DIFFERENTIAL PHYSIOLOGICAL RESPONSES OF BEEF CATTLE TO PROLONGED TEMPERATURE STRESS

By
HERNANDO GUTIERREZ DE LA R.

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
1969

ACKNOWLEDGEMENTS

The author wishes to express his sincere gratitude to Dr. A. C. Warnick, chairman of his supervisory committee for his valuable guidance, assistance and encouragement throughout the academic program, experimental investigation and preparation of this dissertation; and to Drs. R. L. Shirley, J. F. Hentges, Jr., J. A. Himes and H. R. Wilson for serving as members of the supervisory committee and for giving freely of their time and knowledge toward the completion of this work.

The author is grateful for the assistance given him by the faculty members, particularly Dr. J. E. Moore of the Department of Animal Science for his advise in the preparation of the rations, and to Drs. F. G. Martin and D. E. Franke and Miss Sara J. Harr for their assistance in the statistical analyses of the data.

Sincere appreciation is also expressed to Dr. Jerry Cowley and Messrs. M. Fields, M. Crooks and other fellow graduate students for their help in the collection of the samples. Special recognition is given to Mr. Dean Pogue for the care of the experimental animals and the help in the handling of the animals.

The financial support of the Rockefeller Foundation, which made this work possible is gratefully acknowledged. Appreciation is also expressed to the Institute Colombiano Agropecuario (Colombian Institute of Agriculture) for the granting of the study leave under which this program was possible. Special thanks are given to Dr. J. Hentges, Jr.

of the Purebred Cattle Experimental Unit at the University of Florida and to Dr. D. W. Beardsley of the Everglades Experiment Station for supplying the experimental animals.

Thanks and appreciation are also due to Mrs. Lucy Salazar for the typing of the first manuscript copy and to Mrs. Lillian Ingenlath for typing this dissertation.

To my parents for their constant guidance and support, to my wife Beatriz for her patience and understanding during the period of academic hardship, and to my daughters, for being the source of inspiration, this work is dedicated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The increasing demand for protein in the world has imposed upon the animal scientists the task of increasing the productivity of those areas of the world which, by virtue of their climatic conditions, were believed to be unfitted for animal production. Among these areas, the hot, humid tropics appear to have adverse effects on many biological systems.

Homeothermy, the characteristic whereby most higher organisms are able to maintain a constant internal environment, is in reality the expression of the ability of the organism to "adapt" its functions to the variations imposed by the complex external environment, or climate. Homeothermy, however, is not by any means a simple process. It involves a complex series of adaptive mechanisms which come into play at specific times within a sequence. A failure in one or more of these steps would result in abnormal processes which would eventually be reflected in any of the outward expressions of the phenotype.

It is this measure of the outward reactions or expressions, which has been of concern to the animal physiologist, as the possible measure of the capacity of an individual to tolerate adverse conditions. There have been many indices studied, which could give an indication of the ability of the animal to maintain its internal environment. Not all of those studied can be used as a reliable single-index, but it is possible that a combination of individual physiological characteristics could result in one single measure of response. Unfortunately this

might not be that simple. A living organism is "a very complex system of individual systems." Its ultimate expression and performance is the result of the interactions of the genotype, which it possesses, with the environmental conditions which surround it. It is through this relationship, throughout the whole evolutionary process, that species differentiated and became what we know at the present. Thus, there are two species of beef cattle commonly used throughout the world. The two species originated under two widely different conditions of environment, and therefore are different in their response to climatic changes. Species and breed of an individual are not the only factors responsible for its performance. It appears, also, that the sex of the organism has an influence on its response to environmental conditions.

With these bases in mind, a series of determinations was initiated to study the differential responses of the two species of beef cattle, as represented by the Hereford (Bos taurus) and the Brahman (Bos indicus) breeds, to varying environmental conditions of temperature and humidity. It was also the objective of this experiment to evaluate various physiological responses, as affected by sex, and the possible interactions which might exist between the breed and the sex of the experimental animals.

REVIEW OF LITERATURE

Work on the environmental physiology of cattle as well as of other domestic species, in relation to heat stress, has been carried out in psychrometric chambers and under natural climatic conditions.

Many physiological parameters have been used by different investigators in an attempt to measure the responses and assess the adaptability of an animal to a given set of environmental conditions. These responses comprise those which measure physiological as well as productive adaptability.

Environment and Hematology

As early as 1934, Manresa and Reyes studied the characteristics of the blood of cattle in the Philippines. These authors compared the hemoglobin, erythrocyte and leukocyte content of blood of Nellore, Native, Holstein and Hereford cattle, and concluded that the degree of hardiness of each of these breeds was correlated with the hemoglobin content of their blood. They further stated: "... The hemoglobin content of the blood may, therefore, be used as a guide in selection for adaptability to a given set of environmental conditions." Manresa *et al.* (1939) demonstrated a slight negative correlation (-0.226 ± 0.04) between atmospheric temperature and the hemoglobin content of the blood of Indian Nellore cattle. However, no effect of humidity was demonstrated. Upon further study by Manresa and Falcon (1939) the authors postulated that the relative humidity did not affect the hemoglobin of animals which had become acclimated to a given environment.

Bazett et al. (1940) measured plasma volume, packed cell volume, hemoglobin and erythrocytes in the blood of man after several days of hot weather. They found that, in man, changes in the volume of plasma developed more rapidly than those in the cells, and thus the initial changes in blood volume were consequently associated with temporary changes in the opposite direction in hemoglobin concentration and packed cell volume. Contrary to previous reports, Brody et al. (1949) found no significant differences in erythrocytes, leukocytes, packed cell volume or hemoglobin of Holstein and Jersey cows under climatic chamber conditions, varying from 50° to 100°F, during a period of four months.

Seasonal differences in erythrocytes, hemoglobin and packed cell volume were found in sheep by Mehrotra et al. (1954). These authors found an increase in the values of these parameters during the winter months. Also working with animals under ambient conditions, Rusoff et al. (1954) found highly significant differences in hemoglobin, packed cell volume, erythrocytes and leukocytes of Holstein, Guernsey and Jersey bulls with variations in environmental temperature. The authors, however, found that these hematological parameters increased as the environmental temperature increased over 80°F. Contrary results were later reported by Weldy et al. (1964) who found a highly significant reduction in packed-cell volume in the blood of Hereford heifers exposed to 90°F constantly for two to twelve weeks. These results of Weldy are not in agreement with the results of Bianca (1957), Findlay and Whittow (1966), and Whittow (1968), all of whom reported significant increases in packed cell volumes as a result of hyperthermia in cattle of different ages and breeds.

Hematological values, however, are also affected by the breed, age and sex of the animal, as well as by other factors such as nutritional state, health, etc. It is now a commonly accepted fact that animals of the Bos indicus species have higher values for hemoglobin, packed cell volume and erythrocytes, as well as blood and plasma volumes, as reported by Howes et al. (1963). These authors also found highly significant differences due to age, as well as significant interactions between age and species. Similar results, indicating higher hematological values for cattle of Bos indicus origin, were reported by Manresa and Reyes (1934) who demonstrated significant differences between hemoglobin values of Native and Indian Nellore breeds and the values of European breeds. These authors also found that there seemed to be no difference in the hemoglobin content of young animals when compared to more mature animals of the same breeding. Some of these hematological differences due to breed were also confirmed by Rusoff et al. (1951). These authors reported significantly higher values of hemoglobin and packed cell volume in the Red Sindhi-Jersey crossbred daughters than in their purebred Jersey dams, when studying these animals on a monthly basis for a period of 2 years. Contrary to previous reports, however, Long et al. (1952) working with the Angus, Hereford and Shorthorn breeds of beef cattle, failed to show any significant breed differences in the hemoglobin content. Their data for erythrocytes also failed to show any consistent difference between breeds. It was suggested by the authors that the variation in the number of erythrocytes was the result of the nutritional state of the animal at the time of the blood sampling.

It is a generally accepted fact that in man such hematological values as hemoglobin, packed cell volume and erythrocytes are higher in

the male than in the female. In cattle, on the other hand, the available reports are somewhat conflicting. Manresa and Falcon (1939) on one hand did not find any significant differences in hematological values between males and females of the Indian Nellore breed of cattle in the Philippines. Byers et al. (1952) on the other hand, reported a difference in the hemoglobin content of the Holstein breed, as affected by sex, but failed to show any appreciable differences, either in the winter or the summer, in the blood of males and females of the Guernsey, Brown Swiss and Jersey breeds. An interaction between environment and sex, as affecting the hemoglobin of Bos indicus cattle, was demonstrated by Mullick (1960). He reported a decrease in the content of hemoglobin of females from 10.4 gm. to 9.8 gm. per 100 ml. of blood, when going from periods of low humidity to periods of high humidity. This change, however, was not observed in the males.

Environment and Body Temperature

Body temperature is probably among the most commonly studied physiological characteristic in relation with animal response to environmental temperature. In fact, some of the so-called "Adaptability Indices" are based directly or indirectly on body temperature.

Manresa et al. (1939) reported a positive correlation of 0.476 ± 0.038, between atmospheric temperature and rectal body temperature of Indian Nellore cattle. McDowell et al. (1953) however, reported that body temperature was found to vary markedly with the season of the year, with a maximum in February and also in August and a minimum in May-June. The repeatability coefficient was low.

The increase in body temperature upon increase in environmental

temperature appears to take place only after other thermoregulatory mechanisms have been "overloaded." Thus, Worstell and Brody (1953) reported that the temperature trends for the various physiological reactions indicated there was virtually no call on the homeothermic mechanisms for physiological adjustments, between about freezing and 60°F. An increase in environmental temperature, above 60°F, affected profoundly most of the physiological reactions, with an increase in rate of vaporization being first affected before any rise in body temperature.

The relation of rectal temperature and apparent heat tolerance of cattle was reported by Quazi and Shrode (1954). These authors conducted a series of 24-hour studies with Jersey, Holstein and Brahman-Jersey females, and measured rectal temperature as well as pulse and respiratory rate. Their results indicate that, based on the measured parameters, there was a relative heat tolerance order of the three groups with respect to one another, the Brahman-Jersey crossbreds being highest, followed by the Jersey and finally the Holstein.

The importance of rectal temperature in the studies of environmental physiology was pointed out by Bianca (1959). This author exposed calves five hours daily to 45°C temperatures for 21 consecutive days and found that the initial body temperature of the animals was gradually reduced with repeated exposures. He concluded, among other things, that rectal temperature might be the most meaningful single criterion for judging the animals' heat tolerance, since rectal temperature indicated the ability to maintain homeothermy.

The regulation of body temperature by higher centers of the central nervous system was clearly demonstrated by the experiments of Andersson and Larsson (1961). These authors, experimenting with goats,

determined the anatomical location of a "Heat Loss Centre" in the preoptic hypothalamic region. This center apparently possesses thermoreceptive nerve terminals, which react to the temperature of the blood reaching the hypothalamus. It reacts to heat by initially promoting a temporary increase in body temperature which results in polypnea and peripheral vasodilation, and thus ultimately leads to a fall in body temperature.

The average increase in rectal temperature of cattle under hyperthermic stress has been studied. Thus, Bianca (1959) reported average increase of 2.2° to 2.6°C upon exposure to environmental temperature of 45°C. Weldy et al. (1964) exposed cattle to 90°F for 2 to 12 weeks and observed a rise in rectal temperature of at least 2°C.

The use of rectal temperature, as a means of determining the degree of stress or of acclimatization to high environmental temperature, has been attacked and criticized on many grounds. One of these is the fact that body temperature appears to be more drastically affected by increases in the moisture content of the environmental air than by increases in ambient temperature alone. This is apparently more true for studies in climatic chambers than for those under field conditions, as indicated by McDowell (1958). This was further corroborated by studies of Beakley and Findlay (1955), who determined that increasing the humidity of environmental air in chambers, from 23.6°C to 27.0°C (wet bulb), was equivalent to an increase of 4°C when the temperature was 30°C (dry bulb). At higher temperatures (35°C dry bulb), the same increase in humidity was equivalent to an increase in ambient temperature of 9°C, as measured by a response in rectal temperature increase. The effect of humidity was also demonstrated more

recently by Robertshaw and Whittow (1966). Studying the effects of hyperthermia on the sympatho-adrenal system of cattle, these investigators found that an increase in relative humidity caused an increase in both respiratory rate and rectal temperature from that under the same environmental temperature, but with lower humidity.

Also important in responses to hyperthermia as measured by rectal temperature appears to be the age of the animals at the time of exposure to high temperature. Thus, Casady et al. (1956) exposed dairy bulls to high temperature at 17-19 months of age and again at 30-32 months of age. These authors found that rectal temperature showed an increase at temperatures of 80°F and above during the first exposure, but only after 90°F in the second exposure. These researchers concluded that this seems to be an indication of better thermoregulation in bulls of an older age.

The range of temperature application also seems to be of importance in the measurement of responses in rectal temperature. Whittow (1962) demonstrated that an increase in environmental temperature of 25°C, from 20° to 45°C, had a significantly greater effect on raising rectal temperature than the same absolute temperature increase from - 5° to 20°C. Measurements of rectal temperature appear to have a nycthemeral or 24-hour cycle. This was demonstrated by Berman (1968), who showed that the lowest rectal temperature occurred at 0430 hours. Maximum values in spring were at 1630 hours, while in the summer they were measured at 1230 hours. This author also pointed out the effect of feeding pattern on the response in rectal temperature. He found that the highly significant increase in rectal temperature from spring to summer was augmented by ad libitum feeding.

As is the case of the hematological responses, body temperature is also dependent, to a certain degree, upon individuality as well as upon the breed and sex of the individuals. Worstell and Brody (1953) demonstrated that the upper limit of heat dissipation by vaporization (limit where body temperature may begin to rise) was found at a rather low environmental temperature for Holstein and Jersey cattle (70°F) and at higher levels for Brown Swiss and Brahman, 85°F and 95°F, respectively. They further stated that, after body temperature began to rise, there was little difference in evaporative cooling ability among the breeds studied. Contrary reports are also found. Johnston and Branton (1953) in a study to evaluate climatic effects on dairy bulls, found no significant breed differences in rectal temperature of bulls of the Holstein, Jersey and Guernsey breeds under field conditions, when measured in the summer as compared to the winter months. As a general rule, however, most workers agree on the fact that differences in thermoregulatory responses, as measured by body temperature, are influenced by the breed of the animal. More striking effects are observed in studying cattle of different origin. Cartwright (1955) reported a study of 366 animals of different breeds and sexes, conducted in environmental chambers. The author found that within the sexes the ranking of the breeds according to the degree of heat tolerance, as measured by rectal temperature and other parameters, was in reality a ranking of the percentage of Brahman breeding of the animals. Similar results are reported by Badrelding and Ghany (1954), Johnston et al. (1958) and by Johnston et al. (1963).

The effect of sex on the thermal response of cattle to temperature stress has not been fully studied. Only a few reports are found in the literature, and these are on occasions conflicting. Cartwright (1955),

studying the responses of animals of different breeds and sexes to the increase in temperature in environmental chambers, found highly significant differences in rectal temperature due to the sex of the animals, the females having a higher rectal temperature than the males. Significant sex by breed interactions were also found in this study with respect to body temperature in the chambers.

Environment and Endocrinology

Although the information available in some aspects of endocrine function is insufficient, other aspects have been more thoroughly studied. Not until the advent of the modern techniques of radio-isotopes, and the even more recent of radio-immuno-assays, had it been possible to study, in some detail, the true involvement of the endocrine system in the physiological adjustments of homeotherms exposed to high-temperature conditions. The entire endocrine system can and possibly is affected not only directly by the action of heat on a particular endocrine gland, but also and more importantly, through the effects of stress on the hypothalamus. Among the many endocrine glands and so-called systems the thyroid gland is probably one of the most studied, as far as its response and involvement in heat stressed animals is concerned. The thyroid gland is sensitive to the direct effects of high temperature, as reported by Bogart and Mayer (1946a). Their work with rams indicated that temperature affected the reproductive mechanisms through the thyroid gland and that the sensitivity of the reproductive organs to slight temperature changes was the reflection of the sensitivity of the thyroid gland to changes in environmental temperature. The involvement of the thyroid in the reproductive pro-

cesses was further investigated by the same authors. In another report, Bogart and Mayer (1946b) demonstrated that the "summer sterility" observed in rams during the summer months could be reversed by the administration of exogenous thyroxine. The authors substantiated these results with the histological examination of the appropriate tissues. Kamal et al. (1958) studied two groups of heifers reared at 50° and at 80°F for 1 year and exposed them to rising temperatures from 35° to 95°F. The results indicated that the higher values of plasma sodium, water retention, blood glucose, plasma protein and others, in the 80°F-reared group, could be attributed to greater adrenal and thyroid activity. Johnson and Ragsdale (1960) studied further the effects of hyperthermia on heifers raised at the two environmental temperatures (50° and 80°F). These authors found a striking decline in thyroid activity on exposure to high temperature. They also reported a negative correlation between the activity of the thyroid gland and environmental temperature. They corroborated the results of Kamal and co-workers in that those animals reared at 80°F had a higher thyroid activity than those reared at 35°F, when all animals were exposed to high temperature. Andersson et al. (1962) determined the anatomical location of a hypothalamic center for the regulation of thyroid gland activity. These authors reported a release of thyroid hormone upon cooling of the preoptic area of the hypothalamus. They concluded that the release of thyroid hormone upon cooling of the preoptic area was mediated through the hypothalamo-pituitary axis. Their data suggested that the preoptic "heat loss centre" exerted a tonic inhibition on thyroid activity. The effects of rearing temperatures on some of the components of the endocrine system, including the thyroid, were demonstrated by Harrison (1963). This investigator raised different strains

of mice under either 21°C or 32°C environmental temperature. It was found that heat-reared animals of all genotypes initially grew faster than their control-reared littermates. At increased ages, however, the trend was reversed, and the heat-reared animals were ultimately smaller than the control-reared ones. Post (1965) in Australia, studied the seasonal variation in thyroid activity of European and Zebu crossbred steers under grazing conditions. He reported that thyroid activity did not follow the smooth seasonal changes in day length and ambient temperature. Rather, it was highest in November, when pasture quality was at its best, and lowest in September when pasture was poorest. He indicated that the superiority of the Zebu crossbreds could not be attributed to the way their thyroid reacted to stressful conditions. Further proof of the effect of the environment of thyroid activity, as well as the influence of other modifying factors, was given by Yousef and Johnson (1966). Studying the effects of temperature 1° to 35°C, on thyroid function of ad libitum and control-fed cows, the authors indicated that high temperatures caused a decrease in thyroid function, regardless of the level of feeding. From these results, it was concluded that feed intake was not the primary factor which altered thyroid activity.

The involvement of other endocrine glands, in this case the gonads, in the overall physiological response to heat, will be reviewed under a separate section on reproduction.

Environment and Reproduction

Among the most important characteristics to be considered in dealing with biological systems, reproduction must hold a very prominent position. The reason for this is basically the fact that the species

must be propagated in order for natural or artificial forces to take place, such as selection, migration, etc., thus allowing evolutionary processes to continue. The reproductive processes of higher vertebrates, even though clearly defined processes, are by no means simple. As are many systems the reproductive patterns of many species are controlled primarily by exteroreceptive factors, including social as well as bioclimatological aspects. The survival of species in an environment with seasonal fluctuations require a series of physiological mechanisms which are responsible for the initiation of critical functions of adaptation at the appropriate season. Again, similar to some but unlike other bodily functions, the reproductive processes must in some aspects be dealt with under the two different categories of sex classification.

Moore (1924), studied the application of heat and its effect on testicular tissue. He applied graded amounts or exposure times of heat to the testicles of guinea pigs (47° - 47.3°C for 10 min.). Sections of the testes, removed 12 days after heat exposure, showed a peripheral area of degeneration along one side, involving tubules to a depth of approximately 1/5 of the diameter of the cross-section. It was found that in this area all of the tubules were devoid of a germinal epithelium, aside from the single layer of basal cells. Deeper than the peripheral area and continuing toward the opposite side of the testis, it was possible to find gradations from completely degenerate tubules to normal ones. Ogle (1934) studied the adaptation of the sexual activity of mice to environmental changes. An increase in fertility was found when mice were housed under a 60° to 68°F environmental temperature compared to a second group at 88° to 92°F.

Casady et al. (1956) studied the effects of high temperature on the process of spermatogenesis in the bull. It was found that bulls subjected to graded environmental temperatures, from 70° to 98.9°F, had a decline in the concentration of spermatozoa in the ejaculate after the exposure to severe heat (98.9°F). This decline was detected in the period following exposure, with recovery except in one case where low concentration persisted for a longer period of time. These authors also reported a decline in initial motility of the spermatozoa and in total number of cells in the ejaculate.

That the effects of hyperthermia on semen quality are generally observed after the high temperature periods was confirmed by Johnston and Branton (1953). They found, in dairy bulls studied under field conditions, that fertility was generally high during winter, spring and early summer, and began to drop in late July and early August. It was found that the lowest average initial spermatozoan motility was 50%, occurring during late summer and early fall.

Other semen characteristics are also severely affected by high temperatures. This is the case of the percent number of live cells found in the ejaculate. Austin et al. (1961) found that insulation of the testicles of Hereford bulls by the use of plastic bags and glass-wool elevated the scrotal temperature to a point where the treated group had a highly significant decrease in the percentage of live cells in the ejaculate, as compared to control bulls, 50% and 78%, respectively. Similar results were also reported for the number of normal cells. The decrease in these characteristics took place two weeks after insulation and remained low for four to five weeks.

Johnston et al. (1963) studied the responses of Holstein and Brown Swiss, as well as of Red Sindhi crossbred bulls to hyperthermia. It

was found that, in general, high temperatures and humidities resulted in an overall highly significant ($P < 0.01$) decrease in initial motility, concentration, and total number of spermatozoa, with a more marked decrease in the purebred animals. It was found, however, that when measured during the period of stress, all bulls showed a significant ($P < 0.05$) increase in spermatozoa concentration and total counts. A study of the semen indicated that the first spermatic abnormality to appear was the bending of the mid-piece, with appearance of several pyriform spermatozoa within a two-week period after stress. Minimum concentration was observed within three weeks after thermal stress.

Several other authors, among others, Waites and Setchell (1964), Hafez (1965), Ulberg and Burfening (1967) and Dutt (1959) also have indicated the deleterious effects of high temperature on the quality of semen of various animals.

Physiological responses of mammals, like many other organisms, are rather variable. Thus, results somewhat different from those previously indicated have also been reported. Anderson (1945) studied the seasonal variation of semen characteristics in bulls in South Africa. He found that semen quality was associated with a higher maximum temperature, a high average number of hours of a temperature above 75°F per day, a low minimum temperature, a large diurnal range in temperature and a high number of daily hours of sunshine. Patrick et al. (1954) studied the effects of high temperature and humidity on the quality of semen of dairy bulls in Louisiana. Animals confined to an environmental chamber at 80°F during the months of June through August had a breeding efficiency of 73.6% as compared to those maintained at ambient temperatures as controls, which had a breeding efficiency of 71.9%. It was also reported that 91% of the samples of

semen, collected from the animals in the 80°F chamber, were suitable for artificial insemination while only 47% were suitable from the control group. They concluded that a temperature of 80°F was not detrimental to semen quality, even if maintained continuously for 90 days.

El Skeikh and Casida (1955) reported that the semen of rabbits subjected to 110°F temperature for 30 to 60 minutes did not show any change in motility of the spermatozoa. When fertility tests were made at different intervals after treatment, a reduction in the percentage of fertilized ova was found by seven days after treatment (from 96.2% in the controls to 73.5% in the treated group). By 12 to 14 days it was reduced further to 53.1% in the heat treated group. The authors concluded that high temperature may bring about a lowering of spermatozoa fertility without affecting or depressing motility. Their assumption was later confirmed by Ulberg (1958), who stated also that high temperature caused a decrease in semen fertility before it caused a decrease in semen quality. He further stated that the possibility also existed that estimated semen quality as measured by motility, abnormal sperm, etc. might not be a measure of fertility.

Vandemark and Ewing (1963) studied the metabolic reactions and activity of testicular tissue after the application of heat by transplant into the body cavity, in rabbits, for 2, 6 and 24 hours. The tissue of the testis that remained in the body cavity for 24 hours, had a reduction of 12% in glucose content, and a 27% decrease in lactate content. It was concluded by the authors ".... This preliminary finding suggests that cellular degradation of testicular tissue could have been due to depletion of the energy reserves of the tissue." The 2 and 6-hour experiments indicated that the effects of temperatures higher

than the scrotal temperatures on spermatogenesis were preceded by a transient increase, and then a decrease, in metabolic activity of testicular tissue.

The "residual effects" of temperature stress on the spermatozoa have been summarized by Ulberg and Burfening (1967), who stated that a slight increase in temperature for a short period of time, acting on either the spermatozoa before fertilization or on the ovum immediately after fertilization, caused the resulting embryo to die sometime during its development. The amount of embryo development before death was dependent upon the very critical factor of stage at which stress was applied, and also on the magnitude of the stress.

A study by Brooks and Ross (1962) of the effect of thyroxine therapy on semen quality of rams, demonstrated that doses of 0.4 mg. of thyroxine did not have any significant effects on semen quality. It was indicated that thyroxine administration was detrimental to semen quality under high temperature conditions. The authors also reported that the rams recovered in 4 to 5 weeks after being subjected to 80°F temperature and 73% relative humidity for a period of 2 months. This indicated that spermatogonia had been relatively unaffected by heat, but cells in more mature stages were probably destroyed.

As opposed to the climatic effects on the male reproductive characteristics, those on the female reproduction are not as clearly defined. Most of the work on female reproduction has been directed towards the effects, either immediate or latent, of heat on the survival of the embryo. There are however some studies, which deal more directly with the effects of high environmental temperatures on the functional integrity of the reproductive system of females. Of influence on the life time reproductive efficiency of female livestock is

the age at which they reach puberty. It is thus important to study the effects of climatic effects, on the appearance of puberty of livestock. Bonsma (1949) considered both males and females when he stated that since the function of reproduction was intimately associated with growth and growth was affected by heat stress, all animals whose growth had been considered retarded, revealed repressed sexual activity. Clinical examinations, as well as observations upon slaughter, proved the sexual organs of animals, particularly the ovaries and wombs, to be infantile. The author further stated that, under heat stress, "....apart from the increased metabolism, and the hyperthermal condition, which makes it difficult for the animal to put on weight, the development of protein tissue, which is the most important tissue associated with the weight increases in the early stages of growth, is still more difficult, since the evolution of one molecule of protein tissue involves the addition of eight molecules of water." Very similar conclusions were reached and reported by Dale et al. (1959). These workers studied the effects of constant environmental temperature on beef calves and found that, in terms of predicted mature value, puberty was delayed in the Brahman calves reared at 80°F, a breed where growth was more rapid at 80°F than at 50°F. In the Santa Gertrudis calves, however, puberty did not seem to be much affected by climatic conditions. It was concluded that the results indicated that in so far as environmental temperature affected growth rate, it affected the age at which puberty appeared. Hafez (1965) indicated that season of birth affected the age at which puberty appeared mainly in seasonal breeders, thus indirectly affecting a part in the reproductive cycle.

The effects of high environmental temperatures and other climatic

factors associated with them have been studied in mature females. Ogle (1934) found that the fertility of female mice, which had been raised under cool conditions, was drastically reduced by mating them in a hot room with a temperature of 88° to 92°F and relative humidity of 75%. Not only was apparent fertility decreased by hot conditions, but it appeared to be increased by cool conditions (60° to 68°F). The author concluded that the sexual functionings under the various conditions studied were apparently dependent on changes in the interrelationships between gonadal, hypophyseal and adrenal systems. It is believed that the gonads are affected indirectly, with the adrenals probably initiating the responses to changes in environmental stimulation.

Presently available evidence indicates that high environmental temperature does not have a very marked effect on ovarian function, but rather on the uterus during the "preparatory" stages for pregnancy as well as during the initial development of the embryo. Yeates (1953) subjected ewes to environmental temperature of 105°F (dry bulb), 87°F (wet bulb) to obtain a rise of 1.5°F in rectal temperature and of 100 respiratory movements per minute. The ewes showed no adverse effects of temperature on occurrence of oestrus. When they were mated with fertile rams and maintained in the hot room, the number of young produced were significantly reduced. Similarly, Alliston and Ulberg (1961) found that temperatures of 70° or 90°F did not significantly alter the ovulatory rate or estrus but reduced the number of embryos. Ryle (1961) conducted a very comprehensive study on the effects of high environmental temperatures on the physiology, as well as the anatomy, of the female reproductive organs. She designed a factorial experiment using 2 levels of each of 4 factors: environmental temper-

ature, thyroxine, vitamin A and progesterone. The high temperature used was approximately 40°C and was used for 4 to 8 hours daily. She found 83% live embryos in the control ewes and only 62% in ewes at high temperature at slaughter. The author indicated that this might be due to fewer ova, higher embryonic mortality or lower fertilization rates. There was no indication, however, that high temperature tended to cause temporarily a complete suppression of ovulation or oestrus. It was also reported that the proportion of ewes in the hot room with live embryos was increased due to thyroxine. In a second report by Ryle (1962) it was indicated that no significant treatment effects or interactions were found on any of the histological indices used to detect uterine response. In the hot room, however, the uterine gland epithelial height was associated with rectal temperature. There was no substantial depression of ovarian endocrine activity, either due to pituitary insufficiency or to any other heat-induced disturbance. This suggested that early embryonic loss in hot environments, as reported earlier, could not be attributed to ovarian malfunction. A later report by Ryle (1963) indicated a reduction in ovarian weight, from 1,344 mg. for the controls to 1,159 mg. for the ewes in the hot room. There were not, however, significant treatment effects on mean corpus luteum diameter and there was no suggestion of an effect due to either environmental temperature or thyroxine status. Follicle size and number of follicles in the ovaries did not show any significant effect of temperature. These results again tended to indicate that the effects of the experimental treatments on embryonic mortality were not mediated primarily via ovarian hormone production.

