RESEARCH BULLETIN 785 JANUARY, 1962

UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

E. R. KIEHL, *Director*

Environmental Physiology and Shelter Engineering

With Special Reference to Domestic Animals

LVIII. Metabolic Reactions During Thermal Stress (35° to 95°F.) In Dairy Animals Acclimated at 50° and 80° F.

T. H. KAMAL, H. D. JOHNSON, AND A. C. RAGSDALE

(Publication authorized January 15, 1962)

COLUMBIA, MISSOURI

CONTENTS

ACKNOWLEDGEMENTS

This project is part of a broad cooperative investigation between the Departments of Dairy Husbandry and Agricultural Engineering of the Missouri Agricultural Experiment Station, University of Missouri, and the Agricultural Engineering Research Division of the United States Department of Agriculture. This bulletin is a report on the Department of Dairy Husbandry research project no. 125, "Climatic Factors."

Acknowledgements are due to M. M. Jones for cooperation on the engineering phase of the work; to C. W. Gehrke for his advice in the chemical phase of this investigation and for help in chemical analyses; to C. L. Gregory for help in statistical analyses; to Sam Barrett for assistance in water measurements; to Veterinary Clinic Staff for veterinary care; to Roy Rumans and Shirley Sturm for care and feeding of animals; to Ed Paschang for assistance in taking measurements; to the State Church Farm in Jefferson City for use of several Church Farm cows for this experiment; and to Donna Gosnell for typing of the manuscript.

Environmental Physiology and Shelter Engineering

With Special Reference to Domestic Animals

LVIII. Metabolic Reactions During Thermal Stress (35° to 95° F.) In Dairy Animals Acclimated at 50° and 80° F.

T. H. KAMAL, H. D. JOHNSON, AND A. C. RAGSDALE

Department of Dairy Husbandry, University of Missouri, Columbia

INTRODUCTION

Environment, particularly ambient temperature is a determinant factor in the production and reproduction of farm animals all over the the world. In various regions where extreme heat or cold may prevail most of the year the dairyman is confronted with difficult production problems. These high and low environmental temperatures are systemic stressors which evoke particular group of changes in the biological characteristics of homeotherms (Selye, 1950). In cattle these particular changes, or the so-called G-A-S* which constitute the "stress", have not as yet been investigated with exposure of cattle to heat or cold. It is well-known, however, that when cattle are exposed to temperatures outside the comfort zone, a vast array of physiological reactions takes place which constitute the generally called thermal stress in cattle (Kamal *et al.*, 1959b). In this investigation, however, the possible occurrence of "stress" in cattle has been presented.

Long exposure to cold or heat may allow compensation for the thermal induced changes. The animals may restore the biological displacements that occurred at the beginning of the thermal exposure (Kamal *et al.*, 1958a; Bianca, 1959), and may be able to withstand a thermal environment more severe than the one at which they were raised (Kamal *et al.*, 1958b; Hart, 1958).

ORIENTATION AND OBJECTIVES

This bulletin presents the metabolic changes that occurred in the 50° and 80°F. reared groups of Brown Swiss, Holstein, and Jersey heifers during their exposure to rising environmental temperatures of 35°, 50°, 70°, 80°, 90°, and 95°F. Each temperature level was of 2 weeks duration. Sixteen biological characteristics are presented including protein, electrolyte, and water metabolism, as well as, blood glucose and plasma protein expressed as total nitrogen.

*General Adaptation Snydrome.

This phase of the research has five major objectives. The first objective was to study the effect of rising temperature on these aforementioned characteristics in attempt to clarify the influence of heat and cold on carbohydrate, protein, water, and electrolyte metabolism in cattle as compared with other species of animals. In this objective it was hoped to understand some facts about the hormonal changes that are associated with these metabolic alterations under thermal stress. An endeavor has never been undertaken before in cattle, using such an extensive program, handling the data statistically, and eliminating the interference of lactation, gestation, growth, and all other environmental factors except temperature.

