acid in Serum by a Carbonate Method. Amer. J. Clin. Path. 25:840.

Ferro, P. V. and A. B. Ham. 1960. Lab-Trol Manual. Revised Ed. Miami, Dade.

Fiske, C. H. and Y. Subbarow. 1925. The Colorimetric Determination of Phosphorus. J. Biol. Chem. 66:375.

Folin, O. and H. Wu. 1920. A simplified and Improved Method for the Determination of Sugar. J. Biol. Chem. 41:367.

Fransen, J. M. and F. N. Andrews. 1958. Blood Plasma Cholesterol Levels in Normal and Dwarf Beef Cattle. Amer. J. Vet. Res. 19:332.

Hawk, P. B., B. L. Oser and W. H. Summerson. 1954. "Practical Physiological Chemistry". 13th ed. Blakiston, New York.

Kamal, T. H., H. D. Johnson and A. C. Ragsdale. 1959. The Effect of Long Exposure and Environmental Temperatures of 50 ° and 80° F. on Glutathione, BEI 131 and Growth Rate of Dairy Calves. Mo. Agr. Exp. Sta. Res. Bul. 710.

Marsh, H. and K. F. Swingle. 1960. The Calcium, Phosphorus, Magnesium, Carotene and Vitamin A Content of the Blood of Range Cattle in Eastern Montana. Amer. J. Vet. Res. 21:212.

Means, T. M. and F. N. Andrews. 1958. The Influence of Season on Blood Plasma Cholesterol in the Ewe. Amer. J. Vet. Res. 19:295.

Miettinen, T., V. Ryhanen and H. Salomoa. 1957. Observations on the Determination of Glucuronic Acid. Annales Med. Exp. Biol. Fenn. 35:173.

Munday, K. A. and G. F. Blane. 1960. Changes in Electrolytes and 17-Oxosteroids in the Rat Subjected to Cold Environment. J. Endo. 20:266.

Patterson, T. B., R. R. Shrode, H. O. Kunkel, R. E. Leighton and I. W. Rupel. 1960. Variations in Certain Blood Components of Holstein and Jersey Cows and Their Relationship to Daily Range in Rectal Temperature and to Milk and Butterfat Production. J. Dairy Sci. 43:1263.

Randolph, T. G. 1949. Procedure for Direct Counting of Eosinophils. J. Lab. Clin. Med. 34:1696.

Reinhold, J. G. 1953. Total Protein, Albumin and Globulin. "Standard Methods of Clinical Chemistry". Vol. I. Miriam Reiner (Ed.) Academic Press, New York. pp. 88-91.

Saifer, A. and Shirley Gerstenfeld. 1958. The Photometric Microdetermination of Blood Glucose with Glucose Oxidase. J. Lab. Clin. Med. 51:448.

Spurr, G. B. and G. Barlow. 1959. Plasma and Electrolyte Sodium, Potassium, Chloride and Water in Hyperthermia and Hypothermia in Dogs. Amer. J. Physiol. 197:648.

Stevenson, T. D., B. L. McDonald and S. Roston. 1960. Colorimetric Method for Determination of Erythrocyte Glutathione. J. Lab. Clin. Med. 56:157.

Yoshimura, H. 1958. Seasonal Changes in Human Body Fluids. Japanese J. Physiol. 8:165.