Fallon (1962) studied the relationship between body temperature

and fertilization in the cow. He postulated that oestrus imposed a heat load on the cow and that it is not readily dissipated. It was found that of 934 records 18.1% were above 102.3°F body temperature; the incidence of high temperature being directly related to the stage of oestrus. It was therefore concluded that increased body temperature *per se* was not inimical to fertility, but was of importance insofar as it indicated the stage of oestrus at which the cow was bred.

Comparison of ewes maintained continuously at 70° or at 90°F indicated that exposure to high temperature significantly reduced fertility, as measured 25 to 30 days after mating (Woody and Ulberg, 1964). It was further demonstrated that a larger proportion of the decrease in fertility from one temperature to another, occurred when high temperature acted prior to the detection of the end of estrus, rather than afterwards.

That temperature stress was more unfavorable through its effect on the uterine environment than directly on the ovum was reported by Woody and Ulberg (1964), who studied it by the use of ova transferred from donors maintained at either 70° or 90°F to mated recipients in constant 70° or 90°F environments.

Warnick *et al.* (1965) compared the ovulation rate, conception rate and embryonic survival up to 25 days of pregnancy in gilts which were kept at 60° or 90°F. The authors reported no significant differences in average ovulation rate at second estrus. There were no significant differences in the number of live embryos due to temperature treatments up to 3 days postbreeding. These results indicate that there is a species difference which must be taken into consideration in the evaluation of the responses of domestic animals to the stress

conditions of high temperature and humidity. To further emphasize this species difference is the work of Pennycuik (1967) who reported that, in mice, the number of females able to carry a litter to 19 days of gestation fell very gradually as the temperature increased from 21° to 36.1°C. At 36.7°C, only 1 female in 18 was able to complete gestation.

Nutrition and Other Physiological Indices

The nutritional status of an individual appears to have influence on the responses to environmental stress. At the same time, the environmental conditions have a direct, as well as indirect, effect on the nutrition of the animal. Wayman et al. (1962) reported a drastic reduction in feed intake of Holstein cows as soon as temperature stress (88°F) was applied. Milk production of the cows followed a similar pattern to that of feed intake. The consumption of hay decreased rapidly under high temperature stress, but concentrate intake remained fairly normal. It was also found that heat tolerance is greater when the ration was lower in fiber and higher in readily available energy. Allen et al. (1963) reported some physiological responses to moderate heat (70° - 80°F) and high heat (103°F) by two Jersey and two Zebu heifers, 10 to 16 months of age, when alternating between normal feed intake and fasting. At the moderate temperature there were diurnal rythmes for both breeds in feed and water consumption, skin and rectal temperature, sweat rate and respiratory rate. The diurnal rythm for skin temperature, sweat rate and respiratory rate disappeared in the hot room. Feed intake was depressed in Jerseys in the hot room, but was not depressed in the Zebu. When fed, the Jersey animals failed

to maintain body temperature in the hot room, despite reduction in appetite and a large increase in respiratory and sweat rates. Winchester (1964) reported that the relationship between intake and temperature was linear and that metabolic energy declined rapidly from 70°F until 100°F at which time it paralleled that of feed intake.

Lundgren and Johnson (1964), in a study to separate the effects of temperature from those of feed on thyroid activity of lactating cows, found a reduction in feed intake due to high temperature, from 44 lb. to 30 lb. per day. Weldy et al. (1964) reported a reduction of rumen volatile fatty acid production in Holstein cattle, while there was a slight increase in Hereford cattle under conditions of heat stress. The variation appeared to be in the proportion of acetic acid. The authors indicated an increased rate of passage through the digestive tract due to heat stress. This increased rate was associated with a lowered acetate propionate ratio, which would help the animal to withstand stress. Contrary results were, however, reported by Kelly et al. (1967). These authors found that even though the total volatile fatty acids declined from 153.05 to 66.27 mEq/liter as the temperature increased from 1.6° to 37.7°C, the acetate : propionate ratio was actually increased. This was due to a greater decline in propionic acid than in acetic acid (72% and 50%, respectively). The authors further stated that heat tolerance was greater when the animals did not have a change in VFA concentration.

Cassuto and Chaffee (1966) demonstrated that the feed intake of rats subjected to 35°C, 50% relative humidity was depressed during the first 4 weeks of exposure. After the fourth week, however, feed intake increased for the next 2 weeks and then leveled off. A similar recovery

trend in cattle was reported by Johnson et al. (1967). These authors indicated that lactating cattle exposed to 29°C constantly showed evidence of partial acclimation or compensation to a stabilized state in milk production at 3 to 5 weeks after initial exposure. More complete recovery of a depressed feed intake was attained at 4 to 5 weeks, although full recovery or acclimation was not demonstrated.

Many physiological characteristics have been used, individually and in combination, in an attempt to assess the adaptability of cattle to high environmental temperature. Benezra (1954) utilized a combination of rectal temperature and respiratory rate, and proposed a formula which gives appropriate recognition to respiration as an effort to maintain body temperature. Dowling (1956) studied 300 Shorthorns under the semiarid conditions of Queensland. The animals were classified according to coat characteristics, and skin moisture and rectal temperature were measured under shade, sun and after walking. The author concluded that it appeared reasonable to attribute some of the variation in body temperature to differences in hair coat cover.

Vernon et al. (1959) studied the possible relations between heat tolerance determinations as measured by rectal temperature and respiratory rate, and productivity of beef cattle as measured by dam weight and progeny weight. The authors concluded that although the correlations were positive their value was too low to justify using respiratory rate as a measure of heat tolerance and as an index for selection for production under stress conditions.

Howes et al. (1957) studied several physiological parameters in yearling heifers of the Brahman and Hereford breeds in relation to heat tolerance. They found that Hereford heifers gained weight at a

faster rate than the Brahman, from February to July, irrespective of the level of protein intake. Blood volume, packed cell volume, erythrocyte numbers, leukocyte numbers, hemoglobin concentration and mean corpuscular volume, all were higher in the Brahman heifers. Erythrocyte numbers declined with age in both breeds.

MATERIALS AND METHODS

This research was carried out during the year of 1967. It was designed to study the effects of prolonged exposure to high environmental temperature and humidity on the physiology of both male and female cattle of the Brahman and Hereford breeds.

Experimental Animals and Design

The animals used were 18 bull calves and 18 heifer calves of the 2 breeds, Brahman and Hereford. The animals were obtained from the Purebred Beef Cattle Experimental Unit at the University of Florida, and from the Everglades Experiment Station at Belle Glade, Florida. All animals were approximately 11 mo. old when transferred to the Animal Physiology Unit at the University of Florida. Twelve of the animals (3 of each breed and sex), previously selected as replacements for the Purebred Beef Cattle Unit, were separated by sex and placed on pastures, to be used as ambient control group. The 24 remaining animals were then placed in individual stanchions in an open barn at the Physiology Unit. This was done so the animals became accustomed to being tied-up, and handled. After a handling period of approximately 4 weeks, the animals were divided at random into 2 groups, each of which was made up of 3 animals of each breed and sex. The 2 groups were then housed in 2 controlled-environment chambers, where each animal was placed in an individual stanchion. Table 1 shows the distribution of the animals under the different environmental conditions.

TABLE 1.--EXPERIMENTAL DESIGN

Treatment	Hereford		Brahman	
	Males	Females	Males	Females
	No.	No.	No.	No.
I. Ambient Conditions.....	3	3	3	3
II. 32° Centigrade 96% Rel. Hum.....	3	3	3	3
III. 21° Centigrade 65% Rel. Hum.....	3	3	3	3

Description of the Controlled Chambers

The 2 controlled-environment chambers used were 30 x 22 x 11 feet, insulated with 4 inches of styro-foam. Each chamber had a capacity of 12 animals in individual 3-feet wide standard dairy barn stanchions, and the stanchions were closed at the back by individual gates.

The hot chamber was heated by a 4 Kw. and a 3 Kw. wall-type heater, both thermostatically controlled. The cold chamber was cooled by two 7-1/2 tons heat pump compressors, also with thermostatic control. Both chambers had a Moeller constant-recording apparatus where both temperature and relative humidity were recorded during the entire experimental period. The floors of the stanchions were covered with a 1-inch thick rubber mat, where the animals were to stand or lie-down. The heated chamber was equipped with a 15-inch exhaust fan on the door, to promote air movement in the room. In addition, each chamber was supplied with a portable 20-inch variable speed fan in order to increase air movement and also to help dry the concrete floors.

Management and Nutrition

During the pre-experimental period, both outside and inside the chambers, the animals were fed hay and a concentrate ration. This period was used to calculate the amount of hay which the animals were consuming, so that this amount, 12 lb. per animal per day, was fed when they were moved into the chambers. This amount was fed for a period of 6 mo., when it was then increased to 16 lb. The hay was a Coastal Bermuda-Alyce clover (*Cynodon dactylon-Aysicarpus vaginalis*) hay, obtained from a commercial supplier. The composition of the

supplemental concentrate ration is described in Table 2. Initially it was calculated that the animals would consume 7 lb. of concentrate per animal per day. All three treatment groups were fed this amount, during the first 6 mo. of the experiment. The amount was increased to 8 lb. per day during the last 6 months. Hay was supplied to the ambient control group only during the winter period, and was discontinued when green forage (pasture) was available. In addition, all animals had access to trace-mineralized salt, and water was available at all times in semi-individual automatic drinking cups. The feeding was calculated to supply the required amount of nutrients for the animals, under the N.R.C. (1963) recommended standards.

The animals of the ambient control group were managed according to the management practices established at the Purebred Beef Cattle Unit, with only the feeding program being altered to fit the experimental conditions. The animals in the chambers were located at random within the chamber, in individual stanchions. The temperature of the cold chamber was then set to reach a temperature of 21°C and was maintained at this temperature for the remainder of the experimental period. In the hot chamber the temperature was gradually increased, over a period of 2 weeks, to reach a maximum of 32°C, which was maintained for the remainder of the experiment. Relative humidity, measured after attaining the desired temperatures, was 65% for the 21°C chamber and 95% for the 32°C. The animals were then allowed a period of 4 weeks, after final temperatures were reached, as a period of acclimatization. Figure 1 shows the average maximum, minimum and mean environmental temperature as reported by Prine and Mickelson (1968).

TABLE 2.--COMPOSITION OF THE SUPPLEMENTAL RATION*

Ingredient	Percent of the ration
Ground yellow corn	62
Soybean meal, 50% C.P.	10
Dehydrated alfalfa meal, 17%	10
Cane molasses, standard	10
Urea, 45% nitrogen	3
Trace mineralized salt	2
Defluorinated phosphate	2
Vitamin A and D premix**	1

*Animals offered hay ad libitum in treatments II and III (32° and 21°C). Animals in treatment I were grazing.

**Vitamin premix: 2,000 gm. PermaDual 30A; 200 gm. PermaDual 16 D_2.

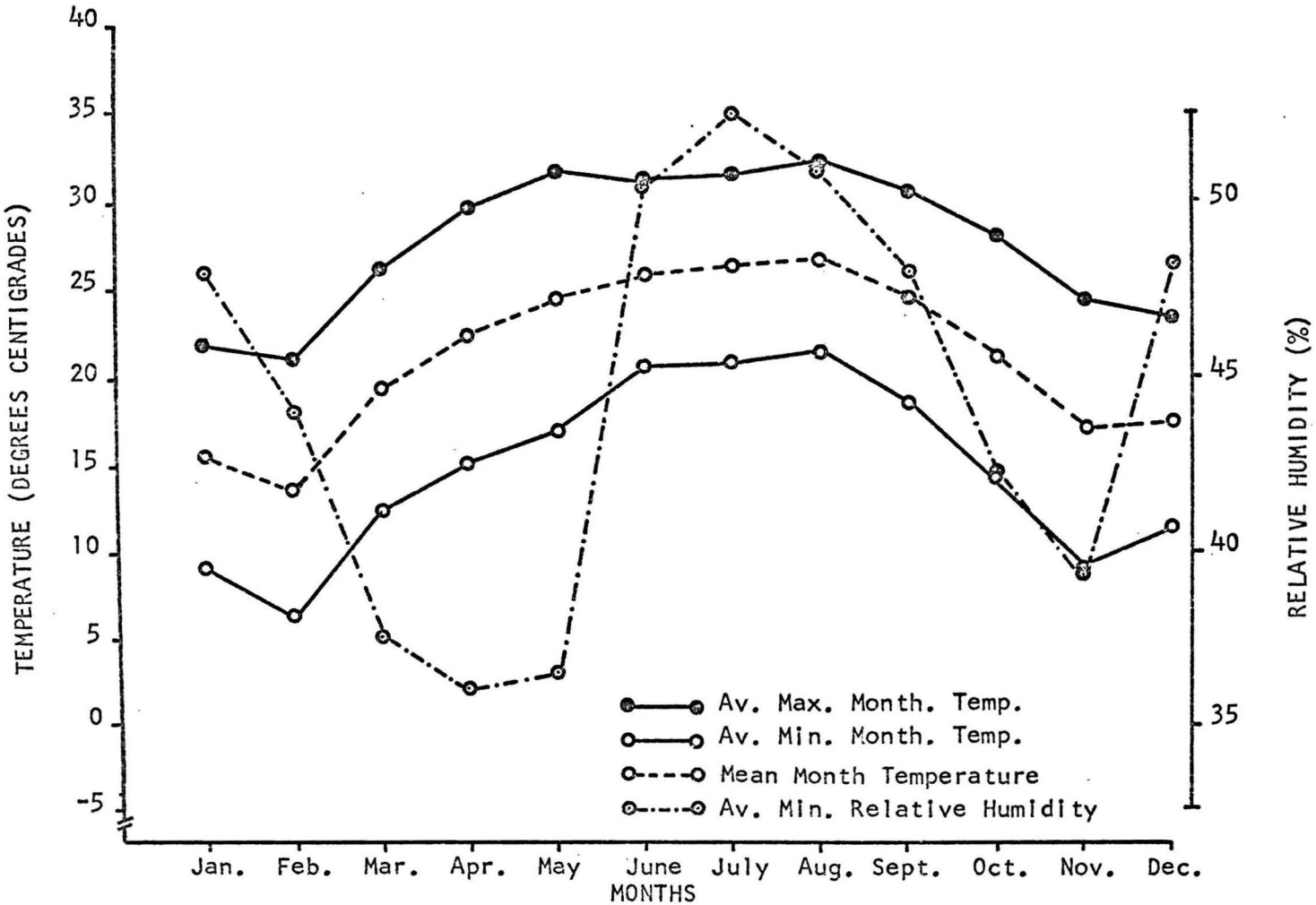

Figure 1.--Average maximum, minimum and mean environmental temperature.

Data and Sample Collection

The experiment was divided into 12 individual periods of 28 days each, and the first sampling was carried out on February 10, 1967 for the females, and on February 17, 1967 for the males, except for libido testing which was initiated on February 24, 1967. These dates were 56, 63, and 70 days after the housing date, for females the first, and males the last two. This step-wise sampling procedure was necessary because of the time involved in the collection of data and samples and in the laboratory analyses of the blood and semen. The physiological parameters measured are listed in Table 3, with the units in which they are expressed. Samples and data were collected every 28 days in the morning, and continued until the last data collection on December 18, 1967.

The following are the procedures for the collection of the data and the samples for those parameters which were common for both males and females, keeping in mind that they were measured at 1-week intervals between the sex groups. The animals in each treatment group were moved to a holding pen and data and samples were collected individually after the animal was restrained in a chute.

Body weight was obtained on a Fairbanks-Morse dial scale, the animals were then moved into the chute and the following data and samples collected:

a) Body temperature was taken by rectal insertion of a thermistor probe connected to a YSI-Telethermometer.

b) Blood was collected in the amount of 8.00 ml. by puncture of the jugular vein, using Vacutainer tubes, containing Sodium-EDTA as anticoagulant.

TABLE 3.--PHYSIOLOGICAL PARAMETERS STUDIED AND UNITS OF MEASURE

Parameter	Unit
Average daily gain	Lb./day
Rectal temperature	Degrees centigrades (^{o}C)
Erythrocytes (RBC)	Millions/cu. mm. blood
Leukocytes (WBC)	Thousands/cu. mm. blood
Hemoglobin (Hb.)	Gm./100 ml. blood
Packed cell volume (PCV)	Volumes per cent
Mean corpuscular hemoglobin (MCH)	Micromicrograms/RBC (uug.)
Mean corpuscular volume (MCV)	Cubic microns (u^3)
Semen volume	Ml.
Initial spermatozoa motility	Per cent of the cells
Spermatozoa concentration	x 10^3/cu. mm. semen
Total spermatozoa in semen	x 10^6/ejaculate
Libido 1/	Score
Ovarian follicles	Number
Corpora lutea	Number

1/ See Table 4 for Libido Scoring System.

c) The females were palpated via-rectum and the size of each ovary was estimated. Also recorded were the number, size and location of any follicles and/or corpora lutea.

d) Semen from the males was obtained by the use of an electro-ejaculator (Nicholson Trans-jector), collected into graduated centrifuge tubes, and the volume, color and appearance were observed and recorded. Hair covering the preputial skin was clipped and the area of the abdomen was washed and cleaned previous to the collection of semen.

e) Libido was determined in the bulls, 1 week after semen collection. Each bull was moved out of the chamber or pasture, individually, and allowed to remain 5 minutes in a small pen with a previously estrogenized heifer or cow. The sexual response was graded according to Table 4.

Laboratory Analyses

Immediately after collection, a drop of semen was placed on a warmed slide and observed under the microscope for evaluation of initial motility and observation of gross spermatozoa abnormalities. Motility was recorded as percentage of total cells that presented forward motility as observed under 100 x magnification. The blood and semen were taken immediately to the laboratory for analysis. The semen was analyzed for spermatozoa concentration by the use of an electronic particle counter (Coulter Counter, Model F) as described by Jones and Wilson (1967), modified for dilution and filtering procedure. Glover and Phipps (1962) reported that the determination of the concentration of spermatozoa in semen made with this instrument was found to be highly correlated

TABLE 4.--NUMERICAL VALUES GIVEN TO THE BULLS ACCORDING TO SEXUAL INTEREST FOR LIBIDO EVALUATION.1/

Degree of Sexual Response	Points
No interest	1
Interest by sniffing	2
Attempt to mount	3
Mount but no copulation	4
Complete mating	5

1/ Bulls exposed to estrogenized female for a period of 5 minutes.

($r = 0.96$) with the corresponding estimates obtained from the direct counts made under the standard conditions in hemocytometer chambers under the microscope. Similar results were reported by Iversen (1964) who found a correlation coefficient of 0.94. He concluded that electronic counts were reliable, fast and accurate, and appeared to be the method of choice over other methods, such as hemocytometer, light scattering and absorption or optical density measurements, all of which he compared.

The blood of both males and females was analyzed for hemoglobin content (Hb) by the cyanomethemoglobin method as described by Crosby et al. (1954) with the use of a "Spec-20" colorimeter. Previous to the first determination a standard curve was prepared for the particular colorimeter used.

Hematocrit (Ht) or packed cell volume (PCV) was determined by the microhematocrit method as described by Strumia et al. (1954). The centrifuge used in this experiment was an International table model clinical centrifuge with a microhematocrit head attachment.

Both erythrocytes (RBC) and leukocytes (WBC) were counted with the use of an electronic particle counter. The advantages of the electronic counter for the determination of blood cells have been indicated by several authors, among others Mattern et al. (1957), Richar and Breakell (1959), Wisecup and Crouch (1963) and Gagon et al. (1966).

Diluent for erythrocyte determinations was freshly prepared physiological saline solution (0.9%). The diluent for leukocyte determinations was a cetrimide-citrate-saline solution as described by D'Angelo and Lacombe (1962). Both diluents were filtered in a Gelman, Parabella vacuum-filter funnel, using 47 mm. Gelman, Metricel membrane filters with a 0.20 u pore size. Filtration was repeated until the

solutions had a background count of less than 200 particles per 0.5 ml. The diluent for spermatozoa was the same physiological saline as used for the erythrocytes. The dilution factors for spermatozoa, erythrocytes and leukocytes were 1:40,000, 1:200,000 and 1:500, respectively.

The two Wintrobe erythrocytic indexes used, i.e. mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), were calculated according to the formulas given by Schalm (1965). Also calculated from the data collected were the figures for average daily body weight gain and the total number of spermatozoa in the ejaculate of bulls.

Statistical Design and Analyses

The experimental design was a 2 x 2 x 3 factorial, based on the factors breed, sex and treatment, respectively. The data were analyzed using an IBM 360 - Mod. 50 computer. Analyses of variance by the method of least squares were performed on the data. One of the analyses used periods as replicates and followed the model:

$$Y_{ijkl} = u + T_i + S_j + A_k + (TS)_{ij} + (TA)_{ik} + (SA)_{jk} + E_{ijkl}$$ where

Y_{ijkl} = response of the variable corresponding to the l^{th} individual in the ijk^{th} subclass

u = population mean

T_i = effect of the i^{th} treatment $i = 1, .. 3$

S_j = effect of the j^{th} sex $j = 1, 2$

A_k = effect of the k^{th} breed $k = 1, 2$

$(TS)_{ij}$ = interaction between the i^{th} treatment and the j^{th} sex

$(TA)_{ik}$ = interaction between the i^{th} treatment and the k^{th} breed

$(SA)_{jk}$ = interaction between the j^{th} sex and the k^{th} breed

E_{ijkl} = random errors.

The second analysis used periods as covariates according to the following model:

$$Y_{ijklm} = u + T_i + S_j + A_k + (TS)_{ij} + (TA)_{ik} + (SA)_{jk} + b_1(X_1-\bar{x}) + b_2(X_1-\bar{x})^2 + b_3(X_1-\bar{x})^3 + E_{ijklm}$$

where u, T_i, S_j, A_k, $(TS)_{ij}$, $(TA)_{ik}$ and $(SA)_{jk}$ are the same as described under the first model, and

$b_1(X_1-\bar{x})$ = linear effect of the 1^{th} period

$b_2(X_1-\bar{x})^2$ = quadratic effect of the 1^{th} period

$b_3(X_1-\bar{x})^3$ = cubic effect of the 1^{th} period

E_{ijklm} = random errors.

RESULTS AND DISCUSSION

Hematological Responses

The mean erythrocyte counts for the animals under the three temperature treatments are presented in Table 5. The overall mean erythrocyte count was 8,710,000 cells per cubic millimeter and this value is similar to the values reported by Holman (1955), Alexander et al. (1959) and Schalm (1965). The values are within the range for Brahman and Hereford cattle as reported by Howes (1964). There was a highly significant breed difference ($P < .01$) in erythrocyte numbers and the analyses of variance for this and subsequent variables are presented in appendix tables 20 through 23. The Brahman cattle showed a consistently higher number of cells than the Hereford group regardless of either sex or temperature treatment. This characteristic of higher erythrocytes has been reported by Findlay (1950). Howes (1964) also indicated that the differences in erythrocyte concentration between the two species were consistently significant ($P < .05$ or $P < .01$). The difference found in the present experiment is indicated graphically in Figure 2. These results, however, do not agree with those reported by Long et al. (1952) who failed to show consistent differences between breeds of beef cattle. Their results, were based on turbidity methods for red blood cells determination. The variability between the methods could have accounted for the difference in their results and those in the present experiment.

TABLE 5.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE ERYTHROCYTE COUNTS (x $10^3/mm^3$) OF HEREFORD AND BRAHMAN CATTLE.

Period No.	Breed	TREATMENT 32°C Male	32°C Female	21°C Male	21°C Female	Ambient Male	Ambient Female
1	Hereford	6,174	7,725	6,962	7,712	6,636	8,030
	Brahman	7,852	9,794	4,989	8,519	7,821	10,449
2	Hereford	7,110	8,396	5,782	7,738	8,867	7,547
	Brahman	9,006	9,196	8,939	9,290	9,282	10,330
3	Hereford	7,630	7,882	6,465	8,006	8,359	7,684
	Brahman	9,118	9,167	9,532	8,795	8,872	10,481
4	Hereford	7,929	8,343	6,607	8,346	8,300	7,441
	Brahman	8,732	9,568	8,921	9,129	9,112	9,905
5	Hereford	8,472	7,786	6,711	8,388	8,183	6,962
	Brahman	8,812	9,252	8,893	8,482	9,095	9,724
6	Hereford	8,555	7,938	7,248	8,911	8,349	7,961
	Brahman	9,008	9,384	8,484	8,665	9,261	9,976
7	Hereford	9,022	8,291	7,538	8,992	8,095	7,854
	Brahman	9,134	9,794	9,014	8,657	9,294	10,359
8	Hereford	9,071	8,177	7,388	9,075	7,836	8,639
	Brahman	9,332	9,165	8,532	8,756	9,338	10,583
9	Hereford	8,945	8,232	7,333	9,176	8,902	8,015
	Brahman	9,352	9,233	8,887	8,542	9,824	9,974
10	Hereford	9,196	8,851	7,731	9,541	8,907	8,266
	Brahman	9,364	10,124	9,094	8,822	9,731	10,371
11	Hereford	9,511	9,395	7,742	9,302	9,327	9,371
	Brahman	9,588	10,383	9,637	8,388	10,020	10,510
12	Hereford	9,492	9,448	7,571	9,254	8,959	8,721
	Brahman	9,502	10,127	8,737	8,564	9,462	10,514
Sex Av.	Hereford	8,425	8,372	7,089	8,703	8,393	8,041
	Brahman	9,067	9,599	8,638	8,717	9,259	10,264
	Mean	8,746	8,985	7,863	8,710	8,826	9,153
Treat. Av.	Hereford	8,398		7,896		8,217	
	Brahman	9,333		8,678		9,762	
	Mean	8,865		8,286		8,989	

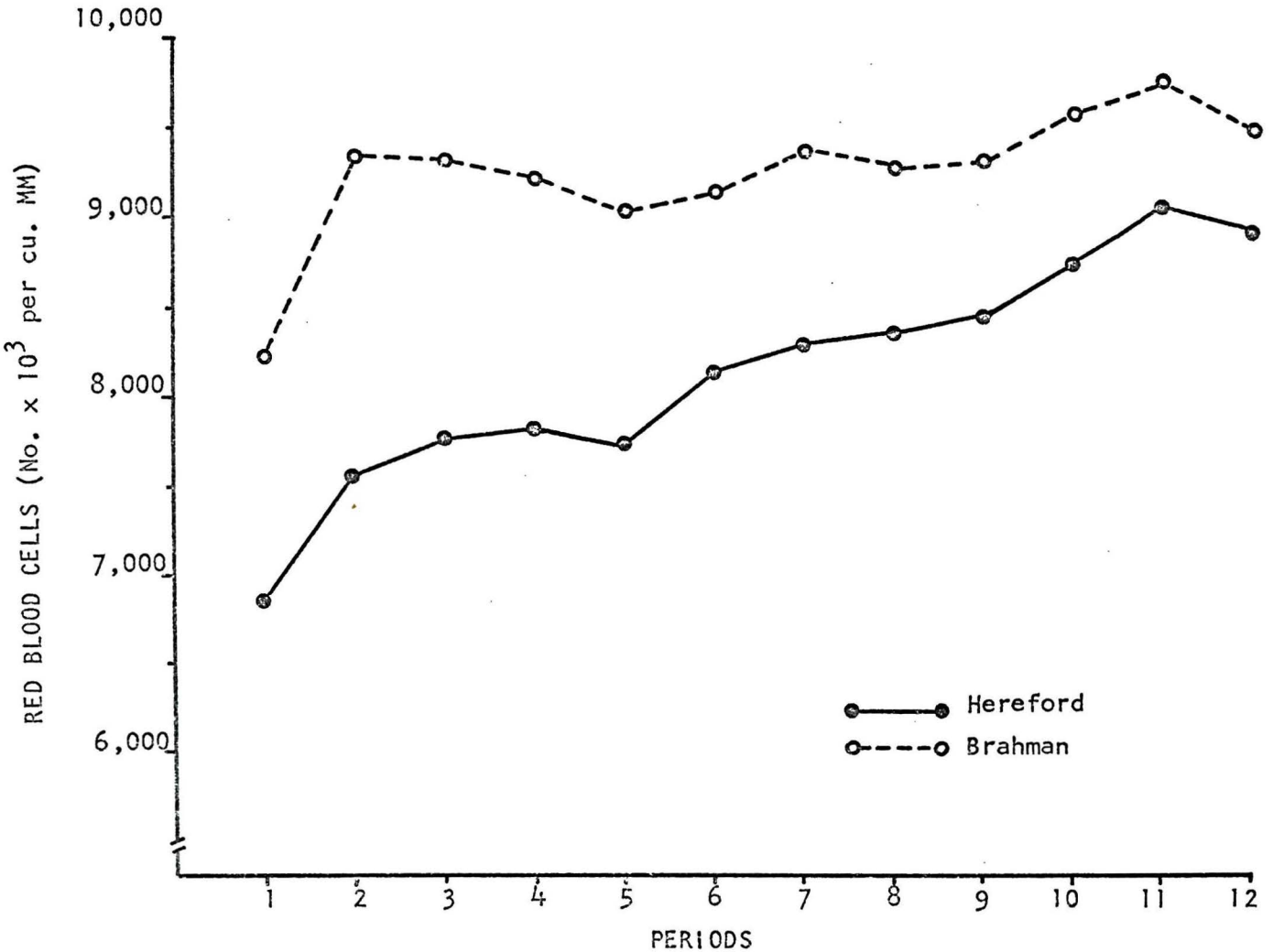

Figure 2.--Effect of breed on erythrocyte counts.