The second objective was to find a reliable index for heat tolerance which is an ultimate goal of most workers in the field of bioclimatology. Studying these aforementioned sixteen biological characteristics which cover most of the major metabolic changes of the body was thought to give a clue to the characteristic of "heat tolerance"; a phenomenon which has long awaited clarification by bioclimatologists.

The third objective of this investigation was to answer the question which was posed in the conclusion of previous publications (Johnson and Ragsdale, 1959, and Kamal *et al.*, 1959). This is, whether the heat acclimation characteristics which are thought to have been acquired by the 80°F. group of heifers would be held by the animals when they were later exposed in this investigation to cold and heat. This question will be answered in this study by comparing statistically the differences in the biological responses of cold and warm acclimated heifers to various environmental temperatures.

The fourth objective was to determine any relation between what we generally call "thermal stress" in cattle (Kamal *et al.*, 1959) and the "stress" as seen by Selye (1950). The frequent usage of the last term recently in cattle with no evidence as yet available regarding the occurrence of the General-Adaptation-Syndrome in cattle has led the writer to devote part of this study for investigating the possibility of the existence of "stress" in cattle under heat and cold.

The lack of information about the levels of many of these aforementioned characteristics in cattle either under comfort temperature or thermal stress has also intensified the need for this study.

The last objective of this report is to present a comprehensive literature review of the biochemical effects of environmental temperature on cattle.

In a previous investigation the hormonal (BEI^{131})**, enzymatic, blood (GSH)***, and physiological (body weight gain) changes during the acclimation of dairy calves to mild cold (50°F.) (Kamal *et al.*, 1959), and heat (80°F.) were studied in order to clarify the thermal acclimation in cattle more precisely. Exposure of the cold and warm acclimated cattle to adverse environmental temperatures (35° to 95°F.), and studying the changes in protein, carbohydrate,

**BEI^{131} = butanol extract-I^{131}.

***(Blood GSH) = blood reduced glutathione.

electrolyte, and water metabolism in both groups, was thought to provide information about the stability of the acquired thermal acclimation characteristics in cattle.

The fact that milk production in the high producing European cattle is markedly depressed in tropic and subtropic regions of the world has urged most bioclimatologists to find an index of heat tolerance in cattle. This would aid in the attempt to establish a heat tolerant breed that maintains high productivity under hot climates. No agreement has been attained among the investigators on an index or indices for heat tolerance. This investigation will provide new information on the biological changes in cattle at various environmental temperatures and further establish the fundamentals for a biochemical index of heat tolerance.

REVIEW OF LITERATURE

Effect of Environmental Temperature on Endocrine Glands Activity:

When homeothermic animals are subjected to environmental temperatures above or below their thermoneutral zone, a vast array of physiological and biochemical changes take place in their bodies, which in cattle, constitute the so called thermal stress (Kamal *et al.,* 1959b). It is well-known that most of the metabolic reactions in the body are carried out by various types of enzymes. The activities of the latter are to a great extent controlled by the levels of both substrates and hormones. These levels should be carefully considered whenever studies on the metabolic changes in animals are undertaken. With changes in environmental temperature the substrate levels available for cellular metabolic activities and cellular multiplications are altered eventually due to changes in feed consumption. The changes in feed consumption in cattle with the fluctuations in environmental temperature, and the theories about the mechanisms of feed regulation, such as the theory of appetite and hunger centers, or that of the thermostatic or chemostatic regulation of feed intake, have been shown and discussed in details by Brody *et al.* (1948-1957), Johnson (1956), Kleiber (1956), McDonald and Bell (1958), and Brobeck (1960), that nothing need to be added. However, changes in hormonal balance with varying environmental temperature have been studied only in connection with thyroid hormones in most species including cattle and more recently with adrenal corticoids and antidiuretic hormones in man and experimental animals. The levels of these hormones and of others such as insulin, growth hormone, lactogenic hormone and hormones of the posterior pituitary that are responsible to a great extent to the depression in growth, milk production, and reproduction of cattle under hot climates have not as yet been investigated in regard to changing environmental temperature.