Also apparent from the graph is the increase in erythrocyte numbers with increase in age. This is in agreement with the results reported by Alexander et al. (1959), who indicated an increase from 7.7 million to 8.25 million cells per cu. mm. from a weight of 500 lb. to a weight of 800 lb. in the same animals. The data, however, do not agree with those of Howes (1964) who reported a higher red blood cell count for calves than for adult cattle of the same breeding.

The difference in red blood cells was reflected directly on the packed cell volume (PCV) results (Table 6). The average PCV for the Hereford animals was 36.82% while that for the Brahman was 40.90%. These values are within the range of 29.0 to 42.0% given by Brody et al. (1949) and Rusoff et al. (1954). The difference in PCV between the two species was significant ($P < .05$) and it is in agreement with the results reported by Howes (1964) who found a highly significant difference between these two breeds of beef cattle.

An increase in PCV was observed as the animals advanced in age from 11 to 24 months. This increase is in reality the reflection of the increase in erythrocyte numbers. The breed difference and the PCV increase with age are illustrated in Figure 3.

A decrease in PCV with increases in environmental temperature has been reported by Mehrotra et al. (1954), and Bass and Henschel (1956). Reports to the contrary, are also found. Thus, Rusoff et al. (1954) and Bianca (1957) indicated that there was an increase in erythrocyte numbers and in PCV of cattle exposed to hot, humid conditions. The present experiment failed to show any significant differences between temperature groups and agrees with the results reported by Brody et al. (1949) who showed no temperature effects on PCV of Holstein and Jersey

TABLE 6.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE PACKED CELL VOLUME (VOL. %) OF HEREFORD AND BRAHMAN CATTLE.

Period No.	Breed	TREATMENT 32°C Male	32°C Female	21°C Male	21°C Female	Ambient Male	Ambient Female
1	Hereford	28.50	33.77	32.50	33.17	31.33	37.10
	Brahman	33.50	38.77	25.83	35.60	33.50	41.77
2	Hereford	31.00	35.17	26.17	34.17	36.33	34.67
	Brahman	37.00	37.33	40.50	36.83	38.00	41.83
3	Hereford	33.17	32.67	31.83	35.00	37.83	36.67
	Brahman	39.00	38.00	43.33	36.67	40.00	42.83
4	Hereford	31.50	34.57	29.83	37.33	37.33	36.67
	Brahman	36.77	41.83	40.17	39.17	40.00	44.17
5	Hereford	33.50	32.33	30.17	37.33	38.50	35.17
	Brahman	39.77	39.00	41.17	35.67	40.17	43.33
6	Hereford	33.83	33.67	34.50	40.50	39.00	40.17
	Brahman	40.23	39.00	40.67	37.27	43.50	45.83
7	Hereford	35.27	34.67	34.50	41.50	37.83	39.00
	Brahman	41.00	41.83	42.67	38.50	40.33	47.17
8	Hereford	36.33	33.67	35.00	44.67	37.00	42.00
	Brahman	43.23	40.33	41.00	39.17	41.17	48.00
9	Hereford	36.00	35.17	34.67	44.00	42.00	41.00
	Brahman	43.23	39.83	43.17	39.17	43.50	45.83
10	Hereford	36.33	37.50	37.33	46.17	42.33	42.33
	Brahman	41.77	44.17	44.00	40.17	43.33	47.67
11	Hereford	37.50	38.50	37.00	45.67	42.83	42.33
	Brahman	43.50	44.33	44.33	37.50	44.67	48.67
12	Hereford	38.00	38.33	36.00	46.17	41.67	44.17
	Brahman	42.00	42.50	41.67	38.83	40.83	48.17
Sex Av.	Hereford	34.24	35.00	33.29	40.47	38.66	39.27
	Brahman	40.08	40.57	40.70	37.87	40.75	45.43
	Mean	37.16	37.78	37.00	39.17	39.70	42.35
Treat. Av.	Hereford	34.62		36.88		38.96	
	Brahman	40.33		39.29		43.09	
	Mean	37.47		38.08		41.03	

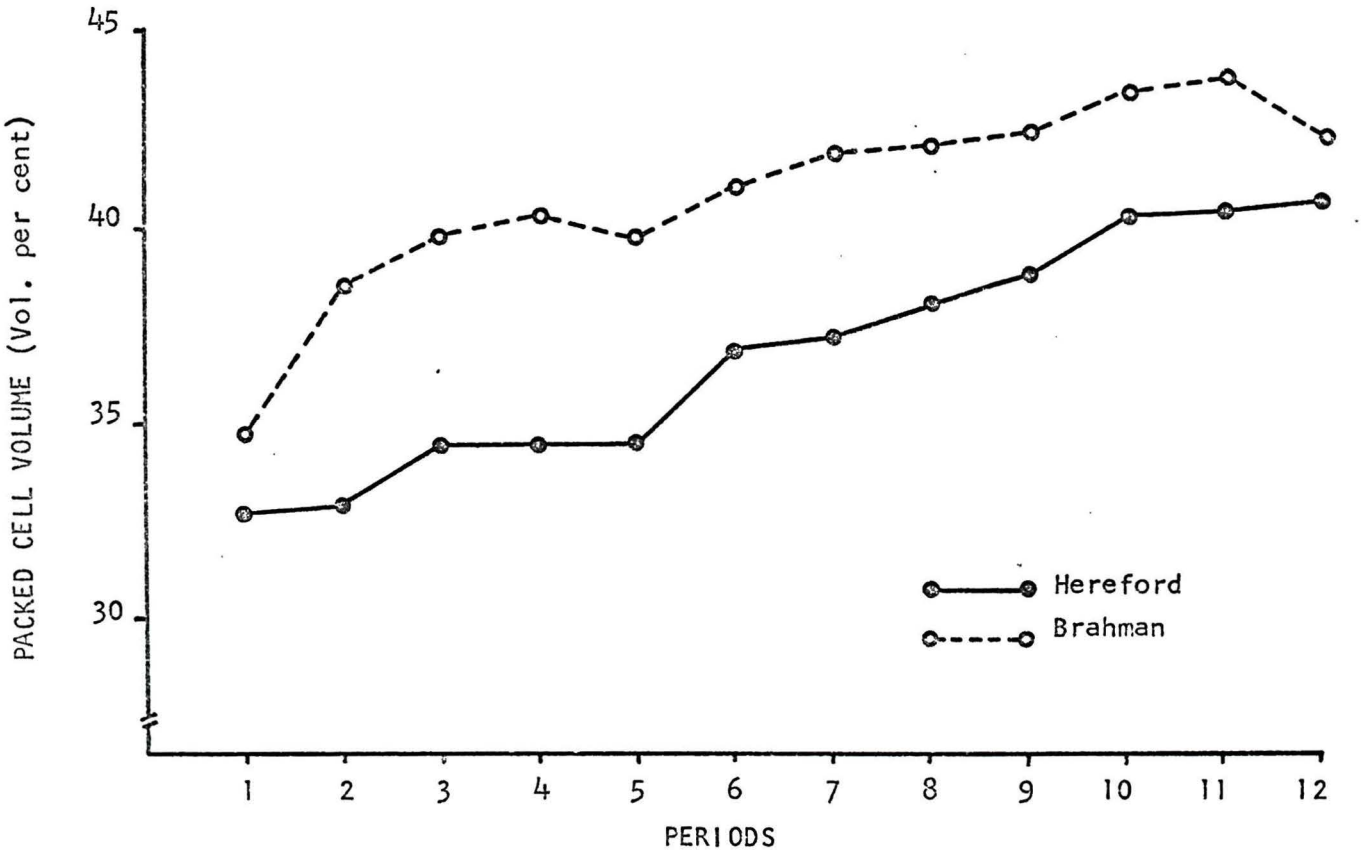

Figure 3.--Effect of breed on packed cell volume.

cattle at 50 to 100°F temperature treatments. It is noteworthy, however, to indicate that as can be observed in Table 6, there is a decrease in PCV from 41.03% for the ambient control group to 38.08% for the 21°C group to 37.47% for the 32°C group. Thus, there appears to be a confounding of the effect of confinement with the effect of temperature increase, as observed from the further decrease in PCV from 21°C to 32°C.

It is also generally agreed that there exists a difference in some hematological indices between the two sexes, with the male having higher erythrocyte number, PCV and hemoglobin (Hb). This was not true in the present experiment, where no statistical differences due to sex could be demonstrated. The trend, however, indicates that the PCV was higher for the females than it was for the males. Less consistent results were found with respect to erythrocytes (RBC). There is no apparent explanation for this since this difference was consistent within treatments, thus indicating an apparently normal condition. Larger numbers of animals should be used in order to reach more definite conclusions.

The overall average value of Hb. in the present study was 13.16 gm./100 ml. with a range of 8.41 to 17.09 gm./100 ml. This variation is extreme but, the highest value of 17.09 gm./100 ml. occurred only once and the low value was due to the presence of anemia in two animals during the first four determinations. Table 7 shows the average Hb. values throughout the experimental period. McCay (1931), Manresa and Reyes (1934), Manresa and Falcon (1939), Brody et al. (1949) and more recently Walker (1958), Mullick (1960) and Bhannasiri et al. (1961) have presented data on the hemoglobin content of the blood of European

TABLE 7.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE HEMOGLOBIN CONCENTRATION (gm/100 ml.) OF HEREFORD AND BRAHMAN CATTLE.

Period No.	Breed	TREATMENT 32°C Male	32°C Female	21°C Male	21°C Female	Ambient Male	Ambient Female
1	Hereford	9.64	12.28	11.41	11.68	11.35	13.12
	Brahman	11.73	13.20	9.88	12.12	11.84	14.50
2	Hereford	10.26	12.07	8.41	11.75	12.15	12.22
	Brahman	11.64	12.45	13.19	12.55	12.30	14.36
3	Hereford	10.72	10.43	10.00	11.49	12.08	12.16
	Brahman	12.58	12.18	14.28	11.55	12.87	13.91
4	Hereford	10.92	11.63	10.36	13.01	12.95	12.70
	Brahman	11.89	13.81	13.42	12.59	13.25	14.71
5	Hereford	11.23	10.48	10.33	12.79	13.03	12.06
	Brahman	12.47	12.52	13.54	11.48	13.14	14.28
6	Hereford	12.04	11.73	11.76	14.99	13.51	14.80
	Brahman	13.92	13.51	14.37	12.76	13.91	16.42
7	Hereford	12.24	11.90	11.87	15.03	12.60	14.54
	Brahman	13.64	14.22	14.39	12.90	13.04	16.91
8	Hereford	12.33	11.50	12.49	16.56	12.89	15.49
	Brahman	14.41	13.78	13.90	12.84	13.42	16.99
9	Hereford	12.16	11.47	12.02	14.96	14.48	14.27
	Brahman	13.94	13.35	14.07	12.64	13.95	15.56
10	Hereford	12.36	12.36	12.94	15.73	14.60	14.99
	Brahman	13.06	14.14	14.40	12.84	13.89	16.22
11	Hereford	13.02	12.56	13.24	15.62	15.36	14.85
	Brahman	14.13	14.52	14.95	12.07	15.13	16.22
12	Hereford	13.26	13.36	12.44	15.83	14.59	15.84
	Brahman	13.94	14.60	13.99	13.16	13.99	17.09
Sex Av.	Hereford	11.68	11.81	11.43	14.12	13.29	13.92
	Brahman	13.11	13.52	13.69	12.45	13.39	15.59
	Mean	12.39	12.66	12.56	13.28	13.34	14.75
Treat. Av.	Hereford	11.74		12.77		13.60	
	Brahman	13.31		13.07		14.49	
	Mean	12.53		12.92		14.05	

and Asiatic cattle. Allcroft (1941) indicated that, of 295 clinically healthy cows, approximately 80% of the Hb. values were between 9.5 and 13.4 gm. per 100 ml. of blood (gm./100 ml.) with approximately 50% within the narrower limits of 10.5 and 12.4 gm./100 ml.

There was a significant ($P < .05$) difference in Hb. content due to temperature treatment. This difference is illustrated in Figure 4, and it appears to be mainly between the animals in the ambient control group and those in the chambers. The average Hb. value for the ambient control group was 14.05 gm./100 ml. while those for the 21°C group and the 32°C group were 12.92 and 12.53 gm./100 ml., respectively. Although the difference between the 21°C and 32°C groups is not statistically significant, the ranking is, nevertheless, in such a fashion as to indicate an adverse high temperature effect, over and above the apparent effect of confinement. These results are in agreement with those of Manresa et al. (1939); Manresa and Falcon (1939) and Bass and Henschel (1956) who reported a decrease in Hb. value of animals under high temperature stress. The mechanism whereby high temperature affects the level of blood hemoglobin is not clearly apparent. It is possible, however, that the heat load imposed upon the animal makes it necessary for the organism to mobilize the energy reserves towards the physiological mechanisms directly involved in heat dissipation, thus removing energy sources from such processes as protein synthesis and thereby, reducing hemoglobin formation. This type of energy shift has been described by Lee and Phillips (1948). A second possible explanation for this result is based upon the action of thyroid hormone. It is commonly known that in hypothyroid cases, there is a reduction in the hemoglobin content of the blood. The anemia which is present in

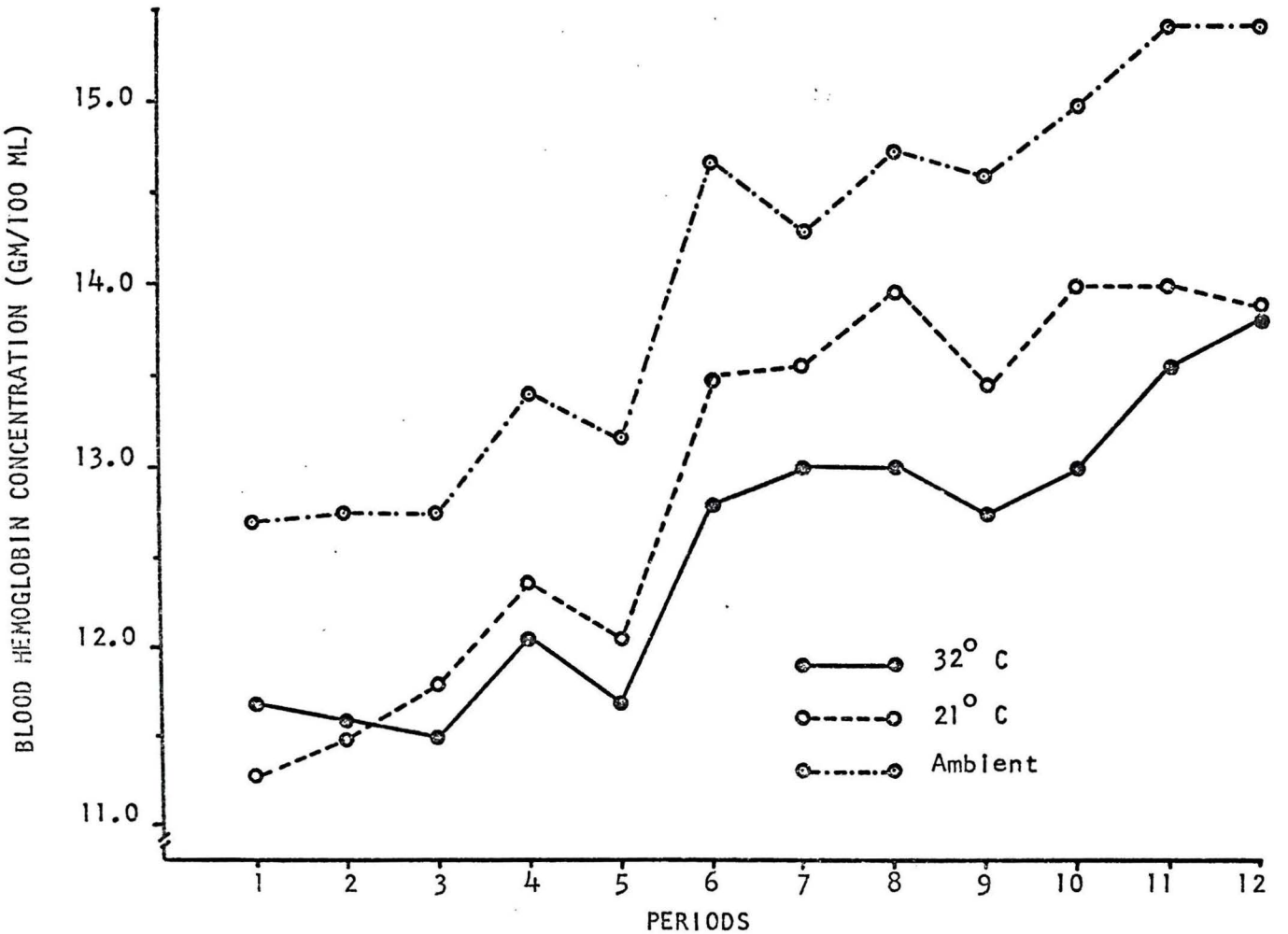

Figure 4.--Effect of treatment on hemoglobin concentration.

these cases is of the microcytic, hypochromic type. It has been sufficiently demonstrated that exposure of animals to heat stress causes a significant depression in thyroid function (Lee and Phillips, 1948; Findlay, 1963; Yousef and Johnson, 1966; and Cowley, 1968). Thus, it could be hypothesized that the significant reduction observed in hemoglobin content of the blood, found under the conditions of the present experiment, was probably mediated by way of the thyroid gland through a reduction in thyroid hormone production.

In an effort to find a more reliable hematological measurement as an index of heat stress, mean corpuscular volume and mean corpuscular hemoglobin values were calculated. The average mean corpuscular volumes (MCV) for the entire experimental period are presented in Table 8. The overall average value found was 44.78 cubic microns (cu. u) which is within the range of values given by Alexander et al. (1959), Bhannasiri et al. (1961) and Howes (1964). Although the differences are not statistically significant, the results of the present experiment indicate that the MCV values for Brahman cattle are lower for both males and females than those for Hereford cattle (43.98 vs. 48.75 and 43.99 vs. 46.26 cubic microns for Brahman and Hereford bulls and for Brahman and Hereford heifers, respectively). Smaller erythrocytes in cattle of Asiatic origin were also reported by Duckworth and Rattray (1948), and Findlay (1950) also believed this to be true. The results of the present study do not agree however with those of Howes (1964), who reported no difference between the erythrocytic size of Hereford and Brahman cattle.

There was a highly significant ($P < .01$) difference in MCV between temperature treatment groups (Figure 5). The animals under heat stress

TABLE 8.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE MEAN CORPUSCULAR VOLUME (u^3) OF HEREFORD AND BRAHMAN CATTLE.

Period No.	Breed	TREATMENT 32°C Male	32°C Female	21°C Male	21°C Female	Ambient Male	Ambient Female
1	Hereford	46.97	43.70	48.10	43.00	47.63	46.10
	Brahman	42.70	39.77	52.20	42.43	42.87	40.00
2	Hereford	43.87	42.13	45.17	44.20	41.03	46.07
	Brahman	41.20	40.80	45.13	40.37	40.97	40.40
3	Hereford	43.50	41.73	49.13	43.67	45.67	48.07
	Brahman	42.93	41.70	45.47	42.33	45.03	40.80
4	Hereford	39.67	41.70	45.27	44.67	45.37	49.30
	Brahman	42.20	43.97	45.27	43.23	43.93	44.57
5	Hereford	39.23	41.67	45.07	44.43	47.27	50.53
	Brahman	45.17	42.63	46.33	42.23	44.20	44.53
6	Hereford	39.50	42.60	47.63	45.40	46.77	50.47
	Brahman	44.70	41.83	48.23	42.97	46.90	45.87
7	Hereford	39.03	42.10	45.73	46.10	46.80	49.67
	Brahman	45.00	43.07	47.40	44.53	43.43	45.50
8	Hereford	39.93	41.47	47.30	49.37	47.20	45.30
	Brahman	46.43	44.90	48.07	44.93	44.07	45.37
9	Hereford	40.03	43.03	47.10	47.93	47.20	51.17
	Brahman	46.30	43.63	48.67	45.93	44.30	45.97
10	Hereford	39.43	42.63	48.17	48.37	47.60	51.30
	Brahman	44.70	43.93	48.43	45.67	44.53	45.97
11	Hereford	39.33	41.33	47.83	49.17	46.00	46.40
	Brahman	45.53	43.10	46.10	44.97	44.57	42.93
12	Hereford	40.00	40.97	47.47	49.93	46.63	50.63
	Brahman	44.33	42.40	47.67	45.67	43.13	45.83
Sex Av.	Hereford	40.87	42.09	46.99	46.35	46.26	48.75
	Brahman	44.26	42.64	47.41	45.27	43.99	43.98
	Mean	42.57	42.37	47.21	45.06	45.13	46.36
Treat. Av.	Hereford	41.48		46.68		47.51	
	Brahman	43.46		45.59		43.99	
	Mean	42.47		46.13		45.75	

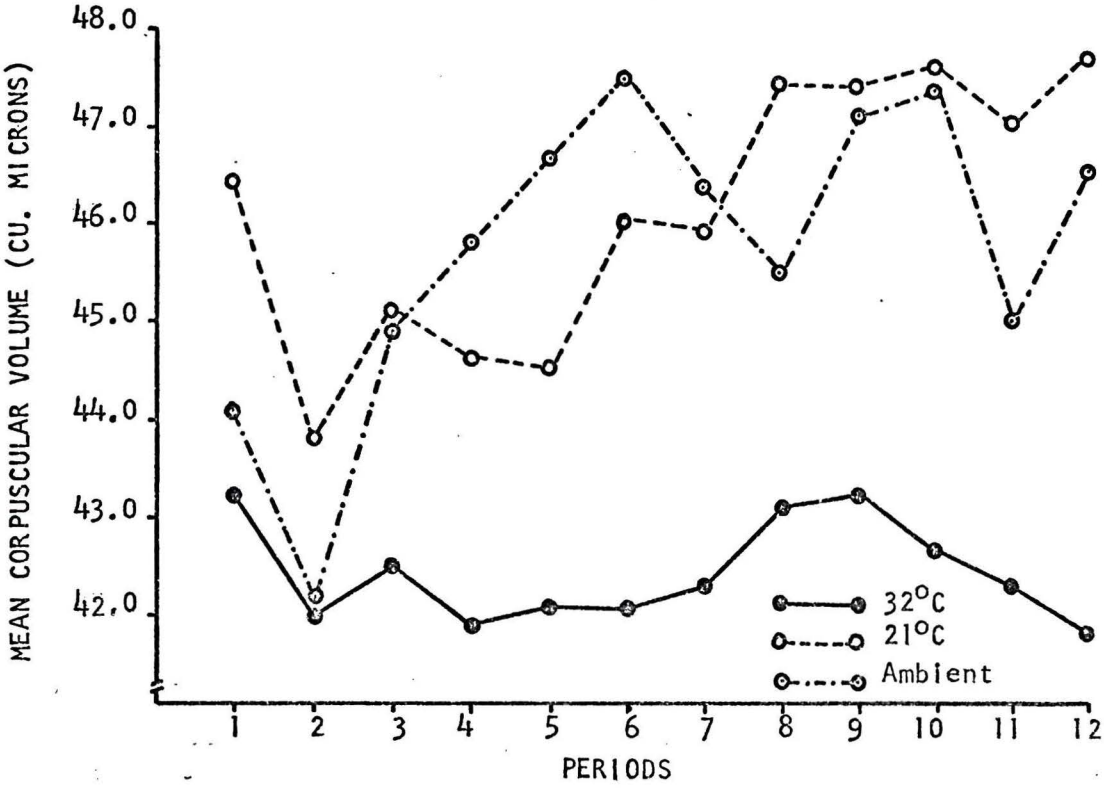

Figure 5.--Effect of treatment on mean corpuscular volume.

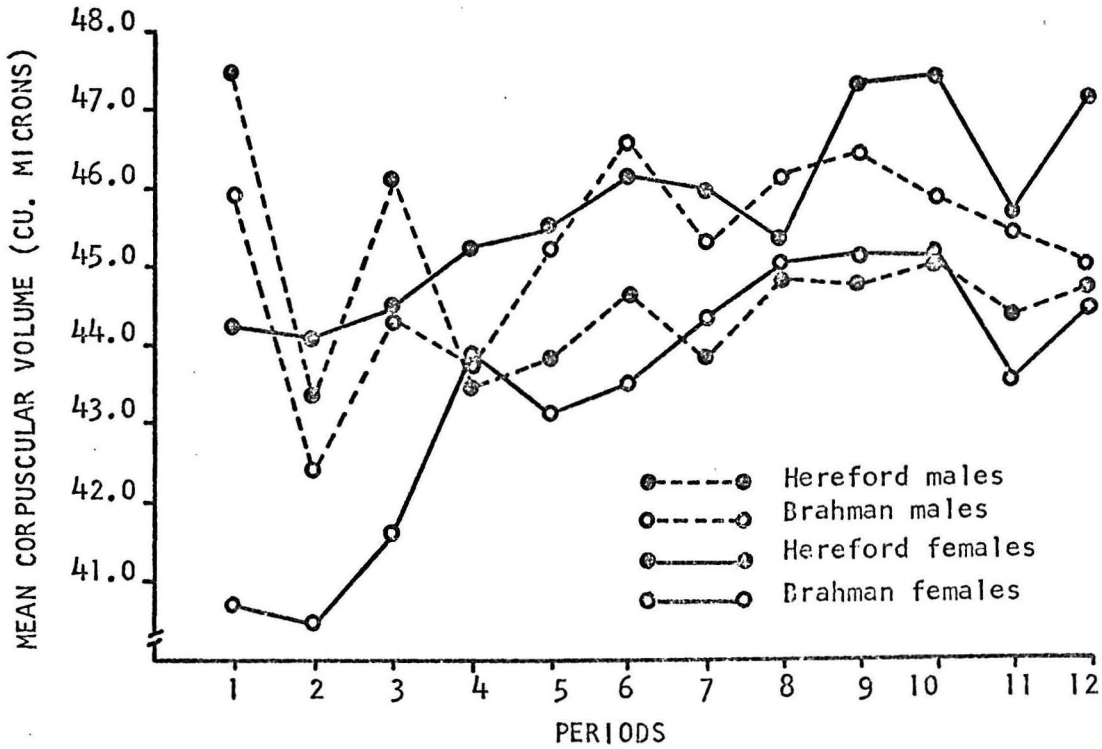

Figure 6.--Effect of breed x sex interaction on mean corpuscular volume.

had lower MCV values than either of the other two groups. Thus, initially, it appeared that MCV could possibly be of use in the assessment of the hematological responses of cattle to stress of temperature and humidity. The largest reduction in MCV was found in the Hereford group at 32°C. The average value for the Hereford breed in the ambient control group was 47.51 cu. u and for those at 21°C was 46.68 cu. u., while the value for those at 32° was 41.48 cu. u. The picture is however complicated and the results do not appear as clear if the various interactions are considered. There was a significant sex x breed interaction ($P < .05$) and this is illustrated in Figure 6. There appears to be two distinct groups. A higher group which is composed of the Brahman males and the Hereford females, and a lower group which included the Hereford males and the Brahman females. The significance of this interaction is obscured even more by the significant ($P < .05$) temperature x sex (Figure 7) and the highly significant ($P < .01$) temperature x breed (Figure 8) interactions. These results thus point out the need to consider sex as well as breed when investigations on the effects of temperature and humidity stress are carried out with animals of Asiatic and European origin.

Mean corpuscular hemoglobin (MCH) varies in Jersey cattle from 8.45 to 14.85 micro-micrograms per red blood cell (uug./RBC) according to Schalm (1965) and this includes animals from 4 months to 14 years of age. Holman (1955) and Bhannasiri et al. (1961) reported a range of 14 to 20 uug./RBC for European beef cattle. An increase in MCH with age was reported by Alexander et al. (1959) and Bhannasiri et al. (1961). The values for MCH found in the present experiment are presented in Table 9. They range from 13.0 to 20.6 uug./RBC and thus fall within

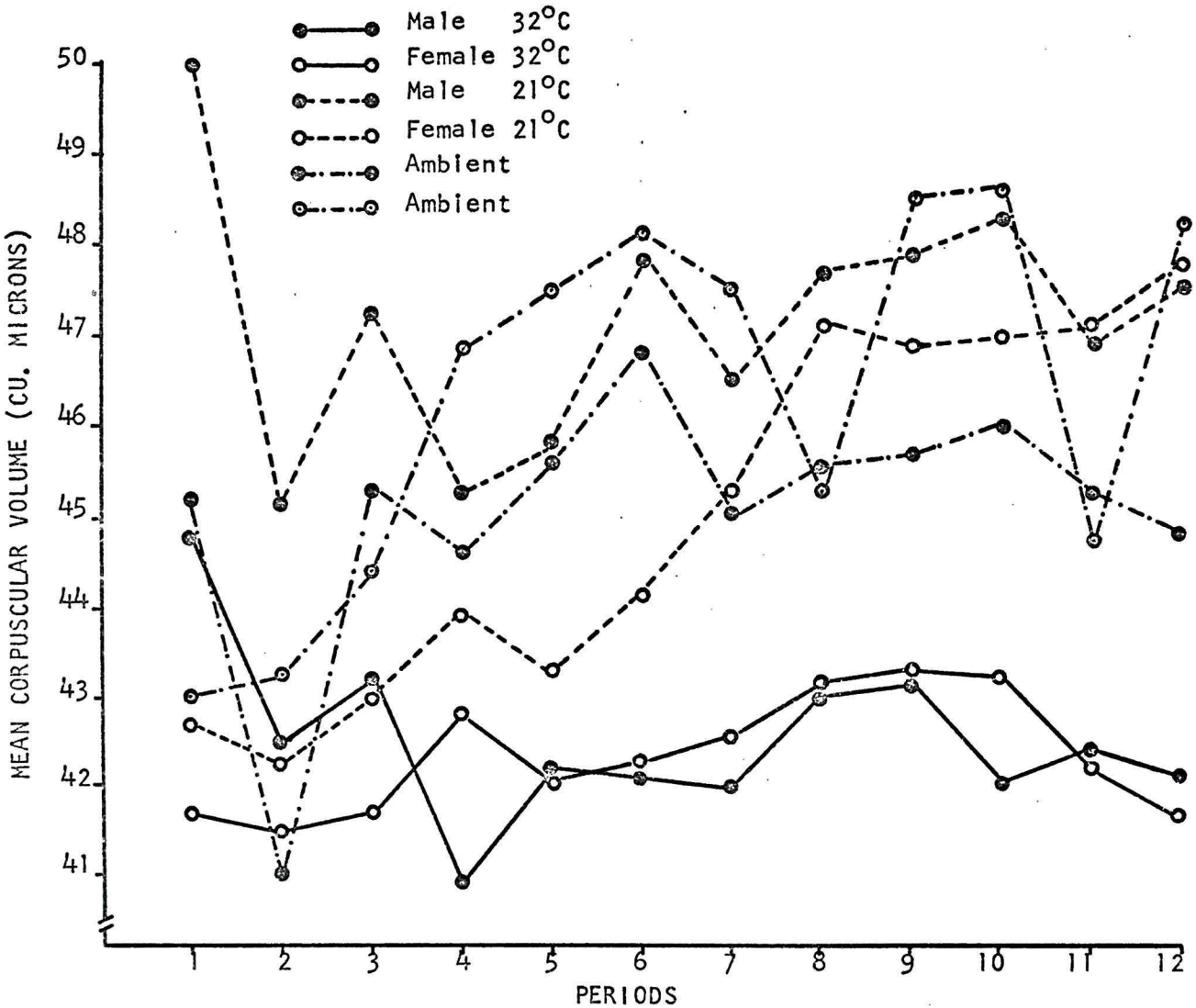

Figure 7.--Effect of treatment x sex interaction on mean corpuscular volume.

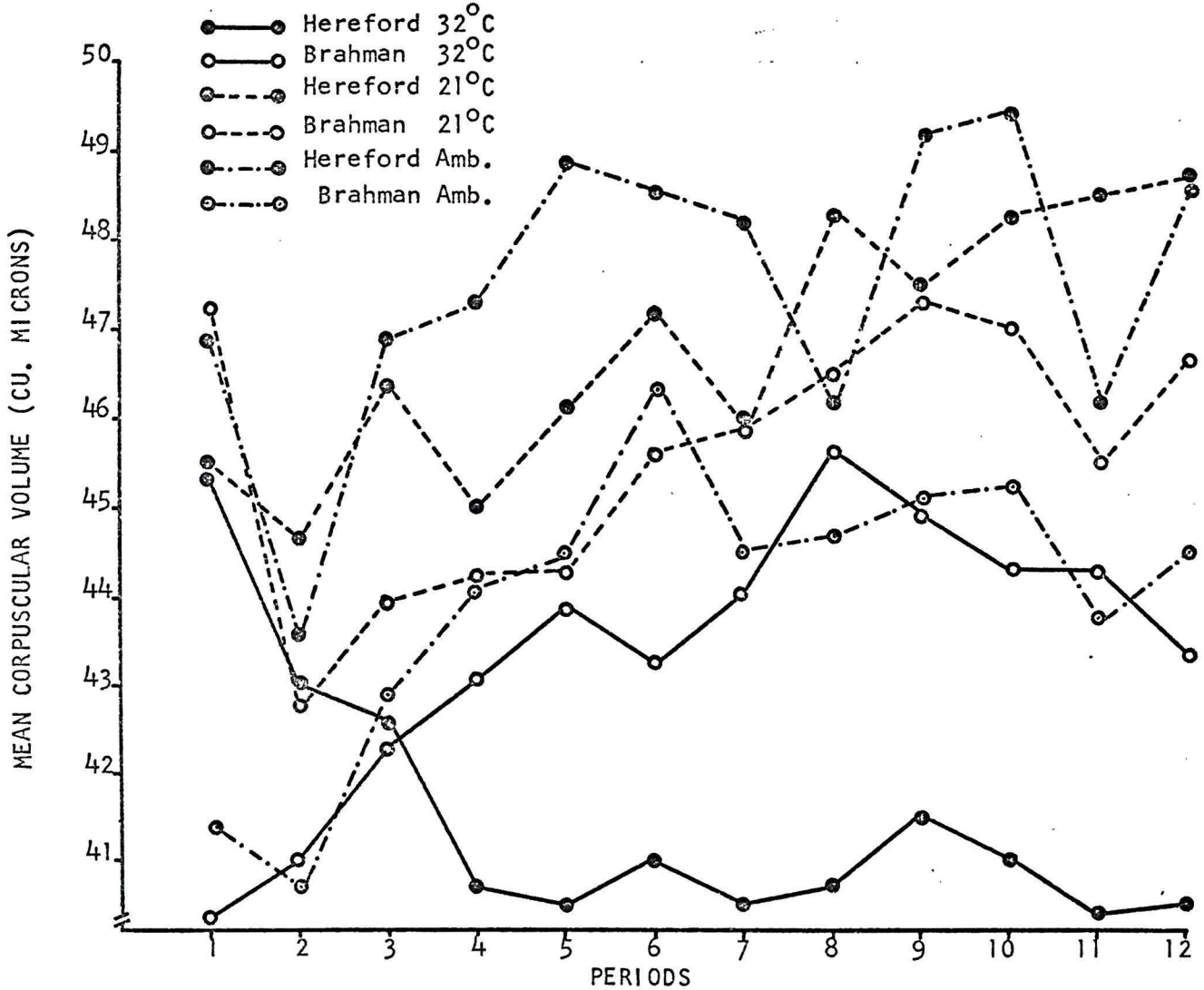

Figure 8.--Effect of treatment x breed interaction on mean corpuscular volume.

TABLE 9.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE MEAN CORPUSCULAR HEMOGLOBIN (uugm./RBC) OF HEREFORD AND BRAHMAN CATTLE.

Period No.	Breed	TREATMENT 32°C Male	32°C Female	21°C Male	21°C Female	Ambient Male	Ambient Female
1	Hereford	15.87	15.93	17.03	15.13	17.30	16.33
	Brahman	14.93	13.53	20.60	14.37	15.13	13.83
2	Hereford	14.47	14.47	14.50	15.20	13.67	16.23
	Brahman	13.00	13.57	14.70	13.70	13.10	13.90
3	Hereford	14.00	13.33	15.60	14.33	14.53	15.90
	Brahman	13.87	13.30	15.00	13.30	14.50	13.27
4	Hereford	13.77	14.03	15.70	15.53	15.73	17.07
	Brahman	13.67	14.43	15.13	13.97	14.57	14.83
5	Hereford	13.17	13.53	15.40	15.20	15.93	17.37
	Brahman	14.20	13.67	15.23	13.60	14.50	14.67
6	Hereford	14.03	14.83	16.13	16.80	16.20	18.57
	Brahman	15.50	14.40	17.13	14.70	15.07	16.40
7	Hereford	13.53	14.43	15.73	16.67	15.53	18.50
	Brahman	14.97	14.60	15.97	14.93	14.07	16.27
8	Hereford	13.57	14.23	16.87	18.30	16.43	18.00
	Brahman	15.53	15.33	16.33	14.70	14.40	16.00
9	Hereford	13.53	14.00	16.30	16.27	16.23	17.80
	Brahman	15.00	14.60	15.87	14.83	14.23	15.60
10	Hereford	13.37	14.03	16.70	16.50	16.40	18.17
	Brahman	14.03	14.07	15.87	14.60	14.30	15.63
11	Hereford	13.63	13.50	17.17	16.80	16.47	16.23
	Brahman	14.77	14.13	15.53	14.47	15.10	15.43
12	Hereford	13.90	14.27	16.40	17.13	16.33	18.17
	Brahman	14.77	14.50	16.03	15.47	14.77	16.30
Sex Av.	Hereford	13.90	14.22	16.13	16.16	15.90	17.36
	Brahman	14.52	14.18	16.12	14.39	14.48	15.18
	Mean	14.21	14.20	16.12	15.27	15.19	16.27
Treat. Av.	Hereford	14.06		16.14		16.63	
	Brahman	14.35		15.25		14.83	
	Mean	14.20		15.70		15.73	

the range reported by other investigators. Contrary to other results, however, the MCH did not show an increase with age and, in fact, the highest average found was at the first determination when the animals were 15 months old rather than at the end of the experimental period when the animals were 27 months old. There was, however, an initial decline after the first determination and a steadily upward trend was observed from the lowest point of 14.21 uug./RBC. This increasing trend reached a peak at about 5 months after the initial decline, and remained at that high level for the rest of the experimental period.

Mean corpuscular hemoglobin was, among all of the hematological indices measured, probably the most sharply defined as far as its response to stress conditions of temperature and humidity. A highly significant ($P < .01$) difference due to temperature treatment was found in this parameter and it is illustrated in Figure 9. The average values for the ambient group and the 21°C group were 15.73 and 15.70 uug./RBC, respectively, while the average value for the 32°C group was 14.20 uug./RBC. These results appear to indicate that stress conditions of high temperature and humidity have an adverse effect on the synthesis of hemoglobin, probably through a reduction in circulating metabolites due to the effect on thyroid function and general metabolism. On the other hand, and even though this index (MCH) appears to be of value in the assessment of the effects of prolonged periods of high temperature and humidity, caution should be exercised in the interpretation of results because any value obtained for MCH is the reflection of those values of hemoglobin concentration and erythrocyte numbers, which are subjected to error in determination and to change due to environmental temperature.

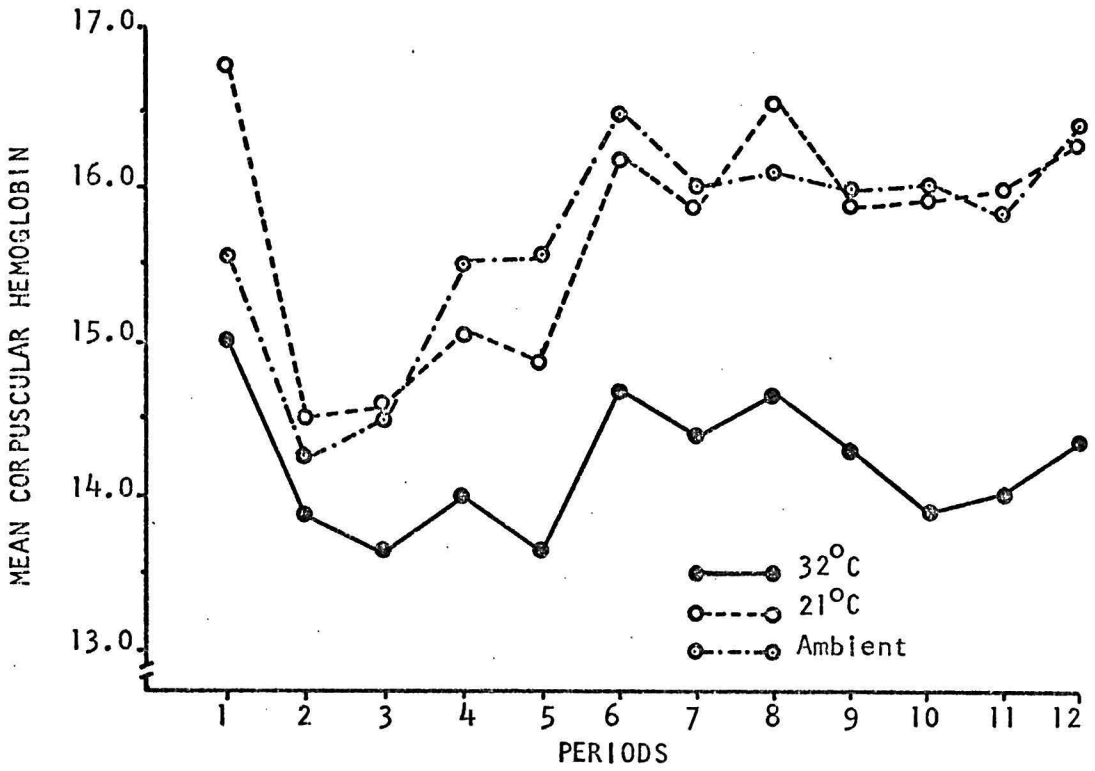

Figure 9.--Effect of treatment on mean corpuscular hemoglobin.

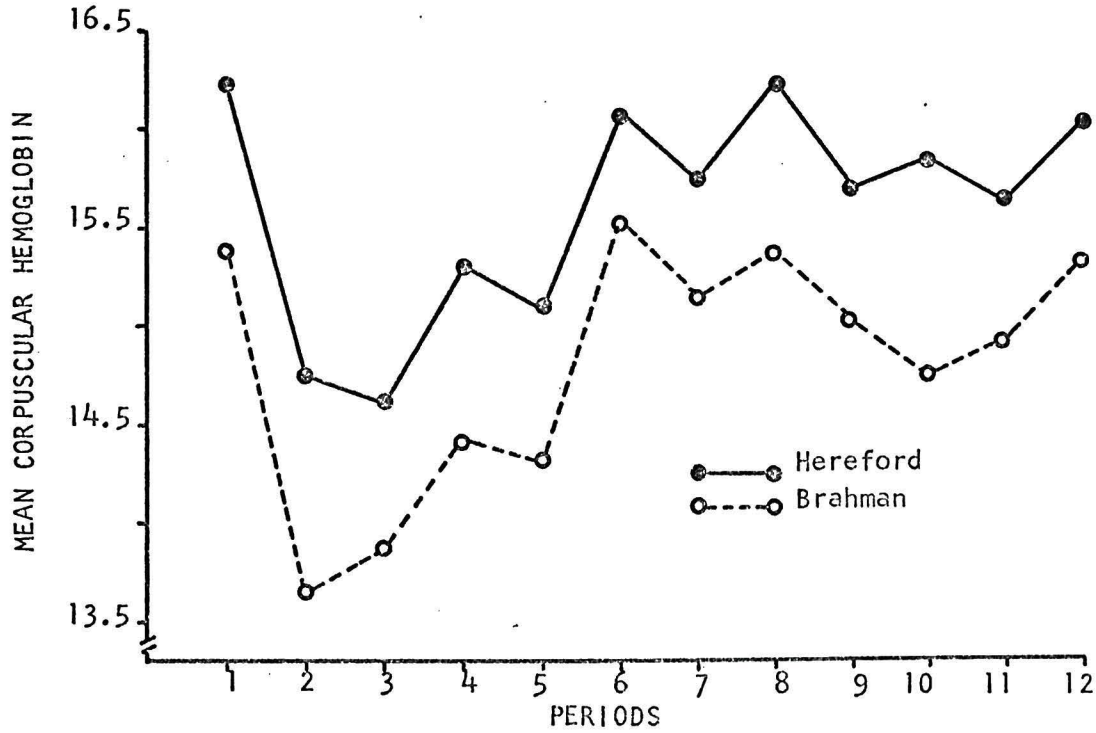

Figure 10.--Effect of breed on mean corpuscular hemoglobin.

A significant difference ($P < .05$) between breeds was found in the MCH values in the present experiment (Figure 10). The Hereford animals had an overall mean value of 15.61 uug./RBC. The results are in accord with those of Howes (1964) who reported highly significant breed differences in MCH between the Brahman and Hereford breeds. His values are, however, somewhat higher than those found in this experiment but this was probably due to the difference in the method of hemoglobin determination and also that of erythrocyte counting.

The number of leukocytes (WBC) in the circulation presented a rather variable picture, with no significant effects due to breed or sex (Table 10). A highly significant difference ($P < .01$) was found due to treatment and this is illustrated in Figure 11. The overall average value of 9,815 WBC/cu. mm. compares favorably with the reports in the literature for beef and dairy cattle (Brody et al. 1949; Rusoff et al. 1954; Alexander et al. 1959 and Bhannasiri et al. 1961). The lack of breed differences in leukocyte numbers also agrees with the results of Duckworth and Rattray (1948), Findlay (1950) and Howes (1964), but is not in agreement with the reports of Rusoff et al. (1954) who found a highly significant breed difference in leukocyte numbers in Holstein, Jersey and Guernsey bulls.

The highly significant treatment difference found in the present experiment appears to be due more to the effect of confinement than to the effect of high temperature. The average WBC number for the ambient group was 11,167 WBC/cu. mm. while those for the 21°C and 32°C groups were 9,266 and 9,010 WBC/cu. mm., respectively. The only apparent explanation for the difference lies in the amount of exercise required of the animals just prior to bleeding. Schalm (1965) reported

TABLE 10.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE LEUKOCYTE COUNTS (NO./mm^3) OF HEREFORD AND BRAHMAN CATTLE.

Period No.	Breed	TREATMENT 32°C Male	32°C Female	21°C Male	21°C Female	Ambient Male	Ambient Female
1	Hereford	9,967	9,631	9,986	9,979	13,131	11,216
	Brahman	13,818	9,841	8,660	11,154	12,537	13,021
2	Hereford	11,140	9,338	9,862	9,349	11,017	9,951
	Brahman	11,543	7,004	9,257	10,612	12,957	11,989
3	Hereford	11,590	9,259	7,742	8,122	11,421	9,501
	Brahman	9,648	7,631	9,933	10,259	14,112	12,454
4	Hereford	9,925	9,718	5,963	10,647	10,946	11,347
	Brahman	7,859	8,449	8,263	10,185	12,532	12,884
5	Hereford	11,208	8,659	6,863	8,576	11,441	11,352
	Brahman	11,009	8,066	9,798	11,026	13,293	11,661
6	Hereford	10,659	8,176	8,789	9,629	9,927	9,686
	Brahman	9,799	7,314	9,236	10,463	11,940	12,379
7	Hereford	10,038	9,434	7,156	11,913	9,428	10,278
	Brahman	8,564	7,970	10,136	10,593	12,580	11,358
8	Hereford	9,313	8,210	7,697	11,038	7,653	12,510
	Brahman	8,644	7,762	8,215	10,293	11,051	11,195
9	Hereford	7,448	7,637	6,397	8,766	9,088	8,325
	Brahman	7,672	6,645	8,605	9,396	12,689	9,918
10	Hereford	7,926	7,027	7,057	8,972	9,129	9,881
	Brahman	7,546	7,175	8,714	10,074	10,410	12,173
11	Hereford	7,989	8,991	6,799	9,476	8,861	8,866
	Brahman	9,630	8,522	9,515	9,249	11,069	11,618
12	Hereford	11,453	8,345	8,911	11,258	9,351	10,945
	Brahman	8,885	8,410	10,166	10,007	10,726	14,239
Sex Av.	Hereford	9,888	8,702	7,769	9,810	10,116	10,322
	Brahman	9,551	7,899	9,208	10,276	12,158	12,074
	Mean	9,720	8,301	8,489	10,043	11,137	11,198
Treat. Av.	Hereford	9,295		8,790		10,219	
	Brahman	8,725		9,742		12,116	
	Mean	9,010		9,266		11,167	

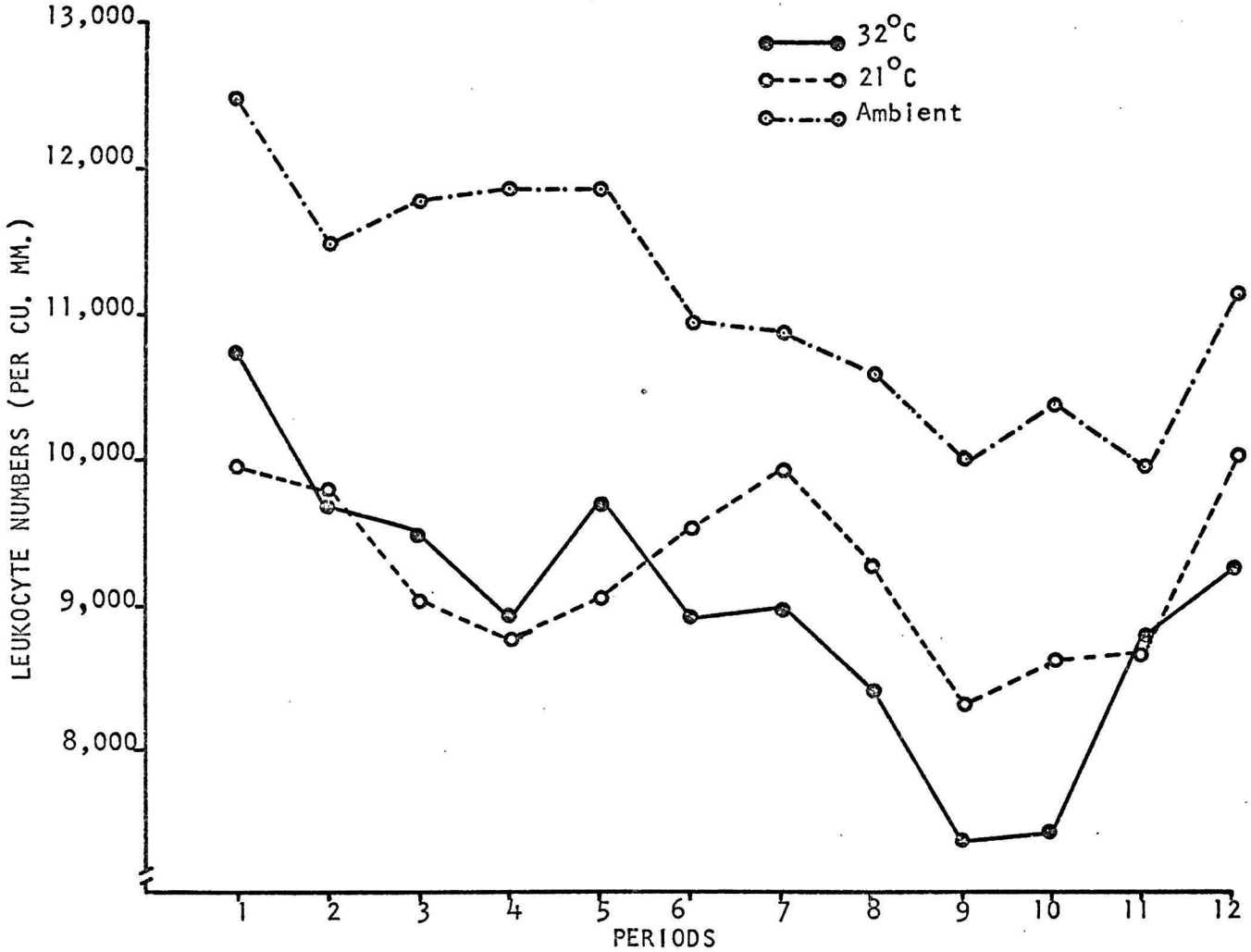

Figure 11.--Effect of treatment on leukocyte counts.

an increase in leukocyte counts in a horse after exercise. The ambient group in the present experiment was moved from the pastures to the holding pens and working area just prior to the collection of the samples. The distance was between 400 and 500 yards while those animals in the environmental chambers were only moved about 50 yards. Thus, it appears that the difference in leukocyte numbers, between the animals in the ambient group and those in chambers, was not due to a reduction in the number of cells in the chamber-groups animals but, rather to an increased number in the animals in the ambient group due to exercise.

Body Temperature Response

The results of rectal temperature measurements are shown in Table 11. The average rectal temperature for the ambient, 21°C and 32°C groups were 39.3°C, 39.0°C and 39.2°C, respectively. There was a highly significant difference ($P < .01$) due to treatment but this difference appears to be caused by two different response patterns (Figure 12). The first pattern is an apparent difference between the ambient group and the other two treatment groups (21° and 32°C) with the ambient group being lower from the 3rd through the 6th experimental period. After the 6th period and until the end of the experiment, the pattern is reversed, with the ambient group having a higher average rectal temperature than the other two groups. At the same time the two chamber groups showed a sudden drop of about the same magnitude each and remained fairly constant for the rest of the experiment (periods 7th through 12th). Also during the last 6 periods the 32°C group showed a higher (0.2°C) rectal temperature than the 21°C group.

TABLE 11.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE RECTAL TEMPERATURE (^{o}Cent.) OF HEREFORD AND BRAHMAN CATTLE.

Period No.	Breed	TREATMENT 32°C Male	32°C Female	21°C Male	21°C Female	Ambient Male	Ambient Female
1	Hereford	39.2	40.0	38.7	40.1	39.5	39.3
	Brahman	38.4	39.5	39.2	38.9	39.7	39.3
2	Hereford	40.1	40.4	40.2	39.9	39.9	40.3
	Brahman	39.8	39.9	40.0	39.1	39.7	39.7
3	Hereford	40.0	40.8	39.2	40.2	39.5	39.8
	Brahman	39.6	39.5	39.0	40.2	39.5	39.3
4	Hereford	41.2	40.4	40.8	40.6	39.7	39.9
	Brahman	39.6	40.1	40.7	39.9	39.5	39.2
5	Hereford	40.5	40.7	40.6	40.2	39.9	39.9
	Brahman	39.6	39.9	40.0	39.8	39.6	39.3
6	Hereford	39.3	40.8	39.2	39.9	38.9	39.9
	Brahman	39.1	40.1	39.4	39.3	38.7	39.0
7	Hereford	38.9	38.8	38.2	37.9	39.5	39.7
	Brahman	38.2	38.2	38.1	37.9	38.7	38.7
8	Hereford	38.7	38.8	38.1	37.8	39.2	39.8
	Brahman	38.1	38.1	38.2	37.9	38.7	38.8
9	Hereford	38.5	38.8	38.1	37.9	39.1	39.1
	Brahman	38.0	38.1	38.1	37.9	38.7	38.6
10	Hereford	38.1	38.8	38.2	37.9	39.0	39.0
	Brahman	38.0	38.2	38.4	37.9	38.7	38.7
11	Hereford	39.0	38.2	38.1	38.3	39.4	38.7
	Brahman	38.4	38.2	38.2	38.0	38.7	38.2
12	Hereford	38.9	38.1	38.2	38.3	39.3	38.8
	Brahman	38.2	38.2	38.1	38.0	38.8	38.6
Sex Av.	Hereford	39.4	39.6	39.0	39.1	39.4	39.5
	Brahman	38.8	39.0	39.0	38.7	39.1	39.0
	Mean	39.1	39.3	39.0	38.9	39.3	39.3
Treat. Av.	Hereford	39.5		39.3		39.5	
	Brahman	38.9		38.9		39.1	
	Mean	39.2		39.0		39.3	

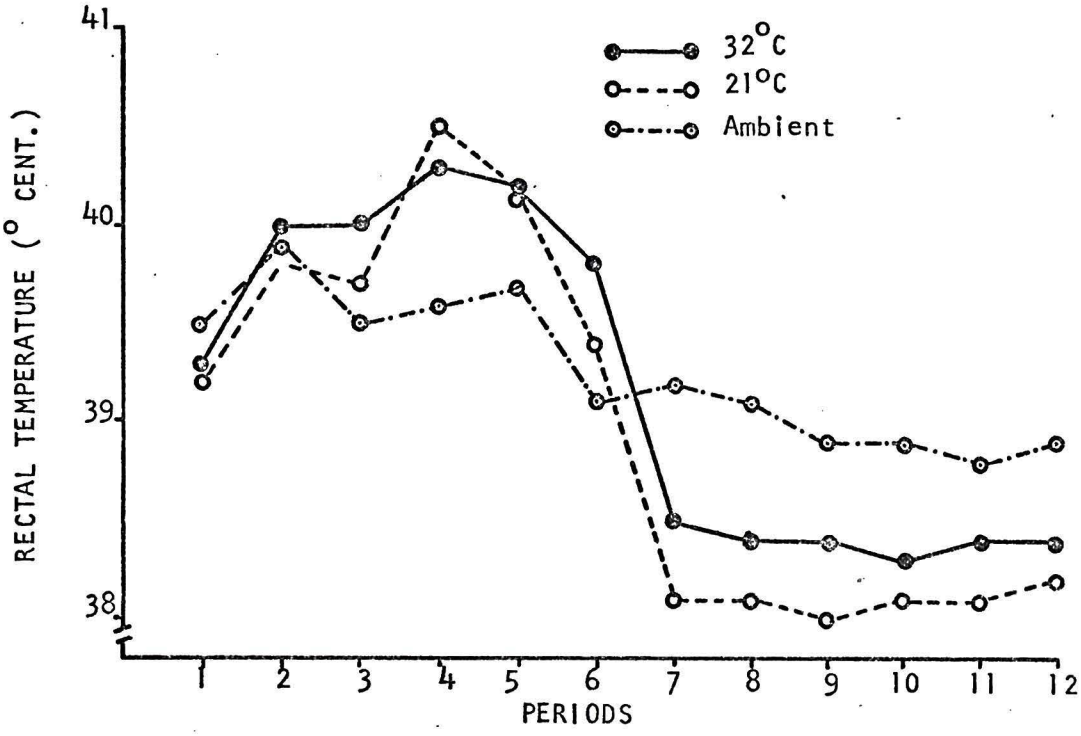

Figure 12.--Effect of treatment on body temperature.