In this review the available information concerning the effect of environmental temperature on thyroid, adrenal glands, and antidiuretic hormones, as well as the mechanism involved in such effect will be discussed. The observed effects of environmental temperature on blood glucose; plasma total nitrogen;

electrolyte metabolism (Plasma and urine sodium, potassium and sodium/potassium ratios); protein metabolism (digestible nitrogen consumption, urinary nitrogen excretion, and nitrogen retention); and water metabolism (water consumption, urine volume, urine specific gravity, and water vaporization) which are studied in this investigation will be reviewed. Particular emphasis, however, have been given on the effect of feed and hormones on those measurements as possible mechanism through which temperature exerts its influence.

Thyroid Gland: A considerable amount of work has been accumulated lately regarding the effect of environmental temperature on thyroid function in different species. This subject has been exclusively reviewed by Blincoe (1955), Johnson (1956), and Premachandra (1958). Among most investigators in this field there is a fairly good agreement about the fact that the thyroid gland activity in different species is increased at moderate cold environment and is depressed at high temperatures. This conclusion was particularly confirmed in cattle by using the conversion ratio, i.e., $\frac{\text{thyroxine - like } I^{131}}{\text{Plasma total } I^{131}}$ by Blincoe and Brody (1955), maximum thyroid uptake of I^{131} (U), thyroid uptake of I^{131} (K_1), and release rate of thyroidal I^{131} (K_4) by Blincoe and Brody (1955a), thyroidal I^{131} release rate by Johnson and Ragsdale (1959), and thyroxine secretion rate by Premachandra, *et al.,* (1958). The last author, however, did not find any relation between the season and the thyroidal I^{131} release rate. The protein-bound iodine techniques as reported in Louisiana progress report (1959) have indicated marked seasonal effects, while attempts to demonstrate differences in PBI among animals due to breed or functional status, however, have not been successful. In this report it was concluded that the PBI is a questionable index of thyroid function where it is necessary to identify small differences among animals. Neither the I^{131} uptake nor the conversion ratio were found later to be correlated with the environmental temperature as reported, respectively, by Swanson *et al.,* (1957), and Blincoe and Brody (1955a). In this respect, Johnson (1959) made a comparison between PBI, thyroxine I^{131} destruction rate, and thyroxine secretion rate. He concluded that PBI method was a questionable index of thyroid function and that, of the various measures used, thyroxine secretion rate was most closely related to metabolic rate. Thyroid release rates were not compared—theoretically release rates may show as good a relationship as so-called secretion rate. The latter is inversely related to environmental temperatures (Johnson and Ragsdale, 1960).

The butanol extract iodine is thought to contain the iodine of "thyroxine-like" compounds (Man *et al.,* 1951; Taurog and Chairkoff, 1948). The former authors found that BEI^{131} correlated more closely than the serum precipitable iodine with the clinical state of patients. However, in cattle no information is available in this concern or regarding the effect of environmental temperatures on the BEI^{131} which is investigated in the present study.

Adrenal Glands: The adrenal gland function in heat and cold has long been investigated in man and smaller experimental animals, while little information in this regard is available on cattle.

The increase in adrenal corticoid secretion in experimental animals upon their sudden exposure to cold has long been recognized as indicated by the decrease in cholestrol content of the adrenals (Sayer *et al.*, 1944; Levin, 1945; Freedman and Gordon, 1955), the decrease in the ascorbic acid content in the adrenals (Sayers, 1948; Kimura, 1954), the adrenal hypertrophy (Selye, 1950; Heroux and Hart, 1954; Clark *et al.*, 1955), or the increase in the incorporation of inorganic P^{32} into the acid-soluble phosphorus of the adrenals (Nicholls and Rossiter, 1955; Nicholls and Rossiter, 1956). In heat, similar conclusions were obtained on cattle and on rats as shown by the adrenals hypertrophy, (Clark *et al.*, 1955) by the decrease in blood or adrenals cholesterol and ascorbic acid (Brody, 1949; Blincoe and Brody, 1951; Langley and Kilgore, 1955) or by the increase in reducing corticoids (Holcombe, 1957).