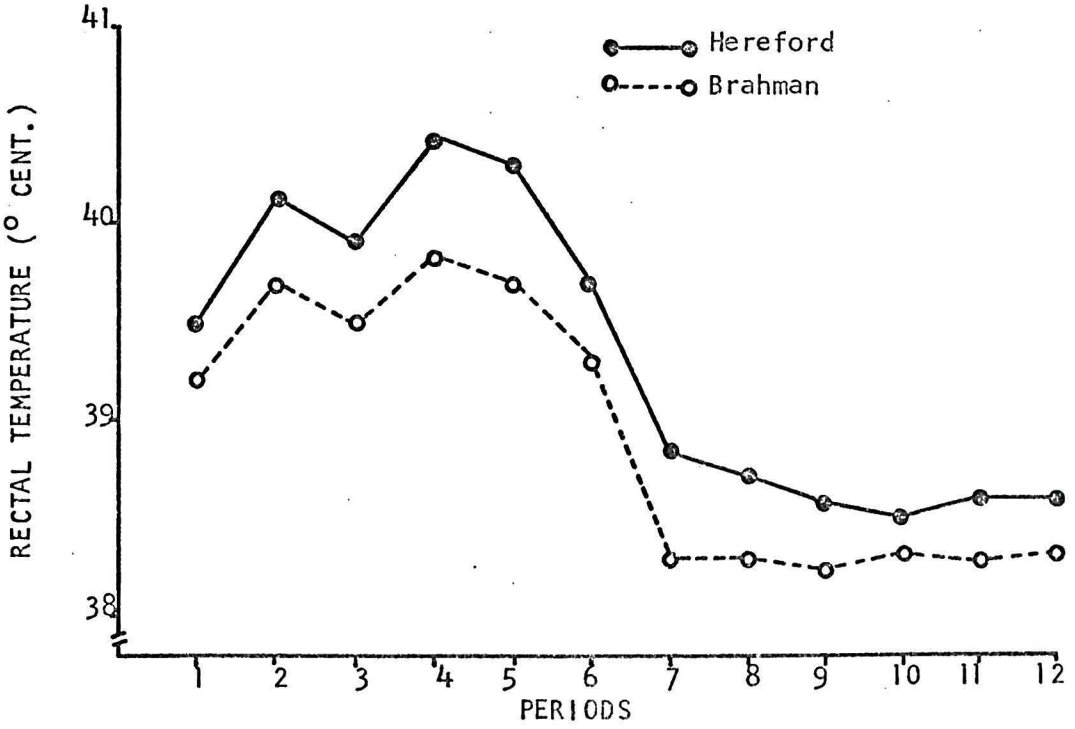

Figure 13.--Effect of breed on body temperature.

The reason for the difference during the two halves of the experiment appears to be the fact that the rectal temperatures of the animals in the 2 chamber groups during the first 6 periods were taken after the animals had been moved to the chute for other samples and data collection. This, however, does not explain the difference between the ambient group and the two chamber groups, since the ambient group was also moved before rectal temperature was taken. During the second half of the experiment, the rectal temperature of the animals in the chambers was taken prior to moving the animals and thus it approximates more the true response to increased ambient temperature as reported by Lee and Rick (1951), Quazi and Shrode (1954), Casady et al. (1956), Weldy et al. (1964) and Gimenez (1966).

A highly significant difference ($P < .01$) in rectal temperature was found between breeds (Figure 13). Hereford animals had an overall average temperature of 39.31°C while the Brahman had an average of 38.91°C. Gimenez (1966) also reported a difference of 0.4°C between the Brahman and Hereford heifers used in his experiments, with the Brahman being lower. Other investigators also have reported significant differences in rectal temperature between Bos taurus and Bos indicus species of beef cattle (Cartwright, 1955; Johnston et al., 1958; Mc Dowell, 1958 and Johnston et al., 1963).

The importance of relative humidity on the responses of cattle to increased environmental temperature has been indicated by Beakley and Findlay (1955), Mullick (1960), Whittow (1962) and Robertshaw and Whittow (1966). This climatic factor appears to be of even more importance when experiments are conducted under environmental chamber conditions (McDowell, 1958). Thus, it appears that, under the conditions of the present experiment, the observed changes in body temper-

ature, and possibly other parameters, are in reality the result of the combined effects of environmental temperature and ambient vapor pressure or relative humidity.

The lower body temperature of the Brahman animals, and therefore the apparent heat tolerance, has been suggested by Cartwright (1955) to be a partially dominant genetic trait. The physiological basis for the lower body temperature increase of the Brahman, under adverse temperature and humidity conditions, appears to be the ability to decrease or lower the metabolic heat production. This is accomplished by a reduction in the level of function of the thyroid gland and therefore decreased production and release of thyroxine (T_4) and triiodotyronine (T_3) (Worstell and Brody, 1953; Johnson and Ragsdale, 1960; Findlay, 1963 and Yousef and Johnson, 1966). There is also the possibility of a decreased rate of utilization of thyroid hormones, as has been postulated by Lundgren and Johnson (1964) and by Yousef and Johnson (1966). This decreased rate of utilization would result in an increased level of thyroid hormones in the systemic circulation. A feed-back control mechanism would then come in to play to regulate (decrease) the rate of synthesis and secretion of thyroid hormones through a reduction in secretion of thyrotropic hormone releasing factor (TRF) from the anterior hypothalamus. The involvement of the higher brain centers and the existence of a TRF center has been reported by Yamada and Greer (1959) and by Kajihara and Kendall (1967). The evidence also exists that the thermoregulatory center demonstrated by Anderssen and Larssen (1961) is thermosensitive not only for the regulation of body temperature but also for the regulation of thyroid function. Anderssen *et al.* (1962) indicated that the pre-optic area's

"heat-loss centre" exerted a tonic inhibition of thyroid activity which could be removed by onset of central hypothermia.

Reproductive Characteristics Responses

The results of the five male reproductive characteristics studied are discussed separately. Data for all of the 12 periods studied is given only for one male characteristic, libido. The other four characteristics provided data suitable for statistical analyses only from the last 6 periods because of the large number of missing values previous to the attainment of puberty.

Results of the libido scores are presented in Table 12. The overall libido score was 1.91 but there was a significant breed difference ($P < .05$), which is illustrated in Figure 14. The Hereford bulls had an overall average of 2.12 while the Brahman bulls had 1.70. This difference was probably the result of the complete absence of sexual interest on the part of two of the Brahman bulls in the ambient group. The reason for this is not clear since other reproductive characteristics were apparently normal. Thus it appears that the individuality of the experimental subjects plays a definite role in the results and therefore the interpretation of data should be made with this in mind. Fields (1968), using the same scoring technique for libido studies, reported an average libido score of 2.9 for 20-month-old Hereford bulls and 1.2 for Brahman bulls of the same age. Crooks (1968) reported values of libido scores of 2.2 for Hereford and 2.0 for Brahman bulls, also at 20 months of age. A highly significant treatment x breed interaction ($P < .01$) was found in the present experiment and this is illustrated in Figure 15. This interaction

TABLE 12.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE LIBIDO (SCORE) OF HEREFORD AND BRAHMAN BULLS.

Period No.	Breed	TREATMENT 32°C	21°C	Ambient
1	Hereford	1.33	1.33	1.67
	Brahman	1.67	1.33	1.33
2	Hereford	1.33	1.33	1.00
	Brahman	1.67	1.33	1.00
3	Hereford	1.00	1.00	1.67
	Brahman	1.67	1.67	1.00
4	Hereford	1.00	1.33	1.33
	Brahman	1.67	2.33	1.33
5	Hereford	1.33	1.33	1.67
	Brahman	1.67	1.33	1.00
6	Hereford	1.33	1.33	5.00
	Brahman	1.67	2.00	2.00
7	Hereford	1.33	1.67	3.00
	Brahman	1.33	1.33	1.67
8	Hereford	1.00	1.67	4.33
	Brahman	1.67	2.33	2.00
9	Hereford	1.67	1.67	4.00
	Brahman	1.33	1.67	2.00
10	Hereford	2.00	2.67	4.00
	Brahman	2.00	3.00	1.33
11	Hereford	3.33	1.67	5.00
	Brahman	2.00	3.33	2.00
12	Hereford	3.67	1.67	5.00
	Brahman	2.00	3.00	1.33
Treat. Av.	Hereford	1.69	1.55	3.13
	Brahman	1.69	2.05	1.37
	Mean	1.69	1.80	2.25

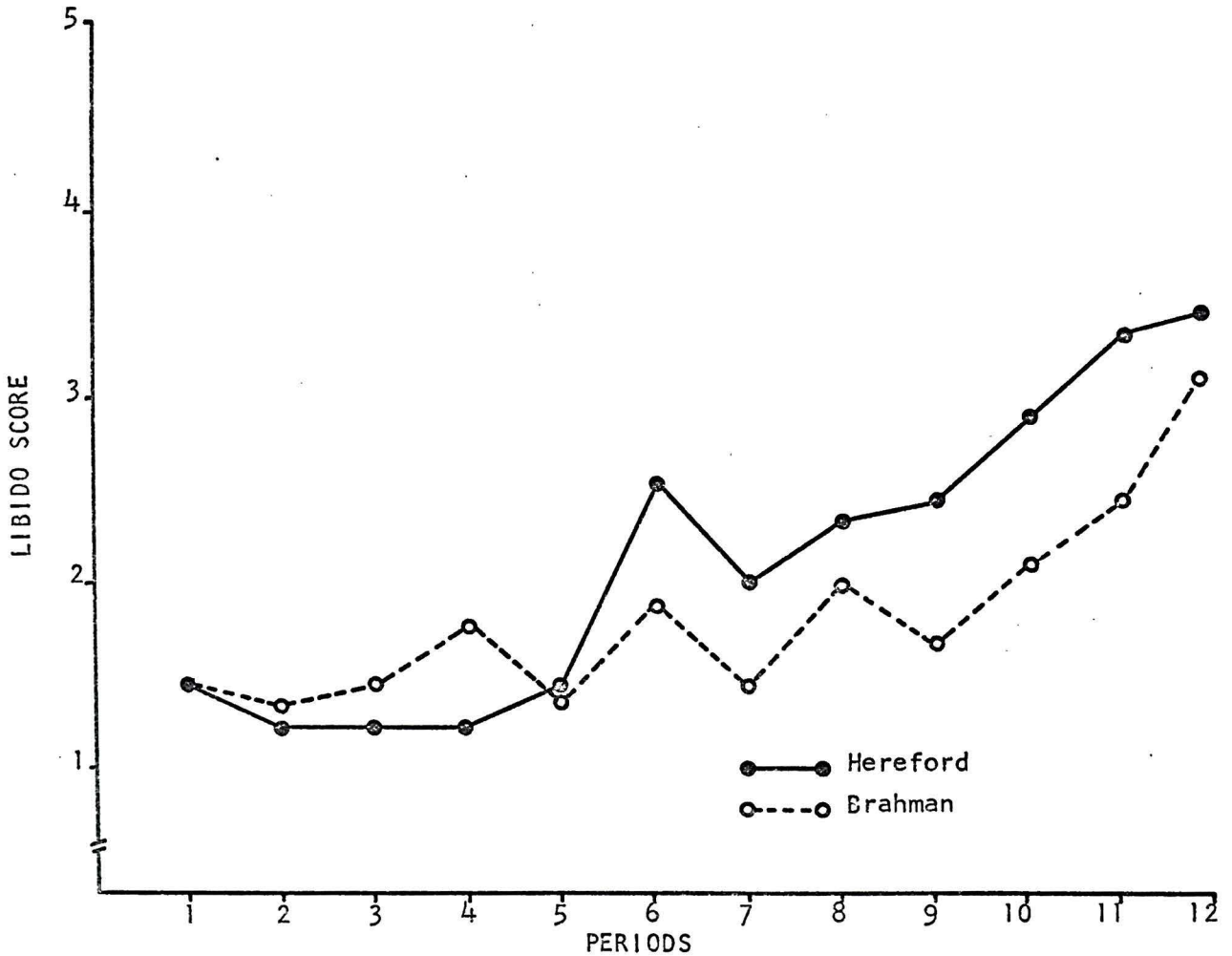

Figure 14.--Effect of breed on libido scores.

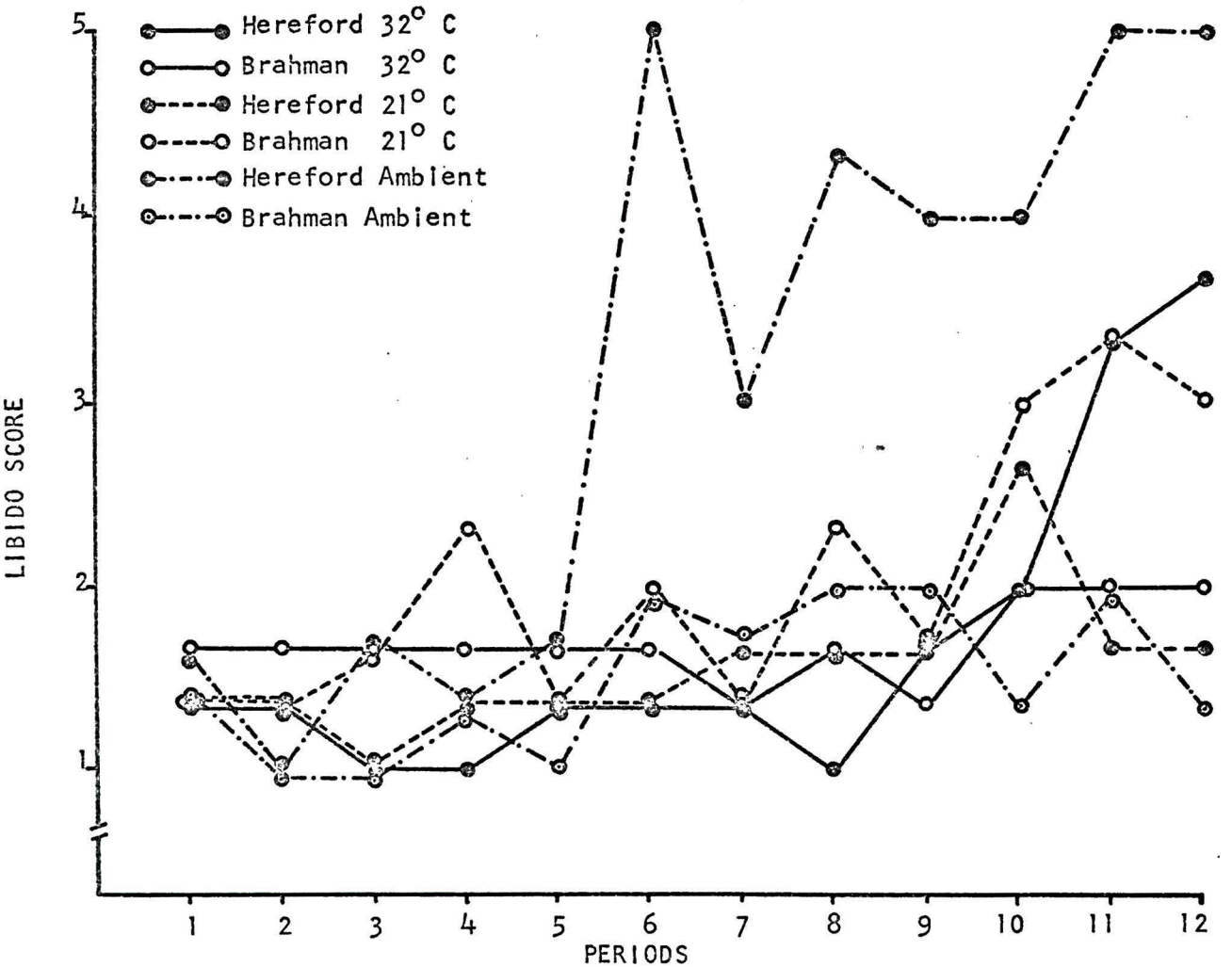

Figure 15.--Effect of treatment x breed interaction on libido scores.

appears to be the result of the higher average of the Hereford bulls in the ambient group (3.13 for the Hereford and 1.37 for the Brahman) in contrast with the higher score (2.05) for the Brahman in the 21°C group as compared to 1.55 for the Hereford in the same group. There was no apparent effect of temperature and humidity on libido of Hereford or Brahman bulls, as indicated by the absence of significance in the statistical analyses. These results do not fully agree with those reported by Casady et al. (1953) who found that bulls exposed to temperatures of up to 98.9°F for as long as 17 days showed some loss of libido, with slow mounting behavior but never failed to mount and thrust. On the other hand, the results obtained here are in agreement with those reported by Pennycuik (1967) who found no significant effect of high temperature on the libido of male mice.

The semen volume results are presented in Table 13. The overall average value of 7.34 ml. compares favorably with results given by other investigators (Fields, 1968 and Crooks, 1968). There was a significant ($P < .05$) effect due to treatment (Figure 16). This difference appears to be due to an initial difference between the ambient group and the two chamber groups, during the 8th and 9th periods. The difference apparently disappeared after this period. It is observed, however, that the reduction in semen volume is much more marked on the Hereford bulls with a decrease from 10.39 ml. in the ambient group to 8.49 ml. in the 21°C group, to 5.45 for the 32°C group. The decrease in the Brahman breed was from 7.89 ml. in the ambient group to 4.98 ml. in the 21°C group, with an increase in the 32°C, over that of the 21°C group, to 5.27 ml. This switching over of the results between breeds among treatments caused the appearance of a highly significant ($P < .01$)

TABLE 13.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE VOLUME OF SEMEN (ml.) OF HEREFORD AND BRAHMAN BULLS.1/

Period No.	Breed	TREATMENT 32°C	21°C	Ambient
1	Hereford	6.00	6.00	7.33
	Brahman	5.73	3.00	5.17
2	Hereford	7.43	8.00	15.67
	Brahman	1.01	4.50	11.67
3	Hereford	3.77	7.50	16.00
	Brahman	7.23	6.40	10.50
4	Hereford	4.50	12.90	10.00
	Brahman	7.50	6.37	7.67
5	Hereford	6.17	10.80	5.37
	Brahman	4.03	5.43	6.00
6	Hereford	4.83	5.77	8.00
	Brahman	6.17	4.23	6.33
Treat. Av.	Hereford	5.45	8.49	10.39
	Brahman	5.27	4.98	7.89
	Mean	5.36	6.73	9.14

1/ Volume of ejaculate and other male characteristics, except libido, were computed only for the last 6 periods due to the large number of missing values during the initial phases of the experiment. See text for explanation.

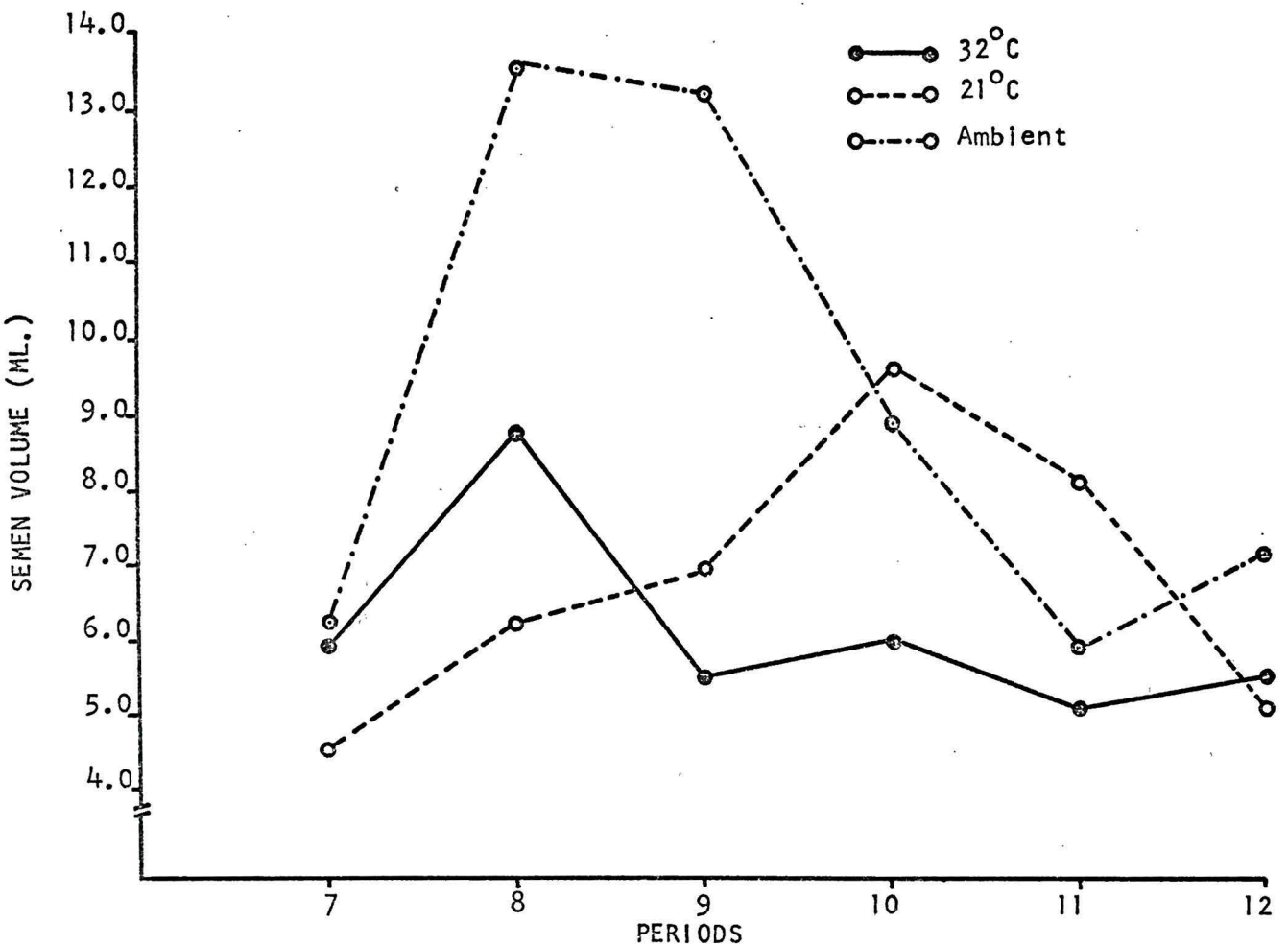

Figure 16.--Effect of treatment on semen volume.

interaction of breed with treatment (Figure 17). There was no definite trend in the variation of the individual breed within treatment, thus making interpretation of the results rather difficult due to the small number of animals per sub-group. The results, however, agree with those of Bogart and Mayer (1946a, b) and Fields (1968) who reported decreased semen volume under high environmental temperature conditions.

More severely affected by heat was the value of initial motility. The results of this parameter are given in Table 14. there was an overall motility of 52.56% which is slightly lower than the values given by Fields (1968) of 68% and by Crooks (1968) of 65% for both European and Asiatic breeds of beef cattle. The overall average is low because of the adverse effects of high temperature and humidity as well as the apparently adverse effects of confinement. There was a similar decline in the average motility from the ambient group to the 21°C (34.8%) and from the 21°C to the 32°C group (37.3%). The absolute motility values, however, were indicative of additive adverse effects of confinement and high environmental temperature and humidity. There was a highly significant ($P < .01$) difference due to treatments and this difference lies between the ambient group at 76.52% and the 32°C group at 31.27% (Figure 18). Two different shapes of curves are observed in this figure also. One shape is formed by the trend followed by the ambient and the 32°C groups which showed a sharp decline from period 7 to 9, followed by a peak at period 11 and again a decline at period 12. This is in contrast to the fairly constant shape of the curve of the 21°C group. There is no apparent explanation for this type of response other than the possible influence of external factors which affected only those animals which had been previously

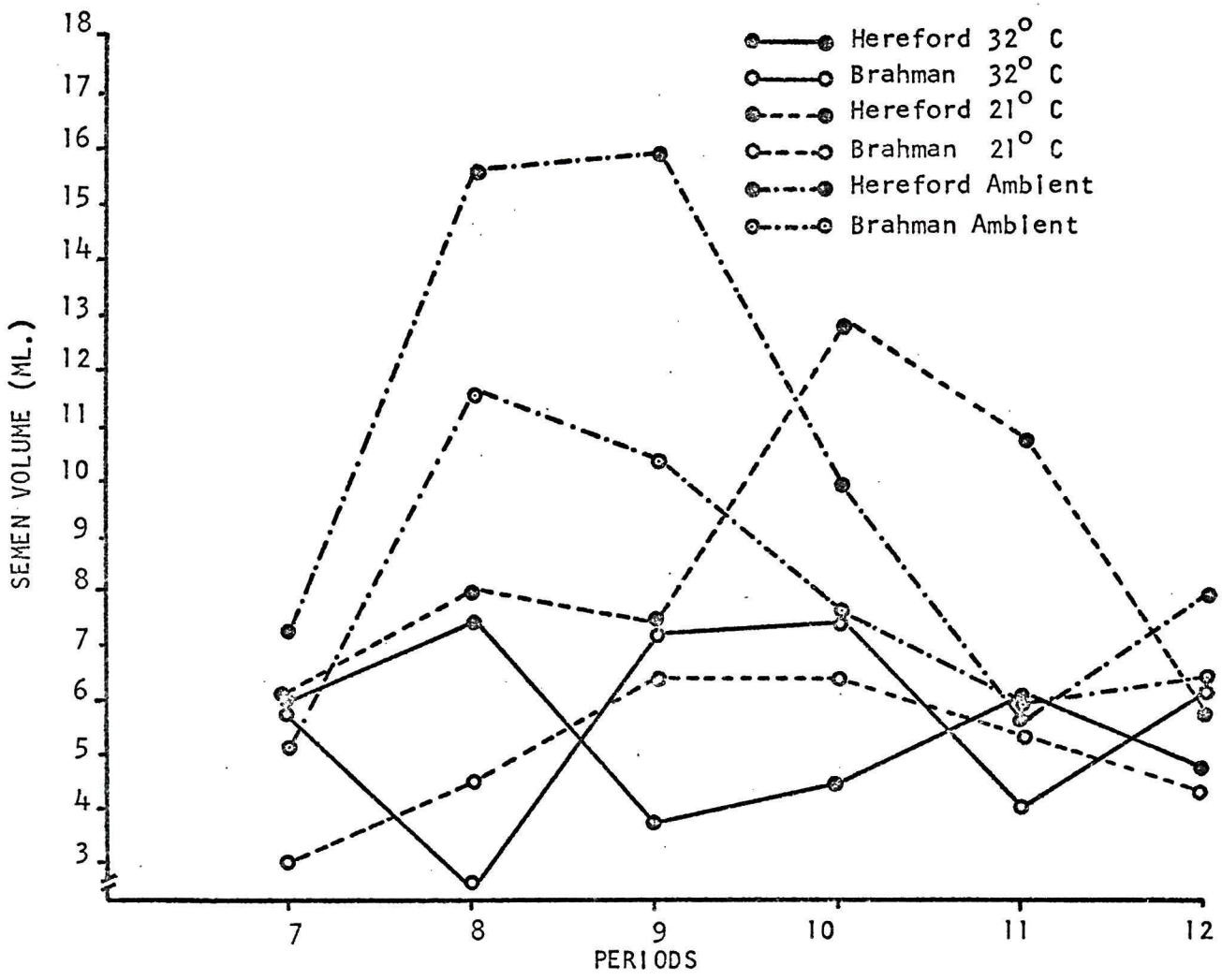

Figure 17.--Effect of treatment x breed interaction on semen volume.

TABLE 14.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE INITIAL MOTILITY OF SPERMATOZOA (% of cells) OF HEREFORD AND BRAHMAN BULLS.

Period No.	Breed	TREATMENT 32°C	21°C	Ambient
1	Hereford	58.33	43.33	91.67
	Brahman	25.00	62.67	81.67
2	Hereford	46.67	73.33	91.67
	Brahman	0	43.33	86.67
3	Hereford	12.67	57.67	43.33
	Brahman	2.67	41.67	50.00
4	Hereford	13.33	48.33	83.33
	Brahman	20.00	48.33	75.00
5	Hereford	56.67	30.00	88.33
	Brahman	77.67	61.67	90.00
6	Hereford	20.00	40.00	56.67
	Brahman	42.33	48.33	80.00
Treat. Av.	Hereford	34.61	48.77	75.83
	Brahman	27.94	51.00	77.22
	Mean	31.27	49.88	76.52

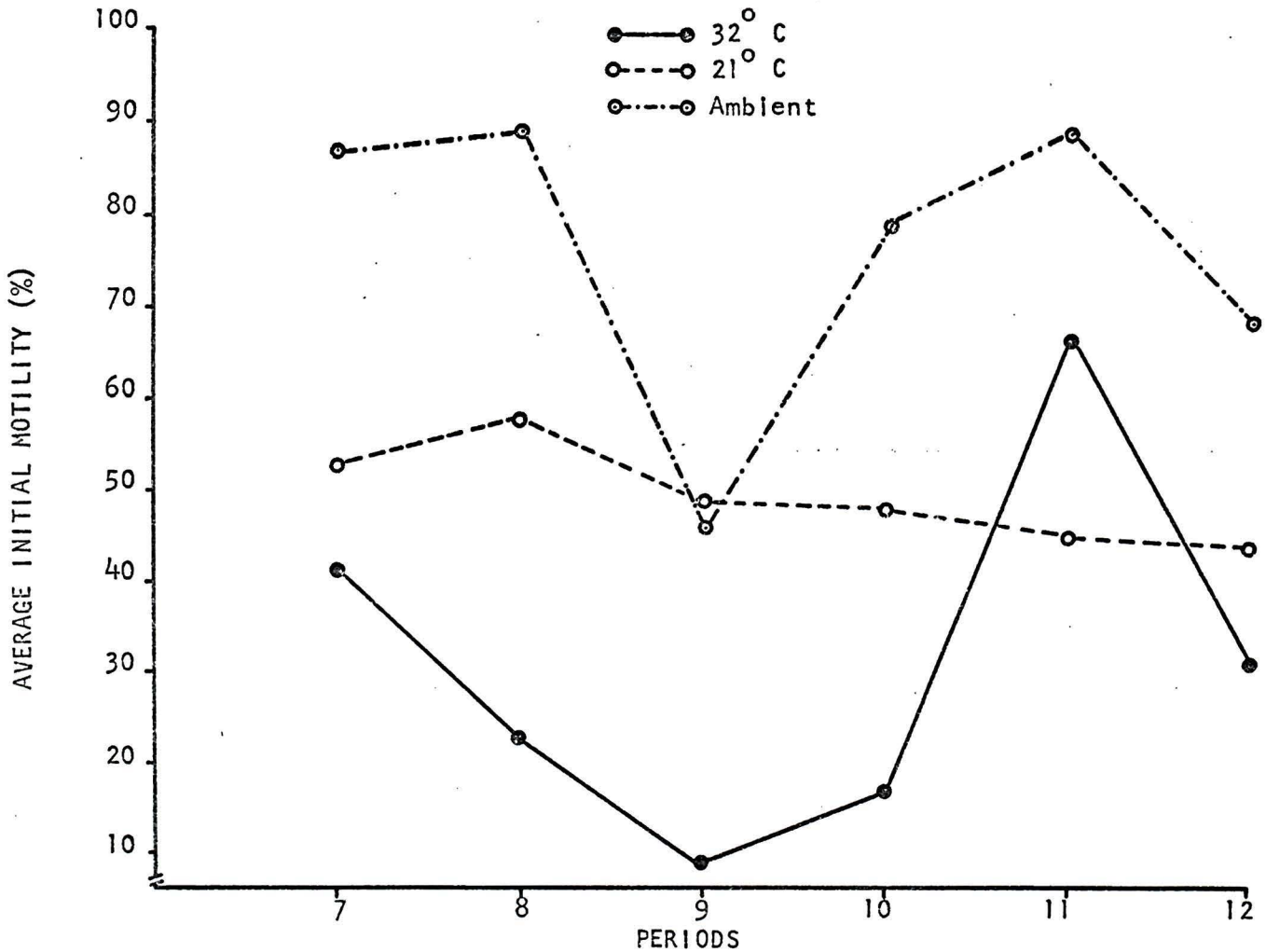

Figure 18.--Effect of treatment on spermatozoa motility.

acted upon by stress of hyperthermia. In support of this line of reasoning are the results of Johnston and Branton (1953) and of Casady et al. (1956) who reported that the adverse effects of high environmental temperature on the quality of semen are very often observed after the stress period of high temperature.

The results of the effects of high temperature and humidity on the spermatozoa concentration of Brahman and Hereford bulls is summarized in Table 15. The average concentration of cells in the ambient group was 518.11 x 10^3 cells/mm^3. This value is higher than the average value of 392 x $10^3/mm^3$ given by Crooks (1968) for Brahman, Santa Gertrudis, Angus and Hereford bulls at 20 months of age. The average, however, is closer to that reported by Fields (1968) of 501 x $10^3/mm^3$ for beef bulls also at 20 months of age. Figure 19 illustrates the highly significant difference ($P < .01$) found between treatment group. The difference appears to be initially between the animals in the ambient group and those under confinement conditions, thus indicating a confinement effect which tended to disappear after period 9. During periods 10 and 11 there was an increase in concentration in the 21°C group which approached the value for the ambient group at period 11 but declined again at period 12. The animals under high environmental temperature conditions maintained a low spermatozoa concentration until period 10 when there was an upward trend. This type of response could possibly be the result of a delayed sexual maturity due to the prolonged hyperthermia. The adverse effects of high temperature on the appearance of puberty has been indicated by several authors (Bonsma, 1949; Dale et al.,1959 and Hafez, 1965).

A significant ($P < .05$) difference in spermatozoa concentration

TABLE 15.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE CONCENTRATION OF SPERMATOZOA ($\times 10^3/mm^3$) IN THE SEMEN OF HEREFORD AND BRAHMAN BULLS.

Period No.	Breed	TREATMENT 32°C	21°C	Ambient
1	Hereford	82.00	169.33	1,147.67
	Brahman	79.00	40.67	246.67
2	Hereford	142.67	299.33	372.67
	Brahman	10.00	55.33	381.33
3	Hereford	216.00	248.00	458.67
	Brahman	8.00	58.67	321.33
4	Hereford	56.33	433.00	466.00
	Brahman	78.00	142.67	352.67
5	Hereford	199.00	703.00	544.67
	Brahman	117.00	206.67	536.00
6	Hereford	215.67	270.00	642.33
	Brahman	277.00	316.67	747.33
Treat. Av.	Hereford	151.94	353.78	605.34
	Brahman	94.83	136.78	430.89
	Mean	123.38	245.28	518.11

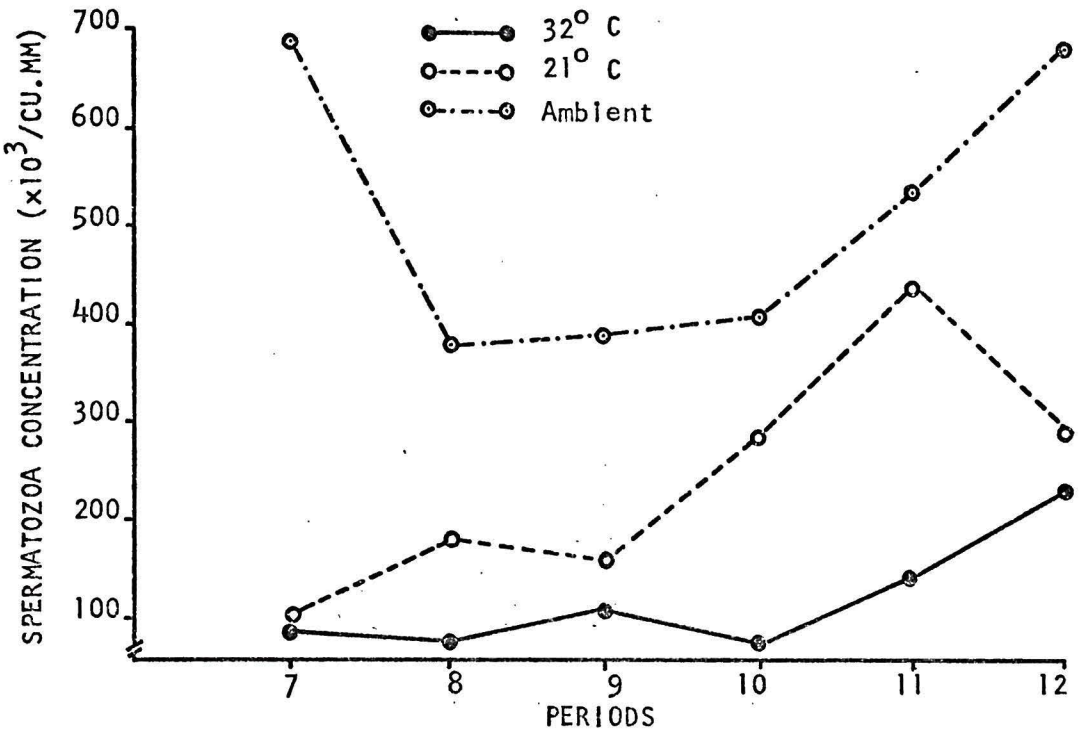

Figure 19.--Effect of treatment on spermatozoa concentration.

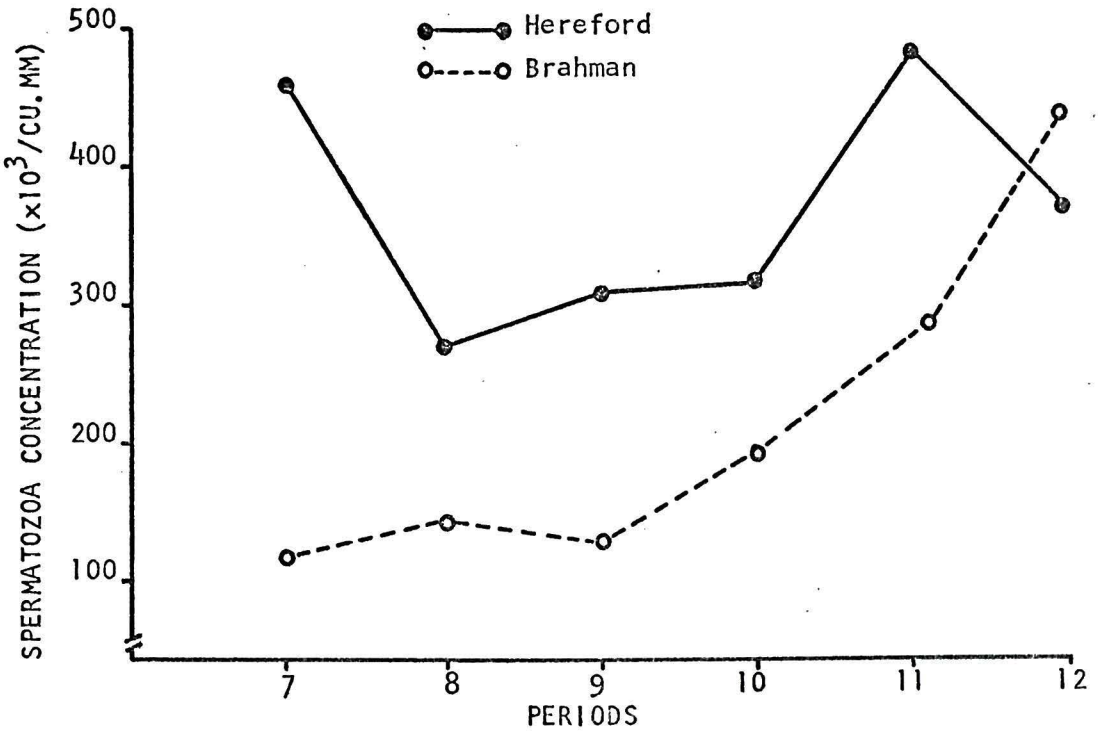

Figure 20.--Effect of breed on spermatozoa concentrations.

was found between Hereford and Brahman bulls (Figure 20). The average values were 370.35 x 10^3 cells per cu. mm. for the Hereford bulls and 220.83 x 10^3 cells/mm^3 for the Brahman bulls. These results are in agreement with those obtained by Fields (1968) and Crooks (1968) who reported lower spermatozoa concentrations in Brahman than in Hereford bulls of the same age.

A significant ($P < .05$) treatment by breed interaction was found in spermatozoa concentration. The 6 treatment-breed combinations possible were grouped into 2 fairly well defined groups, as illustrated in Figure 21. A higher group was composed, as expected, of the Hereford groups at 21°C and ambient conditions and also of the Brahman group at ambient conditions. The lower group was made up of the Brahman and Hereford animals at 32°C and the Brahman at 21°C. Thus the Brahman did not show any superiority as far as heat tolerance is concerned, as measured by sperm cell concentration, and furthermore, was adversely affected by the 21°C temperature. This response appears to be the result of confinement rather than of temperature, especially on the Brahman breed whose behavioral response is somewhat different from that of the Hereford animals.

The last male reproductive characteristic, namely total spermatozoa in the ejaculate, is the product of two other characteristics, already discussed (semen volume and spermatozoa concentration). The results are thus affected by the combined results of the other two parameters, and they are summarized in Table 16. The average total spermatozoa was 2,380.9 x 10^6 cells which is somewhat lower than the value reported by Fields (1968) but similar to that reported by Crooks (1968). Figure 22 shows the treatment effects on this parameter. A highly significant difference was found ($P < .01$) which shows a very marked

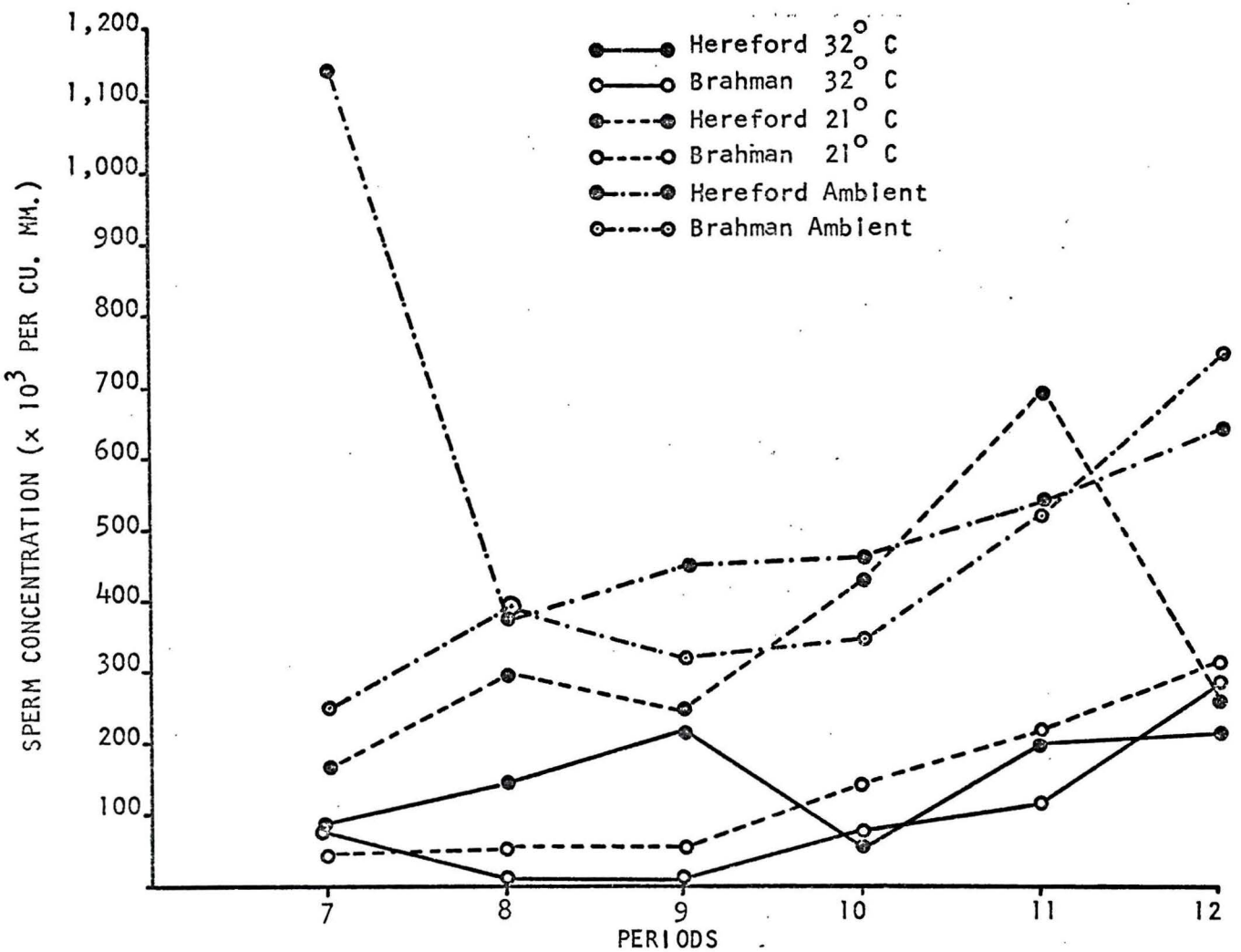

Figure 21.--Effect of treatment x breed interaction on spermatozoa concentration.

TABLE 16.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE TOTAL NUMBER OF SPERMATOZOA (x 10^6/ejac.) IN THE EJACULATE OF HEREFORD AND BRAHMAN BULLS.

Period No.	Breed	TREATMENT 32°C	21°C	Ambient
1	Hereford	610.33	1,447.67	8,235.00
	Brahman	367.67	139.67	1,312.33
2	Hereford	923.00	2,514.33	5,728.00
	Brahman	103.00	259.67	4,132.00
3	Hereford	495.00	2,069.33	7,443.00
	Brahman	35.67	245.33	3,789.67
4	Hereford	251.00	3,779.00	4,545.33
	Brahman	579.67	985.67	2,931.00
5	Hereford	1,139.67	8,741.00	2,979.00
	Brahman	487.00	922.00	3,442.67
6	Hereford	790.67	1,680.67	4,447.67
	Brahman	1,874.00	1,283.00	5,003.00
Treat. Av.	Hereford	701.61	3,372.00	5,563.00
	Brahman	574.50	639.22	3,435.11
	Mean	638.05	2,005.61	4,499.05

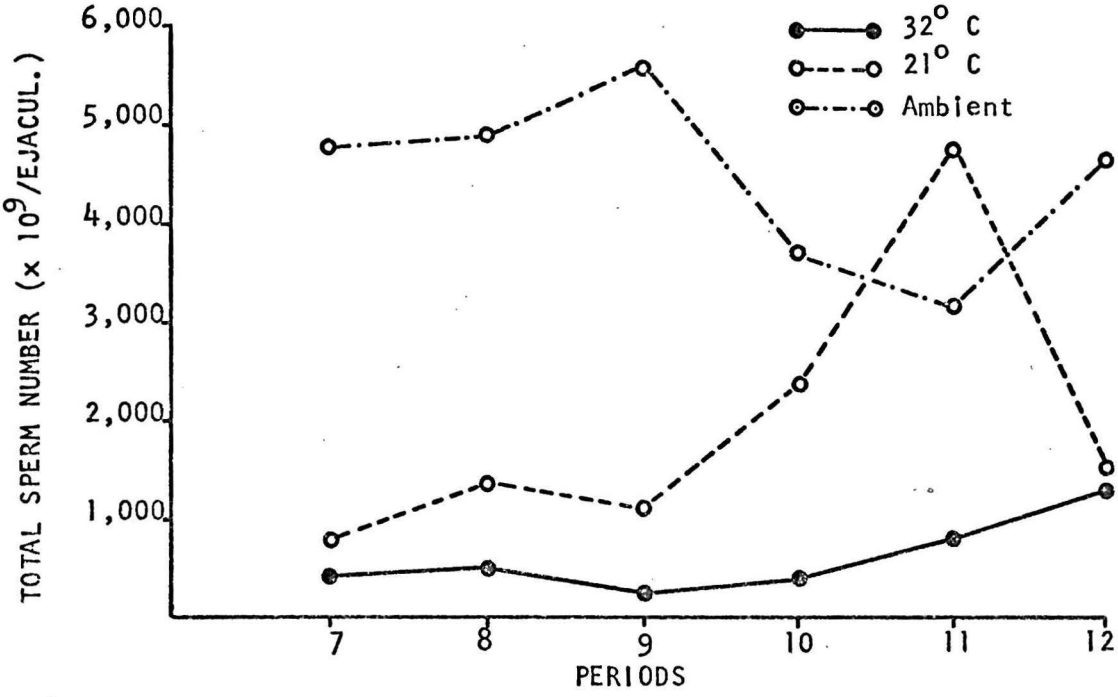

Figure 22.--Effect of treatment on total number of spermatozoa

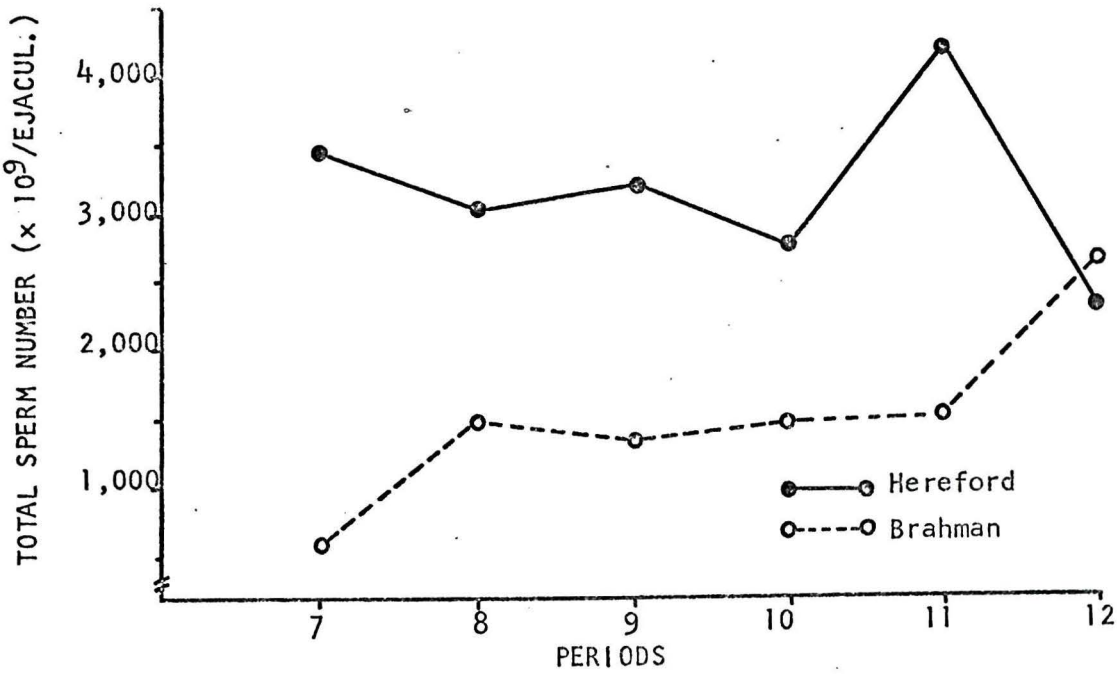

Figure 23.--Effect of breed on total number of spermatozoa.

effect of confinement as demonstrated by the lower values of both 21°C and 32°C chamber groups. As was the case of concentration, the 21°C group appeared to increase after the 9th period and at period 11 it had a higher value than that of the ambient group, but declined sharply again at the 12th period. The adverse effects of high temperature are however clearly observed and appear to result from the additive effects of confinement and hyperthermia. These results are in agreement with the results reported by several other authors (Bogart and Mayer, 1946a; Austin et al., 1961; Brooks and Ross, 1962 and Johnston et al., 1963). The analyses also indicated a significant ($P < .05$) difference in total cells between the Hereford and Brahman breeds (Figure 23). This difference is the result of the lower concentration and semen volume in the Brahman bulls.

Observation of the overall results of male sex characteristics tends to indicate that in the Brahman breed, because of its late sexual maturity as compared to the Hereford breed, it is difficult to evaluate adaptability to conditions of high temperature and humidity using sexual characteristics as the criteria of heat tolerance. This is especially true of animals which by any reason have not attained complete sexual maturity. This appeared to be the case in the present experiment. The results of Vandemark and Ewing (1963), Waites and Setchell (1964) and Findlay and Whittow (1966), who reported decreased metabolism and/or decreased oxygen supply to the testes of animals under hyperthermia, can be used to give support to the assumption that the reduction in quality of semen characteristics observed in the present experiment is the result of a reduced oxygen supply to the testes. This appeared to be especially true in the case of the 32°C chamber in which a high

ammonia content in the atmosphere was highly noticeable by odor. This could have reduced the total oxygen tension thus producing a mild hypoxia which was added to that caused by direct effects of high temperature on the cardiovascular or erythropoietic systems.

The results of the first of the female reproductive responses measured, number of ovarian follicles, are summarized in Table 17. It is readily apparent from the data that ovarian activity in the Hereford heifers in the ambient group started about the 6th period while the Brahman heifers showed a low but steady level of ovarian function as early as the 3rd experimental period (Figure 23). This was not true for the animals in the 2 chamber groups, either Brahman or Hereford, where ovarian function began to show a steady functional level at about period 6th. Thus, it would be safe to assume that puberty in the heifers of both breeds was attained at about 20 mo. of age, the only exception being one of the Brahman heifers in the ambient group who reached puberty at about 17 months. The results indicate a somewhat later sexual maturity age than that given by Dale et al. (1959) of 14 - 16 months, but are in agreement with those results reported by Plasse et al. (1968) who found that sexual maturity of Brahman heifers occurred at a mean age of 19.4 months while that of Brahman-British crossbred heifers occurred at 17 months with a variation from 15 to 20 months.

There was a significant difference ($P < .05$) in number of follicles between the Hereford and Brahman breeds and this is illustrated in Figure 24. The difference became apparent at about the 5th or 6th period, with the Brahman heifers reaching and maintaining a larger average number of follicles throughout the entire experimental period.

TABLE 17.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE NUMBER OF OVARIAN FOLLICLES (NO./ANIMAL) OF HEREFORD AND BRAHMAN HEIFERS.

Period No.	Breed	TREATMENT 32°C	21°C	Ambient
1	Hereford	0.00	0.00	0.33
	Brahman	0.00	0.33	0.33
2	Hereford	0.00	0.00	0.00
	Brahman	0.00	0.00	0.00
3	Hereford	0.00	0.00	0.00
	Brahman	0.33	0.00	0.33
4	Hereford	0.00	0.00	0.00
	Brahman	0.00	0.00	0.33
5	Hereford	0.00	0.00	0.00
	Brahman	0.00	0.67	0.33
6	Hereford	0.00	0.00	0.33
	Brahman	0.67	0.67	1.00
7	Hereford	0.33	0.33	0.00
	Brahman	0.00	0.67	0.33
8	Hereford	0.33	1.00	0.67
	Brahman	1.33	1.33	1.67
9	Hereford	1.67	1.00	0.67
	Brahman	1.33	1.67	2.33
10	Hereford	1.00	0.67	0.00
	Brahman	0.33	1.33	1.67
11	Hereford	1.00	1.00	0.33
	Brahman	1.00	1.67	1.00
12	Hereford	1.00	1.00	2.00
	Brahman	1.67	1.33	2.00
Treat. Av.	Hereford	0.44	0.42	0.36
	Brahman	0.55	0.80	0.94
	Mean	0.50	0.61	0.65

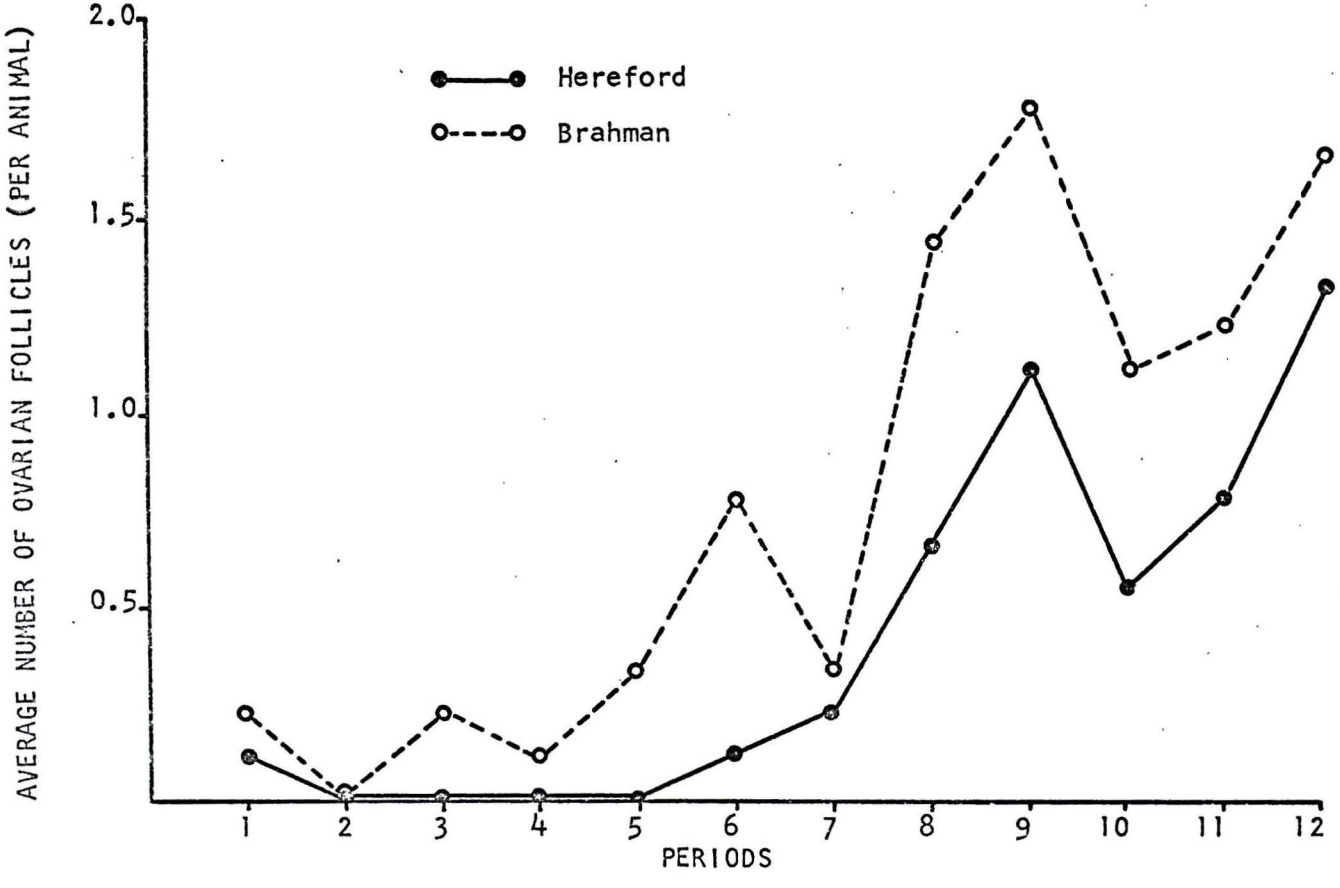

Figure 24.--Effect of breed on the average number of follicles.