The application of indirect methods as shown above for assessing the adrenal cortical activity has shown that both extremes of environmental temperature may increase the hormonal production of adrenal cortex. With the development of the new direct methods of determining the glucocorticoid levels in blood and urine it became obvious to know what types of adrenal corticoids may account for the aforementioned increase in adrenal cortical activity in either cold or heat.

In man, Thorn *et al.* (1955) reported very slight increase in the urinary 17-hydroxycorticoids during exposure to cold. Bass *et al.* (1955) showed that exposure of men to heat for 14 days caused no marked change in the circulating eosinophils or the urinary 17-ketosteroid excretion. There was, if anything, a downward trend in the 17-ketosteroid excretion during the heat period in spite of the occurrence of a negative nitrogen balance. On the other hand, sweat and urine Na/K ratio decreased during this period indicating an increase in mineralocorticoid activity in the heat.

In accordance with the above results, Hellman *et al.* (1956) who carried out an extensive study on urinary 17-hydroxycorticosteroids and aldosterone excretion in men during heat exposure, showed that any stress resulting from the condition of heat and work or heat alone had no significant effect on the outputs of either cortisone and cortisol or of hydrocortisone and tetrahydrocortisol. Aldosterone excretion, on the other hand, was significantly increased during heat exposures as indicated by the urinary Na^{24}/K^{42} method. The increase in aldosterone in heat was accomplished without the mediation of ACTH or an occurrence of sale deficiency.

Reduced excretion of 17-ketosteroids and of 17-ketogenic steroids in human urine in summer and artificial heating and increased excretion of these steroids in winter were reported (Watanabe and Yoshida, 1956; Macfarlane and Robinson, 1957; and Robinson and Macfarlane, 1958). The last authors showed that during the heating period urinary sodium excretion decreased rapidly, while the K/Na ratio rose (presumably the ratio should decline if it has been computed as Na/K) indicating an increase in aldosterone production. The decrease in urinary Na/K ratio in man and the increase of the ratio in sheep when subjected to

heat (Macfarlane *et al.*, 1958) suggested an increase in the mineralocorticoids secretion in man and a decrease of these hormones in sheep during heating periods. The methods of determining the mineralocorticoids are based on measuring the changes in the inert or radioactive Na/K urinary ratio of adrenalectomized rats (Simpson and Tait, 1955b).

Determining the 17-hydroxycorticosteroid levels in blood, however, gave opposite results to those of urine mentioned above. Barlow *et al.* (1956) reported a marked increase in blood levels of 17-hydroxycorticosteroids in dogs subjected to hyperthermic stress. This was later confirmed by Hale *et al.* (1957) in man. They found that although exposure of healthy men to brief periods (45 minutes) to high environmental temperature (50°C.) induced no detectable increase in the titer of ACTH in peripheral blood or in the concentration of plasma 17-hydroxycorticosterone, or corticosterone-like steroids during the heat exposure, yet after two or three hours of the heat exposure two thirds of the subjects exhibited significant increases in the concentration of peripheral plasma 17-hydroxycorticosteroids or corticosteroids.

It can be concluded from the above results, that, from the urine determinations of corticoids, it seems that heat increases the mineralocorticoid and decreases the glucocorticoid production in man. Blood analysis, however, shows that the glucocorticoid secretion may also increase in man during heat treatments. In sheep, differently from man, mineralocorticoids production tends to decline markedly with exposure to heat.

Antidiuretic Hormone: Macfarlane and Robinson (1957) exposed one sheep and four healthy humans for 4 hours to heat (40.5°C.) once every three months during the year. They showed that in both sheep and human plasma antidiuretic activity was higher in the heat treatments (40.5°C.) than in control (24°C.). The values, however, obtained during summer and autumn were higher than those obtained during the winter and spring. They concluded from their results and those of other investigators that seasonal changes in blood composition are closely linked with endocrine activity particularly that of adrenal cortex and pituitary. More recently Macfarlane *et al.* (1958b) observed that urine flow in man and sheep though declined directly upon exposure to heat (41°C.), the antidiuretic substance in the plasma started to appear only after 2 to 4 hours of heating (41°C.). In ten merino sheep it was necessary to heat for 4 hours at 44°C. to induce a release of the antidiuretic substance in the plasma during the summer. Injection of deoxycosterone in sheep and heating for 4 hours, caused release of the antidiuretic substance in the plasma and as the effect of hormone wore off the amount of antidiuretic substance appearing in the plasma at 41°C. was reduced. After 10 days of the injection there was no residual activity.