The lack of a significant effect of temperature on the number of follicles present lends support to the results reported by Ryle (1963) who found that follicle number and size in ewes did not show any significant effect of any of the treatments used, including high environmental temperature. It is also in agreement with the results of Warnick et al. (1965) who reported no significant differences in average ovulatory rate at second estrus for gilts at a 60° or 90°F environmental temperature.

The results of the average number of corpora lutea (CL) are presented in Table 18. It is interesting to observe that this parameter was affected by temperature treatment, as illustrated in Figure 25. There was a highly significant difference ($P < .01$) in number of CL and initially the difference appeared to be mainly between the ambient group and the 2 chamber groups, while at period 12 the 21°C group approached the ambient group value. Thus, the lack of significance in number of follicles due to temperature, coupled with the significant difference in the number of CL, tends to indicate that the effect of hyperthermia acts on the ovary by way of higher centers. It could be hypothesized that high temperature acts on the anterior hypothalamic center which is responsible for a cyclic increase in luteinizing hormone releasing factor (LHRF), (Thatcher, 1969). This LHRF is responsible for the increased release of luteinizing hormone (LH) to produce ovulation, over and above the basal production of the hormone throughout the estrous cycle, necessary for the normal development of the follicles. Several authors (Schiavi et al., 1963; McCann and Ramirez, 1964; Schally and Bowers, 1964 and Nikitovitch-Winer et al., 1965) have indicated that there is indeed a hypothalamic nucleus which controls the cyclic release of LH. This nucleus has been determined to

TABLE 18.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE NUMBER OF CORPORA LUTEA (NO./ANIMAL) IN THE OVARIES OF HEREFORD AND BRAHMAN HEIFERS.

Period No.	Breed	TREATMENT 32°C	21°C	Ambient
1	Hereford	0.00	0.00	0.00
	Brahman	0.00	0.00	0.00
2	Hereford	0.00	0.00	0.33
	Brahman	0.00	0.00	0.00
3	Hereford	0.00	0.00	0.33
	Brahman	0.00	0.00	0.00
4	Hereford	0.00	0.00	0.67
	Brahman	0.00	0.00	0.00
5	Hereford	0.00	0.00	0.00
	Brahman	0.00	0.00	0.33
6	Hereford	0.00	0.00	0.33
	Brahman	0.00	0.33	0.00
7	Hereford	0.00	0.00	0.67
	Brahman	0.67	0.33	0.67
8	Hereford	0.00	0.00	1.00
	Brahman	0.00	0.00	0.00
9	Hereford	0.00	0.00	1.33
	Brahman	0.33	0.00	0.00
10	Hereford	0.00	0.00	1.00
	Brahman	0.00	0.00	0.00
11	Hereford	0.00	0.33	1.00
	Brahman	0.00	0.00	0.33
12	Hereford	0.00	0.67	0.67
	Brahman	0.00	0.33	0.00
Treat. Av.	Hereford	0.00	0.08	0.61
	Brahman	0.08	0.08	0.11
	Mean	0.04	0.08	0.36

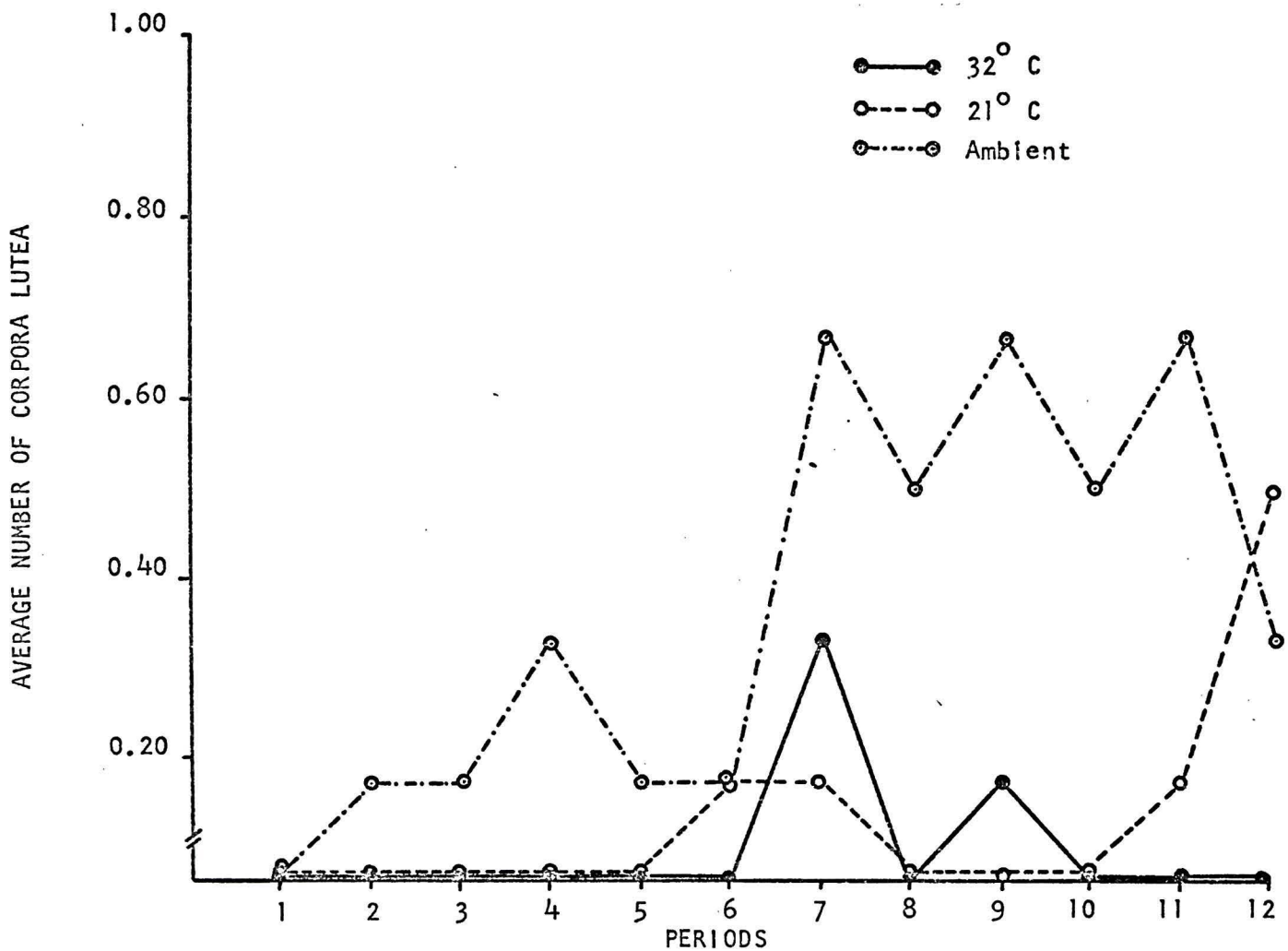

Figure 25.--Effect of treatment on the average number of corpora lutea.

be located anatomically in close proximity to the one controlling the hypothalamo-pituitary-thyroid axis and, as it has been previously indicated, this nucleus is affected by temperature.

A significant treatment by breed interaction ($P < .05$) was found in the number of CL (Figure 26). Part of this interaction is possibly the result of the complete absence of CL in the 32°C-Hereford group and also the low but equal values for both Hereford and Brahman heifers in the 21°C group.

Body Weight Gain and Nutrition Responses

The average daily gain of the animals during the entire experimental period is presented in Table 19. The overall average for all of the animals was 0.88 lb./day with a range from 0.62 to 1.16 lb./day. A highly significant ($P < .01$) difference in average daily gain was found due to treatment group and this is illustrated in Figure 27. There was a sharp difference between the animals in the ambient group and those in the chambers. In fact the overall average daily gains for the 32°C and the 21°C groups were the same (0.78 lb./day) while that of the ambient group was 1.07 lb./day. This difference is probably due to the type of animals which were used as ambient control group in comparison with those used in the two chamber groups, as previously indicated in the materials and methods section. Confinement of the animals also appeared to be responsible for the difference. In a comparison of the 21°C and the 32°C groups, it is observed that the average daily gain of the 21°C group tended to increase slightly after the 7th period, while that of the 32°C group remained constant. This could be the result of the prolonged effects of hyperthermic conditions

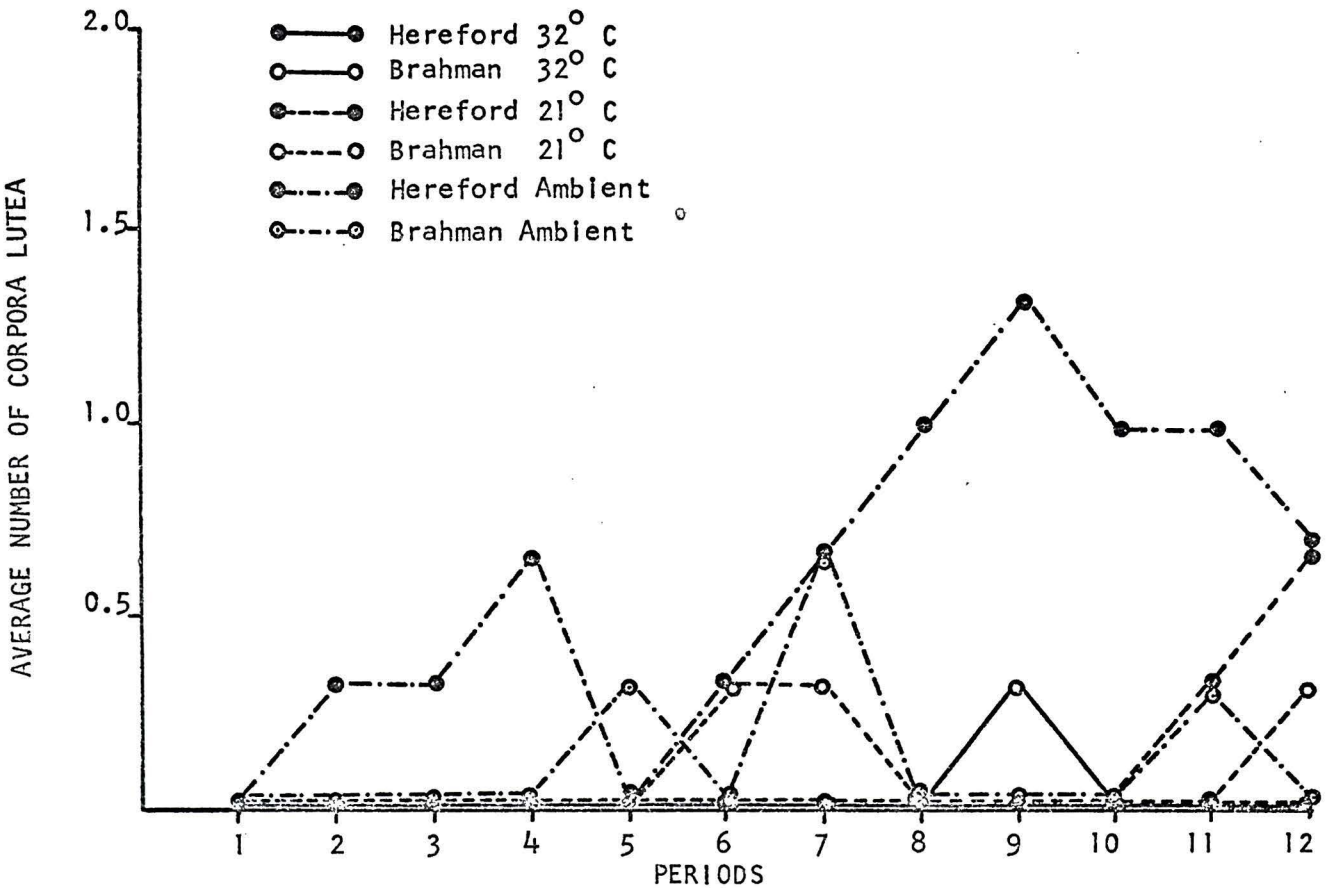

Figure 26.--Effect of treatment x breed interaction on the average number of corpora lutea.

TABLE 19.--COMPARATIVE EFFECTS OF PROLONGED TEMPERATURE STRESS ON THE AVERAGE DAILY GAIN (LB./DAY) OF HEREFORD AND BRAHMAN CATTLE.

Period No.	Breed	TREATMENT 32°C Male	32°C Female	21°C Male	21°C Female	Ambient Male	Ambient Female
1	Hereford	0.76	0.95	-0.39	0.87	0.93	0.57
	Brahman	1.22	1.35	1.10	1.54	0.87	0.62
2	Hereford	0.60	0.84	0.41	0.66	0.66	0.60
	Brahman	0.68	1.11	1.03	1.31	0.50	0.60
3	Hereford	0.58	0.70	0.44	0.59	0.93	0.67
	Brahman	0.72	1.01	0.86	1.14	0.99	0.70
4	Hereford	0.70	0.66	0.47	0.54	1.29	1.07
	Brahman	0.86	1.01	0.79	0.93	1.31	1.04
5	Hereford	0.61	0.69	0.62	0.70	1.52	1.26
	Brahman	0.99	1.05	0.96	1.05	1.49	1.13
6	Hereford	0.66	0.68	0.56	0.58	1.59	1.22
	Brahman	0.98	1.02	0.84	0.89	1.60	1.13
7	Hereford	0.66	0.57	0.67	0.62	1.48	1.15
	Brahman	0.92	0.84	0.87	0.86	1.56	1.05
8	Hereford	0.61	0.52	0.83	0.60	1.47	1.01
	Brahman	0.91	0.86	0.96	0.76	1.41	0.99
9	Hereford	0.59	0.57	0.75	0.70	1.37	1.10
	Brahman	0.88	0.91	0.93	0.83	1.46	1.02
10	Hereford	0.64	0.49	0.85	0.72	1.50	1.09
	Brahman	0.82	0.83	1.01	0.81	1.57	1.07
11	Hereford	0.66	0.52	0.86	0.74	1.28	1.11
	Brahman	0.90	0.82	1.03	0.83	1.39	1.01
12	Hereford	0.65	0.53	0.87	0.78	1.23	1.07
	Brahman	0.86	0.85	1.01	0.86	1.32	0.98
Sex Av.	Hereford	0.64	0.60	0.57	0.68	1.27	0.79
	Brahman	0.90	0.97	0.94	0.91	1.29	0.95
	Mean	0.77	0.79	0.76	0.79	1.28	0.87
Treat. Av.	Hereford	0.62		0.62		1.03	
	Brahman	0.94		0.92		1.12	
	Mean	0.78		0.78		1.07	

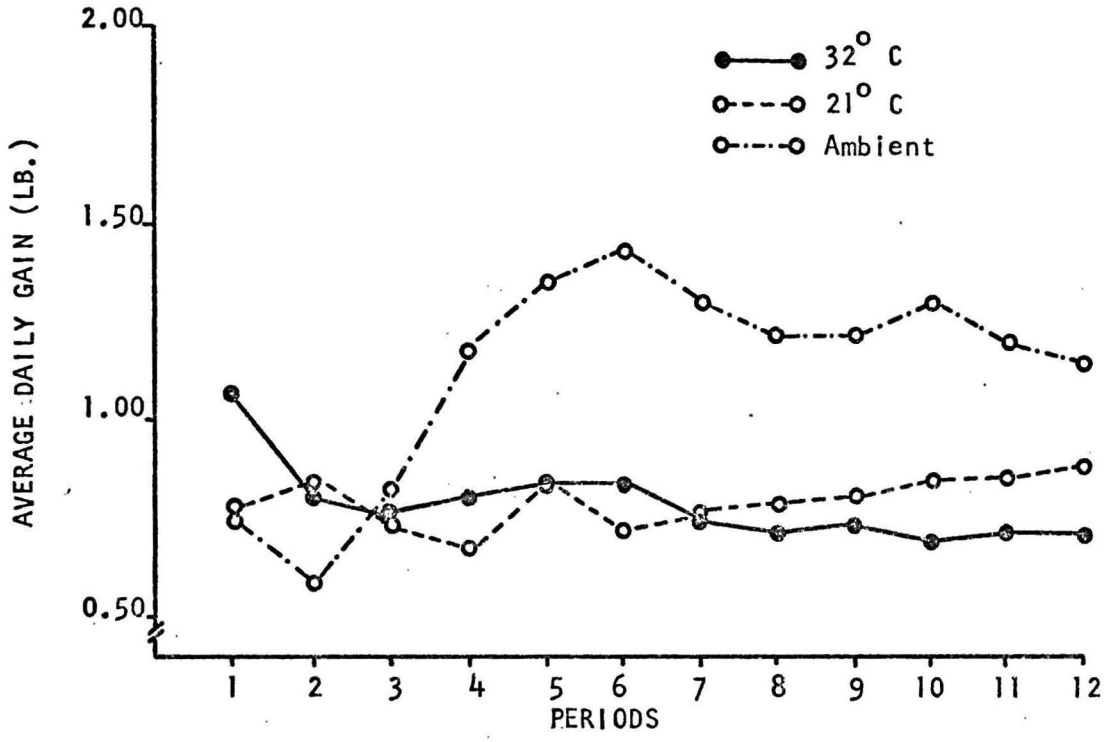

Figure 27.--Effect of treatment on average daily gain.

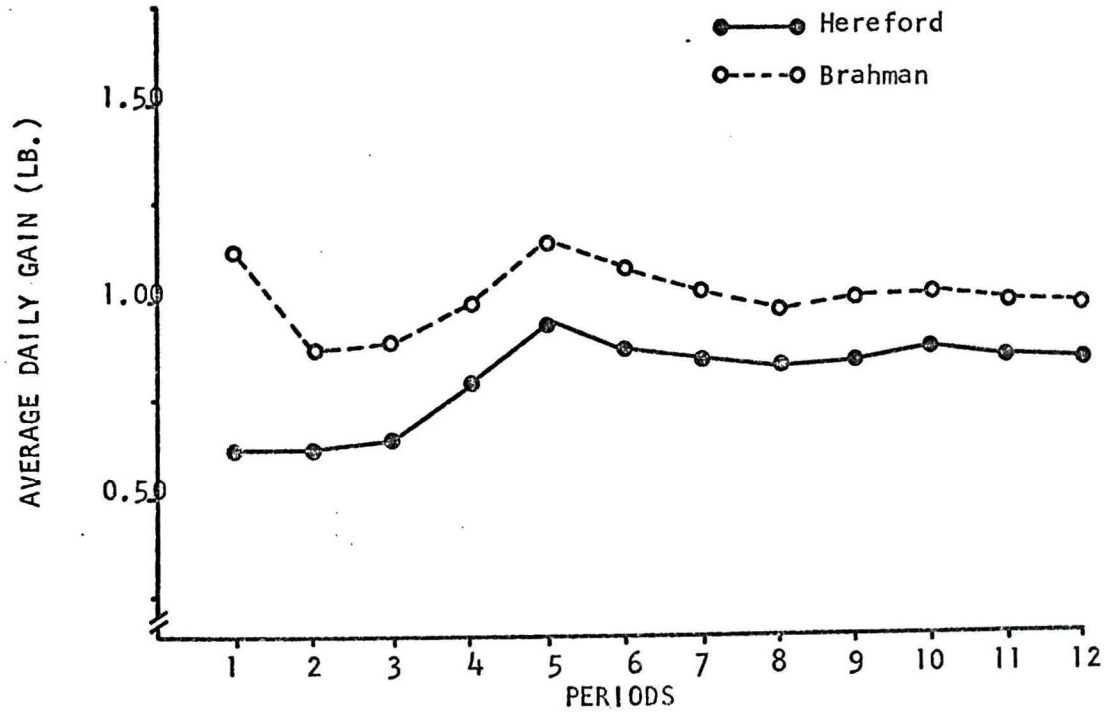

Figure 28.-- Effect of breed on average daily gain.

on the animals acting probably through the decrease in roughage consumption which was observed in the animals in the heated chamber. No reduction in concentrate consumption was observed due to high environmental temperature. A reduction of feed intake has been demonstrated experimentally as the effect of warming the preoptic area of the hypothalamus where the heat loss center was located (Anderssen and Larsson, 1961). Reduction of feed intake has also been demonstrated in animals subjected to high environmental temperature by Wayman et al. (1962), Allen et al. (1963), Weldy et al. (1964), Lundgren and Johnson (1964) and Johnson et al. (1967).

A highly significant ($P < .01$) difference between breeds was found in average daily gain (Figure 28). Brahman had a constantly higher average daily gain than the Hereford, regardless of the treatment group. The difference was, however, more pronounced in the 21°C and the 32°C groups than in the ambient group. A significant ($P < .05$) treatment by sex interaction was found, mainly due to the better performance of both males and females in the ambient group (Figure 29). A significant ($P < .05$) treatment by breed interaction was found and is illustrated in Figure 30. Essentially three groups are observed in this interaction. One group is composed of the animals, both male and female of the two breeds in the ambient group, with a marked superiority over the other groups. A second group is composed of the Brahman animals of the two sexes and under the 21°C and 32°C temperature treatments. This group was somewhat superior to the Hereford males and females at both 21°C and 32°C temperatures and this superiority was indicated previously (Figure 28).

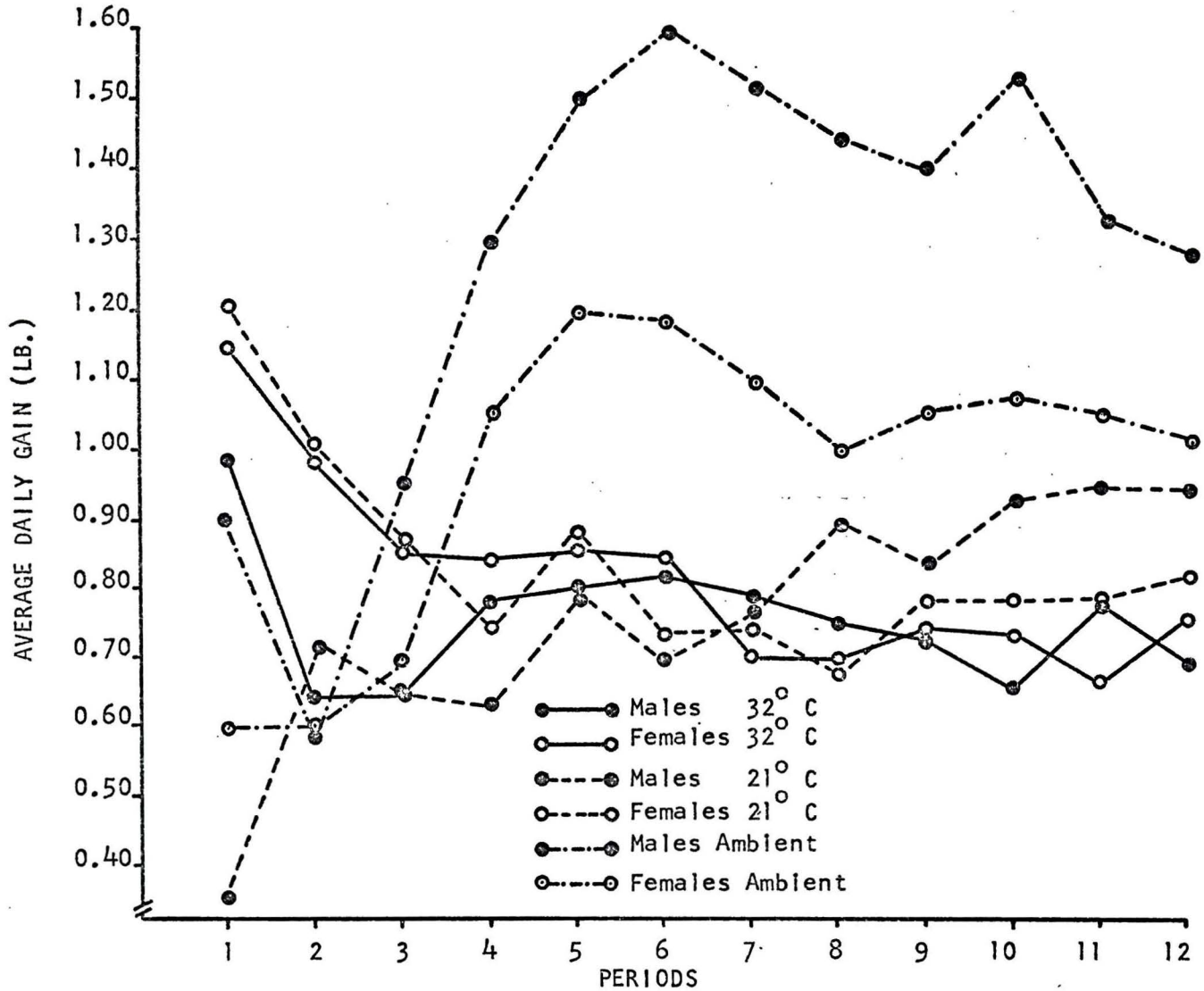

Figure 29.--Effect of treatment x sex interaction on average daily gain.

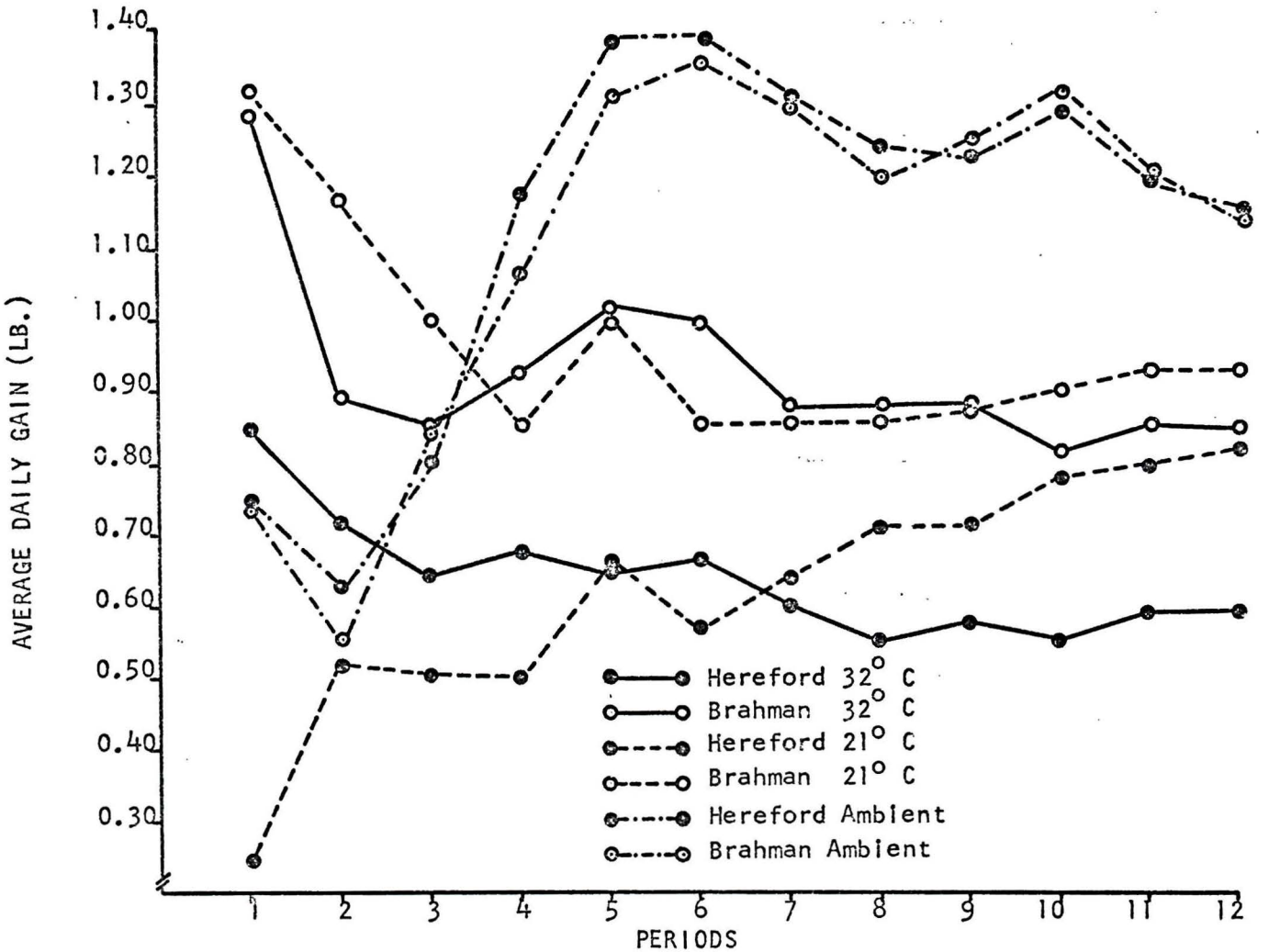

Figure 30.--Effect of treatment x breed interaction on average daily gain.