In rats similar results were obtained by Itoh (1954). The urine excreted by rats, which were injected by serum obtained from rats exposed to cold, was larger in volume than that obtained from heat exposed rats. He also found that the content of antidiuretic hormone in the pituitary gland of rats exposed to heat was lower than that of rats exposed to cold indicating a greater release of the hormone from the former's pituitary gland.

Not only antidiuretic substances were found in plasma of sheep, man and rats exposed to heat, but the occurrence of antidiuretic hormone in their urine was also indicated. Kellman and Weiner (1953) showed that after the exposure of man to heat (100°F.) for 90 minutes and leaving the chamber, significant antidiuretic activity was detected in the urine of these subjects after leaving the chamber, while it was not detected in their urine during the heating period though urine volume was markedly reduced. The authors concluded that the antidiuretic was not stimulated by the heat, per se, but indirectly through the mild degree of "negative" water balance or incipient dehydration caused by heat.

Itoh and Kimura (1953) also showed that antidiuretic substance was excreted in human urine after heavy sweating due to 90 minutes exposure to heat (45°C.). The antidiuretic substance was also found in human sweat though much less than in urine.

Mechanisms Involved in Regulating the Thyroid, Adrenocortical, and Antidiuretic Hormones by Environmental Temperature: Although the mechanisms responsible for thyroid and adrenal cortical glands regulation have been studied by many investigators, yet the mechanism through which cold and particularly heat stimulate these glands has normally been overlooked. When the individual is exposed to temperatures above or below the comfort zone an immediate sensation of warmth or cold is carried all over the body. Sensation of heat and cold, as well as other kinds of sensations are well known to be a direct function of sensory receptors which are in the form of different kinds of nerve endings on tissues surfaces. These receptors set up nervous impulses only when they are stimulated by special kind of stimuli which are plentiful in the body, and particularly near its surface. The nerve fibers that lead from these various kinds of receptors lead in general to the regulatory centers of the hypothalamus and cerebrum.

Definite anatomical structures responsible for temperature awareness, have been identified histologically (Ham, 1957) and physiologically (Oppel and Hardy, 1937; Hardy and Oppel, 1938; Bing and Skouby, 1950; Bazett, 1951; Hensel and Zotterman, 1951; Hensel *et al.*, 1951; Dodt, 1952). These studies indicate that these receptors function as the body's first line of defense against external or internal temperature changes. Ham (1957) has described two types of thermal receptors. The cold receptors (Krause end-bulbs) are of two structurally different types and are found in the skin, but most prevalent in the dermis of the conjunctiva, the mucosa of the tongue and the external genitalia. The heat receptors (corpuscles of Ruffini) lie deeper in the skin or even in the subcutaneous connective tissue. The depth of the thermoreceptors in the tongue tissue of cats was determined by Hensel *et al.* (1951). The presence of peripheral thermal receptors in calves has been indicated by Bligh (1957). A direct response to heat stress ensued in calves, as indicated by the onset of respiration frequency, before any increase in the temperature of the blood supplying the brain was noted. A brief review on the subject was covered by Newburgh (1949), Robinson (1952), and Selle (1952).

Once the hypothalamus receives the temperature stimulant either by impulses from the peripheral thermal receptors or directly by the circulating blood temperature, through unknown mechanism or mechanisms, the hypothalamus influences the adenohypophysis for hypo or hyper secretion of thyrotrophic and adrenocorticotrophic hormones. Definite areas in the pituitary have been indicated (Greer and Erwin, 1956; Harris and Woods, 1957; Greer, 1957). However, the precise mechanism or mechanisms by which this regulatory control is mediated remains unsettled. Sayers *et al.* (1958) from his review on the hypothalamus, adenohypophysis and adrenal cortex have assumed that there is a "connecting link" between hypothalamus and adenohypophysis which represents a "final common path" for the great variety of stimuli which induce ACTH release. The available evidence justifies the popularity of acceptance of the thesis that portal venous system carries a neurohumor from the median eminence to the adenohypophysis that regulate the ACTH. The same may also be true for the TSH secretions.