SUMMARY AND CONCLUSIONS

Some differential hematological, thermal, reproductive and nutritional responses of beef cattle were measured in Brahman and Hereford animals of both sexes under various environmental conditions. There were 3 animals of each breed and sex in each of three environmental groups: ambient conditions; 21°C temperature, 65% relative humidity and 32°C temperature, 95% relative humidity. The experiment was conducted over a period of 1 year and divided into 12 periods of 28 days each. Determinations of the parameters studied were made at the end of each 28-day period.

Statistically significant breed differences were found. Brahmans had higher average erythrocyte numbers, average packed cell volume, average number of ovarian follicles and average daily gain. Herefords had higher mean corpuscular hemoglobin, body temperature, libido scores, spermatozoa concentration and total number of spermatozoa in the ejaculate.

Statistically significant effects due to the temperature treatments were found in average hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, leukocyte numbers, rectal temperature, volume of semen, average initial motility of spermatozoa, average spermatozoa concentration, total number of spermatozoa in the ejaculate, average number of corpora lutea and average daily gain. Physiological advantages were observed in almost all cases, for the ambient control group over the 21°C and 32°C temperature groups where

there was a confounding of the effects of confinement with the effects of high temperature and humidity. Two hematological parameters appeared promising as indicators of hyperthermic response with no apparent effect due to confinement. These were the mean corpuscular volume of the erythrocytes and the mean corpuscular hemoglobin. They were affected by breed and sex as demonstrated by the significant interactions observed. The number of animals was not large enough, however, to allow definite conclusions to be drawn.

The amount of light was drastically reduced for animals under chamber conditions. This environmental factor could be affecting the results to a greater degree than expected, thus adding to the already adverse effects of confinement and high temperature.

The results support the view that rectal temperature, alone is not the best index of adaptability to conditions of hyperthermia and indicates a need for further investigations of the possible indices which in combination might give the best evaluation of responses to hyperthermia and provide an index of heat tolerance.

Variability of hematological parameters was large. It was hypothesized, that a reduction observed in hemoglobin content was mediated by way of the thyroid gland through a reduction in thyroid hormone secretion. This hormonal imbalance could also be affecting the energy balance in such a way that energy normally used for such processes as protein synthesis, including hemoglobin formation would be shifted to physiological processes necessary for heat dissipation.

Individuality in response particularly with libido scores, appeared to be the only explanation for the breed differences observed.

It was hypothesized that high temperature and humidity had an

apparent adverse effect on the number of ovarian follicles which can rupture and ovulate. This ovulatory inhibition could be accomplished through the inhibition of the cyclic release of luteinizing hormone releasing factor (LHRF) from an anterior hypothalamic nuclei thus inhibiting the surge of release of LH from the anterior pituitary, necessary for ovulation.

APPENDIX

TABLE 20.--ANALYSES OF VARIANCE OF THE HEMATOLOGICAL RESPONSES OF HEREFORD AND BRAHMAN CATTLE UNDER PROLONGED TEMPERATURE STRESS.

Source	df	Red Blood Cells	White Blood Cells	Hemoglobin
Treatment (T)	2	20,234,287	200,046,420**	89.49*
Sex (S)	1	23,937,638	464,002	69.39
T x S	2	3,877,691	79,611,613	11.92
Breed (B)	1	127,569,240**	62,380,800	91.14
T x B	2	5,881,333	55,772,486	14.60
S x B	1	501,502	8,975,817	12.73
T x S x B	2	20,181,617	1,137,276	74.47
Animals/TSB	23	10,511,418	38,409,023	19.84

Source	df	Packed Cell Volume	Mean Corpuscular Hemoglobin	Mean Corpuscular Volume
Treatment (T)	2	520.44	109.24**	583.88**
Sex (S)	1	356.07	0.56	14.85
T x S	2	40.29	33.84	103.43*
Breed (B)	1	1,799.12*	69.28*	82.95
T x B	2	97.90	39.54	272.84**
S x B	1	114.91	30.29	208.47*
T x S x B	2	468.89	3.33	0.57
Animals/TSB	23	162.87	7.00	54.60

*Significant at the 5% level.

**Significant at the 1% level.

TABLE 21.--ANALYSES OF VARIANCE OF BODY WEIGHT, WEIGHT GAINS AND RECTAL TEMPERATURE RESPONSES OF HEREFORD AND BRAHMAN CATTLE UNDER PROLONGED TEMPERATURE STRESS.

Source	df	Body Weight	Period Daily Gain	Cumulative Daily Gain	Rectal Temperature
Treatment (T)	2	4,577,722**	10.4327**	5.2634**	3.5585**
Sex (S)	1	797,650**	4.3842**	0.5167	0.3169
T x S	2	177,257*	0.5015	1.5835*	0.7565
Breed (B)	1	546,347**	0.3640	4.5551**	18.1302**
T x B	2	130,178	1.1914**	1.3074*	1.2206
S x B	1	156,142	0.0574	0.0082	0.8802
T x S x B	2	140,527	0.0465	0.0583	0.4459
Animals/TSB	23	42,673	0.2009	0.3059	0.3801

*Significant at the 5% level.

**Significant at the 1% level.

TABLE 22.--ANALYSES OF VARIANCE OF THE MALE REPRODUCTIVE RESPONSES OF HEREFORD AND BRAHMAN BULLS UNDER PROLONGED TEMPERATURE STRESS.

Source	df	Libido	Semen Volume	Sperm Motility	Sperm Cells	
					Concentration	Total
Treatment (T)	2	10.73	91.56*	18,621**	1,470,603**	137,968,770**
Breed (B)	1	16.33	65.33	28	603,606*	74,633,781*
T x B	2	22.53**	59.02*	217	617,138*	16,737,752
Animals/TB	11	3.37	15.54	975	98,798	9,249,210

*Significant at the 5% level.

**Significant at the 1% level.

TABLE 23.--ANALYSES OF VARIANCE OF THE FEMALE REPRODUCTIVE RESPONSES OF HEREFORD AND BRAHMAN HEIFERS UNDER PROLONGED TEMPERATURE STRESS.

Source	df	Follicles	Corpus Lutea
Treatment (T)	2	0.4491	2.1713**
Breed (B)	1	7.0417*	1.0417
T x B	2	1.0139	1.7917**
Animals/TB	12	0.7037	0.2176

*Significant at the 5% level.

**Significant at the 1% level.

BIBLIOGRAPHY

Alexander, G. I., D. A. Price, R. Bogart and H. Krueger. 1959. Rate and efficiency of gains in beef cattle. VII - Hematology of growing Herefords and Angus calves. Ore. Agr. Exp. Sta. Tech. Bull. 47.

Allcroft, W. M. 1941. Observations on the haemoglobin level of cows and sheep. J. Agr. Sci. 31:320.

Allen, T. E., Y. S. Pan and R. H. Hayman. 1963. The effect of feeding evaporative heat loss and body temperature in Zebu and Jersey heifers. Australian J. Agr. Res. 14:580.

Alliston, C. W. and L. C. Ulberg. 1961. Early pregnancy loss in sheep at ambient temperatures of 70°F and 90°F as determined by embryo transfer. J. Animal Sci. 20:608.

Anderson, J. 1945. Seasonal variation in the reproductive capacity of the bull. J. Agr. Sci. 35:184.

Andersson, B. and B. Larsson. 1961. Influence of local temperature changes in the preoptic area and rostral hypothalamus on the regulation of food and water intake. Acta Physiol. Scand. 52:75.

Andersson, B., L. Ekman, C. C. Gale and J. W. Sundsten. 1962. Activation of the thyroid gland by cooling of the preoptic area in the goat. Acta Physiol. Scand. 54:191.

Austin, J. W., E. W. Hupp and R. L. Nurphree. 1961. Effect of scrotal insulation on semen of Hereford bulls. J. Animal Sci. 20:307.

Badreldin, A. L. and M. A. Ghany. 1954. Species and breed differences in the thermal reaction mechanism. J. Agr. Sci. 44:160.

Bass, D. E. and A. Henschel. 1956. Responses of body fluid compartments to heat and cold. Physiol. Rev. 36:128.

Bazett, H. C., F. 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.

Beakley, W. R. and J. D. Findlay. 1955. The effect of environmental temperature and humidity on the rectal temperature of calves. J. Agr. Sci. 45:339.

Benezra, M. V. 1954. A new index for measuring the adaptability of cattle to tropical conditions. J. Animal Sci. 13:1015 (Abstr.).

Berman, A. 1968. Nychthermal and seasonal patterns of thermoregulation in cattle. Australian J. Agr. Res. 19:181.

Bhannasiri, T., R. Bogart and H. Krueger. 1961. Hemoglobin and blood cells of growing beef cattle. J. Animal Sci. 20:18.

Bianca, W. 1957. The effect of repeated short exposures to heat on the volume and hydration of the blood of the calf. British Vet. J. 113:227.

Bianca, W. 1959. Acclimatization of calves to a hot dry environment. J. Agr. Sci. 52:296.

Bogart, R. and D. T. Mayer. 1946a. The relation of temperature and the thyroid to mammalian reproductive physiology. Am. J. Physiol. 147:320.

Bogart, R. and D. T. Mayer. 1946b. Environmental temperature and thyroid gland involvement in lowered fertility of rams. Mo. Ag. Exp. Sta. Res. Bull. 402.

Bonsma, J. C. 1949. Breeding cattle for increased adaptability to tropical and sub-tropical environments. J. Agr. Sci. 39:204.

Brody, S., C. Blincoe, G. Burge, J. Barton, R. Tary and W. Platner. 1949. Environmental physiology III - Influence of amb. temp. 50-100°F on the blood composition of Jersey and Holstein cows. Mo. Agr. Exp. Sta. Res. Bull. 433.

Brooks, J. R. and C. V. Ross. 1962. Effect of ambient temperature and thyroxine therapy on semen quality of rams. J. Animal Sci. 21:700.

Byers, J. H., I. R. Jones and J. R. Haag. 1952. Blood hemoglobin values of dairy cattle. J. Dairy Sci. 35:661.

Cartwright, T. C. 1955. Response of beef cattle to high ambient temperatures. J. Animal Sci. 14:350.

Casady, R. B., R. M. Myers and J. E. Legates. 1953. The effect of exposure to high temperatures on spermatogenesis in dairy bulls. J. Dairy Sci. 36:14.

Casady, R. B., J. E. Legates and R. M. Myers. 1956. Correlations between ambient temperatures varying from 60° - 95°F and certain physiological responses in young dairy bulls. J. Animal Sci. 15:141.

Cassuto, Y. and R. R. J. Chaffee. 1966. Effects of prelonged heat exposure on cellular metabolism of hamster. Am. J. Physiol. 210:423

Cowley, J. J. 1968. *In vitro* measurement of circulating thyroid hormone levels in beef cattle. Ph.D. Dissertation. Univ. of Florida.

Crooks, J. M. 1968. Testicular growth. Semen production and growth rate in beef bulls. M.S.A. Thesis. Univ. of Florida.

Crosby, W. H., J. I. Munn and F. W. Furth. 1954. Standarizing a method for clinical hemoglobinometry. U. S. Armed Forces Med. J. 5:693.

Dale, H. E., A. C. Ragsdale and C. S. Cheng. 1959. Effect of constant environmental temperatures, 50° and 80°F, on appearance of puberty in beef calves. J. Animal Sci. 18:1363.

D'Angelo, G. and M. Lacombe. 1962. A practical diluent for electronic white cell counts. Am. J. Clin. Path. 38:658.

De Alba, J. and J. M. Couto. 1957. Climatic stress on tropically reared breeds of cattle. J. Animal Sci. 16:725.

Dowling, D. F. 1956. An experimental study of heat tolerance of cattle. Australian J. Agric. Res. 7:469.

Duckworth, J. and O. B. Rattray. 1948. The three-quarter-bred Holstein-Zebu heifer. I. Blood changes during the first year of life. Empire J. Exp. Agr. 16:14.

Dutt, R. H. 1959. Temperature and light as factors in reproduction among farm animals. J. Dairy Sci. (Suppl.) 43:123.

El Skeikh, A. S. and L. E. Casida. 1955. Motility and fertility of spermatozoa as affected by increased ambient temperature. J. Animal Sci. 14:1146.

Fallon, G. R. 1962. Body temperature and fertilization in the cow. J. Reprod. Fertil. 3:116.

Fields, M. J. 1968. Effects of breed and age on testicular biometry, growth and semen characteristics in beef bulls. M. S. A. Thesis. Univ. of Florida.

Findlay, J. D. 1950. The effect of temperature, humidity, air movement and solar radiation on the behavior and physiology of cattle and other domestic animals. Hannah Dairy Res. Inst. Bull. 9:54.

Findlay, J. D. 1963. Acclimatization to heat in sheep and cattle. Fed. Proc. 22:688.

Findlay, J. D. and G. C. Whittow. 1966. The role of arterial oxygen tension in the respiratory response to localized heating of the hypothalamus and to hyperthermia. J. Physiol. 186:333.

Gagon, T. E. J. W. AThens, D. R. Boggs and G. E. Cartwright. 1966. An evaluation of the variance of leukocyte counts as performed with the hemocytometer, Coulter and Fisher Instruments. Am. J. Clin. Path. 46:684.

Gimenez, D. M. 1966. Effect of ambient temperature on reproduction, feed intake, gains and rectal temperature of Angus, Brahman and Hereford heifers. M.S.A. Thesis. Univ. of Florida.

Glover, F. A. and L. W. Phipps. 1962. Preliminary study of an electronic method of counting and sizing bull spermatozoa. J. Reprod. Fertil. 4:189.

Hafez, E. S. E. 1965. Environment and reproduction in farm animals. World Rev. Animal Prod. 1965 (1):118.

Harrison, G. A. 1963. Temperature adaptation as evidenced by growth of mice. Fed. Proc. 22:691.

Holman, H. H. 1955. The blood picture of the cows. Brit. Vet. J. 111:440.

Howes, J. R. 1964. The comparative physiology of Bos taurus and Bos indicus cattle on two nutritional levels. Ph.D. Dissertation. Univ. of Florida.

Howes, J. R., J. F. Hentges, Jr. and J. P. Feaster. 1963. Blood volume of Brahman and Hereford cattle as measured by injected radio-iodinated bovine serum albumin. J. Animal Sci. 22:183.

Howes, J. R., J. F. Hentges, Jr., A. C. Warnick and T. J. Cunha. 1957. A comparison of the blood composition and physiology in Brahman and British cattle. J. Animal Sci. 16:1029.

Iversen, S. 1964. Evaluation of the number of spermatozoa in bull semen - a comparison between electronic counting, light scattering and absorptiometry. J. Agr. Sci. 62:219.

Johnson, H. D., L. Hahn, H. H. Kibler, M. K. Shankling and J. E. Edmondson. 1967. Environmental physiology and shelter engineering with special reference to domestic animals. LXXII. Heat and acclimation influences on lactation of Holstein cattle. Mo. Ag. Exp. Sta. Res. Bull. 916.

Johnson, H. D. and A. C. Ragsdale. 1960. The effect of rising environmental temperatures (35° - 95°F) on thyroid - ^{131}I release rate of Holstein, Brown Swiss and Jersey heifers. J. Agr. Sci. 54:421.

Johnston, J. E. and C. Branton. 1953. Effect of seasonal climatic changes on certain physiological reactions, semen production and fertility of dairy bulls. J. Dairy Sci. 36:934.

Johnston, J. E., F. B. Hambling and G. T. Schrader. 1958. Factors concerned in the comparative heat tolerance of Jersey, Holstein and Red Sindhi x Holstein (F.) cattle. J. Animal Sci. 17:473.

Johnston, J. E., H. Naelapaa and J. B. Frye. 1963. Physiological responses of Holstein, Brown Swiss and Red Sindhi crossbred bulls exposed to high temperatures and humidities. J. Animal Sci. 22:432.

Jones, J. E. and H. R. Wilson. 1967. Use of an electronic counter for sperm concentration determination in chicken semen. Poul. Sci. 46:532.

Kajihara, A. and J. W. Kendall. 1967. Evidence for a hypothalamic feedback center controlling TSH release. Clin. Res. 15:123.

Kamal, T. H., H. D. Johnson and A. C. Ragsdale. 1958. Effect of heat acclimation on physiological and biochemical responses of dairy heifers to various temperatures (35°F - 95°F). J. Animal Sci. 17:1227.

Kelly, R. O., F. A. Martz and H. D. Johnson. 1967. Effect of environmental temperature on rumen volatile fatty acids levels with controlled feed intake. J. Dairy Sci. 50:531.

Lee, D. H. K. and R. W. Phillips. 1948. Assessment of the adaptability of livestock to climatic stress. J. Animal Sci. 7:391.

Lee, D. H. K. and R. F. Rick. 1951. The time factor in response of dairy cattle to hot conditions. J. Animal Sci. 10:1077.

Long, R. A., W. A. VanArsdell, R. MacVicar and O. B. Ross. 1952. Blood composition of normal beef cattle. Okla. Agr. Exp. Sta. Tech. Bull. T-43.

Lundgren, R. G. and H. D. Johnson. 1964. Effects of temperature and feed intake on thyroxine - I^{131} disappearance rates of cattle. J. Animal Sci. 23:28.

Manresa, M. and P. R. Falcon. 1939. Hematological studies on cattle in the Philippines. III. Fluctuation in the Hb. of Indian Nellore oxen as affected by seasonal variation. Philippine Agriculturist 28:187.

Manresa, M., F. Gomez and F. O. Santos. 1939. Hematological studies on cattle in the Philippines. II. Variations in Hb. in Indian Nellore oxen as affected by atmospheric temperature, relative humidity, body temperature and certain chemical components of the blood. Philippine Agriculturist 28:79.

Manresa, M. and N. C. Reyes. 1934. Hematological studies on cattle in the Philippines. I. The hemoglobin, erythrecytes and leucocytes in different breeds of cattle in the college of agirculture. Philippine Agriculturist 23:588.

Mattern, C. F. T., F. S. Brackett and B. J. Olson. 1957. Determination of number and size of particles by electrical gating: blood cells. J. Appl. Physiol. 10:56.

McCann, S. M. and V. D. Ramirez. 1964. The neuroendocrine regulation of hypophyseal luteinizing hormone secretion. Rec. Prog. Horm. Res. 20:131.

McCay, C. M. 1931. The hemoglobin and total phosphorus in the blood of cows and bulls. J. Dairy Sci. 14:373.

McDowell, R. E. 1958. Physiological approaches to animal climatology. J. Heredity 49:52.

McDowell, R. E. 1966. The role of physiology in animal production for tropical and subtropical areas. World Rev. Animal Prod. 1966 (1):39.

McDowell, R. E., C. A. Matthews, D. H. K. Lee and M. H. Fehrman. 1953. Repeatability of an experimental heat tolerance test and the influence of season. J. Animal Sci. 12:757.

Mehrotra, P. N., D. N. Mullick and N. D. Kehar. 1954. Seasonal variations in the blood composition of sheep. J. Animal Sci. 13:1026.

Moore, C. R. 1924. Properties of the gonads as controllers of somatic and psychical characteristics. VIII. Heat application and testicular degeneration; the function of the scrotum. Am. J. Anat. 34:337.

Mullick, D. N. 1960. Effect of humidity and exposure to sun on the pulse rate, rectal temperature and haemoglobin level in different sexes of cattle and buffalo. J. Agr. Sci. 54:391.

N. R. C. 1963. Nutrient requirements of farm animals, No. 4. Nutrient requirements of beef cattle. National Research Council, Washington, D. C. Pub. 1137.

Nikitovitch-Winer, M. B., A. H. Pribble and A. D. Winer. 1965. Luteinizing hormone-releasing factor: partial purification. Am. J. Physiol. 208:1286.

Ogle, C. 1934. Adaptation of sexual activity to environmental stimulation. Am. J.Physiol. 107:628.

Patrick, T. E., J. E. Johnston, H. C. Kellgren, J. B. Frye, Jr., G. D'Arensbourg and C. Branton. 1954. Effects of hot weather and high humidity on semen production and fertility of the dairy bull. J. Animal Sci. 13:1028.

Pennycuik, P. R. 1967. A comparison of the effects of a range of high environmental temperatures and of two different periods of acclimatization on the reproductive performance of male and female mice. Australian J. Exp. Biol. Med. Sci. 45:527.

Plasse, D., A. C. Warnick and M. Koger. 1968. Reproductive behavior of Bos Indicus females in a sub-tropical environment. I. Puberty and evulation frequency in Brahman and Brahman x British heifers. J. Animal Sci. 27:94.

Post, B. T. 1965. Thyroid secretion rates in grazing British and Zebu crossbred steers: seasonal effects and relationships with growth. Australian J. Agr. Res. 16:229.

Prine, G. N. and J. E. Mickelson. 1968. 1967 climatological data for Gainesville, Florida and B.R.U. Agronomy Mimeo Series AG 68-4, Univ. of Florida.

Quazi, F. R. and R. R. Shrode. 1954. Variations in rectal temperature, pulse rate and respiration rate of cattle as related to variation in four environmental variables. J. Animal Sci. 13:1028.

Richar, W. J. and E. S. Breakell. 1959. Evaluation of an electronic particle counter for the counting of white blood cells. Am. J. Clin. Path. 31:384.

Robertshaw, D. and G. C. Whittow. 1966. The effect of hyperthermia and localized heating of the anterior hypothalamus on the sympatho-adrenal system of the ox (Bos taurus). J. Physiol. 187:351.

Rusoff, L. L., J. B. Frye, Jr. and G. W. Scott, Jr. 1951. Blood studies of Red Sindhi-Jersey crosses. I. Hb., Ht., Plasma Ca and Plasma inorg. P. values of Red Sindhi-Jersey daughters and their Jersey dams. J. Dairy Sci. 34:1145.

Rusoff, L. L., J. E. Johnston and C. Branton. 1954. Blood studies on breeding dairy bulls. J. Dairy Sci. 37:30.

Ryle, M. 1961. Early reproductive failure of ewes in a hot environment. I. Ovulation rate and embryonic mortality. J. Agr. Sci. 57:1.

Ryle, M. 1962. Early reproductive failure in ewes in a hot environment. II. The uterus. J. Agr. Sci. 58:137.

Ryle, M. 1963. Early reproductive failure of ewes in a hot environment. IV. The ovary. J. Agr. Sci. 60:101.

Schally, A. V. and C. Y. Bowers. 1964. Purification of luteinizing hormone-releasing factor from bovine hypothalamus. Endocr. 75:608.

Schalm, O. W. 1965. Veterinary hematology (2nd ed.). Lea & Febiger, Philadelphia, pp 46-129.

Schiavi, R., M. Jutisz, E. Sakiz and R. Guillemin. 1963. Stimulation of ovulation by purified LH-releasing factor (LRF) in animals rendered anovulatory by hypothalamic legions. Proc. Soc. Exp. Biol. Med. 114:426.

Strumia, M. M., A. B. Samples and E. D. Hart. 1954. An improved microhematocrit method. Am. J. Clin. Path. 24:1016.

Thatcher, W. W. 1969. Personal communication.

Ulberg, L. C. 1958. The influence of high temperature on reproduction. J. Heredity 49 (2):62.

Ulberg, L. C. and P. J. Burfening. 1967. Embryo death resulting from adverse environment on spermatozoa or ova. J. Animal Sci. 26:571.

Vandemark, N. L. and L. L. Ewing. 1963. Factors affecting testicular metabolism and function. II. Effect of temperature elevation in vivo on subsequent metabolic activity of rabbit testicular tissue in vitro. J. Reprod. Fertil. 6:9.

Vernon, E. H., R. A. Damon, Jr., W. R. Harvey, E. J. Warwick and C. M. Kincaid. 1959. Relation of heat tolerance determinations to productivity in beef cattle. J. Animal Sci. 18:91.

Waites, G. M. H. and B. P. Setchell. 1964. Effect of local heating on blood flow and metabolism in the testis of the conscious ram. J. Reprod. Fertil. 8:339.

Walker, C. A. 1958. Studies in the environmental physiology of cattle in Northern Rhodesia. I. The relation between the changes in the hemoglobin index with age and the development of the heat toleration coefficient. J. Agr. Sci. 51:119.

Warnick, A. C., H. D. Wallace, A. I. Palmer, E. Sosa, D. J. Duerre and V. E. Caldwell. 1965. Effect of temperature on early embryo survival in gilts. J. Animal Sci. 24:89.

Wayman, O., H. D. Johnson, C. P. Merilan and I. L. Berry. 1962. Effect of ad libitum or force-feeding of two rations on lactating cows subject to temperature stress. J. Dairy Sci. 45:1472.

Weldy, J. R., R. E. McDowell, P. J. Van Soest and J. Bond. 1964. Influence of heat stress on rumen acid levels and some blood constituents in cattle. J. Animal Sci. 23:147.

Whittow, G. C. 1962. The significance of the extremities of the ox (Bos taurus) in thermoregulation. J. Agr. Sci. 58:109.

Whittow, G. C. 1968. Cardiovascular response to localized heating of the anterior hypothalamus. J. Physiol. 198:541.

Winchester, C. F. 1964. Symposium on growth. Environment and growth. J. Animal Sci. 23:254.

Wisecup, W. G. and B. G. Crouch. 1963. Evaluation and calibration of an electronic particle counter for enumeration of multispecies blood cells. Am. J. Clin. Path. 39:349.

Woody, C. O. and L. C. Ulberg. 1964. Viability of one cell sheep ova as affected by high environmental temperature. J. Reprod. Fertil. 7:275.

Worstell, D. M. and S. Brody. 1953. Environmental physiology and shelter engineering, with special reference to domestic animals. XX. Comparative physiological reactions of European and Indian cattle to changing temperature. Mo. Ag. Exp. Sta. Res. Bull. 515.

Yamada, T. and M. A. Greer. 1959. Studies on the mechanism of hypothalamic control of thyrotropin secretion: effect of thyroxine injection into the hypothalamus or the pituitary on thyroid hormone release. Endocr. 64:559.

Yeates, N. T. M. 1953. The effect of high air temperature on reproduction in the ewe. J. Agr. Sci. 43:199.

Yousef, M. K. and H. D. Johnson. 1965. Some blood constituents of dairy cattle: influence of thyroxine and high environmental temperature. J. Dairy Sci. 48:1074.

Yousef, M. K. and H. D. Johnson. 1966. Blood thyroxine degradation rate of cattle as influenced by temperature and feed intake. Life Sci. 5 (15):1349.

BIOGRAPHICAL SKETCH

Hernando Gutierrez De La R. was born April 14, 1938, in Colombia, South America. In November, 1956, he graduated from San Bartolome School at Bogota, Colombia. In February, 1962, he received the degree of Bachelor of Science in Agriculture with a major in Poultry Science from the University of Florida. That same year he enrolled in the Graduate School of the University of Georgia. He worked as a research assistant in the Department of Poultry Science until November, 1963, when he completed the requirements for the degree of Master of Science with majors in Genetics and Physiology. In June, 1964, he was granted the degree of Master of Science with "Distinction." From 1963 until 1966 he worked as Associate Physiologist and Director of the National Physiology Program of the Colombian Institute of Agriculture, Bogota, Colombia. From September, 1966, until the present time he has pursued his work toward the degree of Doctor of Philosophy.

Hernando Gutierrez De La R. is married to the former Beatriz Botero and is the father of two children. He is a member of Alpha Zeta, Gamma Sigma Delta, Phi Kappa Phi, Sigma Xi, Phi Sigma, the American Society of Animal Science, the Latin American Society of Animal Production and the Society for the Study of Reproduction.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1969

W. A. Broyles
Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:

A. L. Hindman
Chairman

J. A. Hunter

A. R. Wilson

J. Henley Jr.

R. L. Shirley