The regulation of thyroid and glucocorticoid hormones production by the pituitary gland has long been known. The pituitary-thyroid relationship was investigated by Cortell and Rawson (1944) and the regulating effect of pituitary ACTH on adrenal glucocorticoids secretion (Macchi and Hechter, 1954; Querido *et al.,* 1955) and not on the mineralocorticoids secretion (Simpson and Tait, 1955) was demonstrated.

The aforementioned systematic mechanism is probably the main system through which environmental temperatures induce the effects mentioned above in the thyroid and adrenal glands' activities. Nevertheless, there are other factors that may play a big part in regulating the secretion rate of thyroidal and adrenocortical hormones under heat and cold. For example, the rate of synthesis of these hormones depends eventually on the nutritive state of the animal which is known to be affected by climatic factors. Environmental temperature could also affect the thyroid and adrenocortical activities by altering the blood levels of circulating hormones which regulate through feed back mechanism the thyrotropin and corticotropin of the pituitary and consequently the hormones of the thyroid and adrenals. The change in the blood levels of these hormones by alterations in environmental temperature can be brought about by enhancing the hormonal excretion outside the body (Intoccia *et al.,* 1959: Kot and Klitgaard, 1959), by altering the electrolyte metabolism (Isler *et al.,* 1958), by changing the feed and water consumption (Reichlin, 1958), and by affecting the renal function (Rasmussen, 1956; Kalant *et al.,* 1959).

Environmental temperature could affect the activities of thyroid and adrenal glands through enhancing the secretion of one or another of those glands, for example, by increasing the adrenocortical hormones production which assist in depressing the thyroid gland activity or vice versa at high temperature. The increase of 17-hydroxycorticosteroids in cattle under certain acute stress conditions was attributed to the decrease in PBI values (Robertson *et al.,* 1958). The inverse relationship between the ACTH or glucocorticoids and thyroid hormones,

however, has been demonstrated (Epstein *et al.*, 1953: Hechter and Pincus, 1954; Brown-Grant, 1956) while the direct relationship between these hormones under certain conditions was shown by Albert *et al.* (1952), Evans *et al.* (1957) and Beck (1958).

From the above discussion it is illogical to attribute the changes in adrenal and thyroid gland's activities in heat and cold to only one mechanism. A coordination of various factors as shown above including nervous system, hypothalamus, renal function, feed and water consumption, hormonal interrelationships and many others are responsible for environmental induced effects on any particular hormonal secretion. The mechanism by which environmental temperature regulates the release of antidiuretic hormone is likewise dependent on the coordination of the above mentioned factors. That heat, per se, affects the release of antidiuretic hormone (Itoh, 1954) is not accepted by many investigators who showed a delay in its release after heat exposure and after urine volume was already reduced (Kellman and Weiner, 1953; Macfarlane *et al.*, 1958b). It seems, however, that the release of antidiuretic hormone is indirectly regulated by environmental temperature through changes in the hydration-dehydration status, or by hormonal effect, such as glycocorticoids and epinephrine, in the animal. Various hypothesis, regarding the mechanisms involved in the stimulation and inhibition of the release of antidiuretic hormone, have been suggested by Strauss (1957), Lotspeich (1958), and Thorn (1958) which will be dealt with again in the part of water metabolism of this review.

Effect of Environmental Temperature on Electrolyte Metabolism:

Importance of Electrolytes in Climatic Studies: Although plasma protein is the only osmotically active substance at the capillary membrane interface, yet electrolytes contribute to almost all the osmolarity of body fluids. The sodium in plasma and interstitial fluid, and the potassium in intracellular fluid represent, by far, over 90 percent of the cations in electrolytes of these fluids, as well as one half or more of the total osmotic pressure of these fluids (Black, 1957). As loss of plasma protein decreases the plasma osmotic pressure causes leakage of intravascular fluid to the extravascular spaces which may result in edema, the deficiency in electrolytes decreases the osmotic pressure of body fluids with resultant of body water loss through the kidneys, and thus, dehydration of the body.

At high environmental temperature, cattle are under an abnormal balance of salt intake due to their severe decline in feed consumption, especially roughages which are very rich in potassium as compared to sodium. Such imbalance in salts intake may contribute, to some extent, to the fluid shifts in the body and the blood volume alteration showed by Dale *et al.* (1956).

The importance of electrolytes in maintaining the acid-base balance, i.e., the balance between anions and cations, is magnified in cattle when they are exposed to high environmental temperature. The hyperventilation associated with panting that takes place under such circumstances (Kibler and Brody, 1950)

blows off the CO_2 and reduces the H_2CO_3 to $BHCO_3$ ratio, followed by a compensatory loss of alkali reserve as $BHCO_3$ in urine, thus resulting in respiratory alkalosis (Dale and Brody, 1954). As long as a deficiency in electrolytes exists the animal consistently drinks and urinates large amounts of water (Holmes and Cizek, 1951; Cizek *et al.,* 1951) which result in further depletion of electrolytes from the body.

These functions of electrolytes, mainly sodium and potassium, along with others, such as the activation of enzymes, nervous and muscular activities which are of significance only in severe depletion of electrolytes (Stewart, 1957), gave the sodium and potassium a great significance in combating thermal stress in cattle. In the following part of this review emphasis will be given to the average values of plasma and urinary sodium and potassium with comparison with such values of other species. The change in sodium and potassium levels in plasma and urine under thermal stress, and the mechanisms involved in such alterations such as dietary and hormonal factors, will also be considered.

Averages of Plasma and Urinary Sodium and Potassium: The plasma sodium and potassium concentrations in cattle are similar to a great extent to those of other species. In lactating Jersey, Holstein, and Braham cows at comfort environmental temperature (50°F.) the values are 141, 139, and 143 meq./1., respectively, for sodium, while the averages of potassium are 4.78 and 4.86 meq./1. in Holstein and Brahman cows, respectively, (Blincoe and Brody, 1951). In 4 Holstein cows and one Guernsey cow under controlled environmental temperature (60-70°F.) the mean values were 162 and 5.1 meq./1. for sodium and potassium, respectively, (Dale *et al.,* 1954). These values are in accordance with earlier studies of Green and McCaskill (1928). In man the ranges of plasma sodium and potassium were reported as from 130 to 143, and from 4.1 to 5.6 meq./1., respectively, (Gamble, 1954 and Hawk *et al.,* 1954).

The average values for plasma sodium and potassium levels are 141 and 3.8 meq./1., respectively, in 8 dogs (Davis and Ball, 1958) and 134.5 and 5.4 meq., respectively, in rats (Hannon *et al.,* 1958).

The amounts of sodium and potassium excreted vary considerably among animals of different species. Moreover, while sodium predominates over potassium in plasma, the relationship between these electrolytes in urine is variable among species. The amount of urinary sodium and potassium excreted per day under normal conditions are 2.3 and 1.9 meq., respectively, in rats (Eversole and Romero, 1958); 5.4 and 5.6 meq., respectively, in chicken (Brown *et al.,* 1958); 150 and 50 meq., respectively, in man (Bass *et al.,* 1955); 680 and 45 meq., respectively, in 10 liters of man's sweat (Robinson and Robinson, 1954); and 529 and 3658 meq., respectively, in cattle (Dale *et al.,* 1954).

Climatic Influence on Plasma and Urine Na, K, and Na/K: The effect of environmental temperature on electrolyte metabolism in the past was concerned with the changes in chloride excretion, which is assumed to have a close physiological relationship with sodium, in sweat or urine. In this concern Adolph (1947) showed that the man under the hot conditions of the desert whether no

water was drunk or whether it was drunk, *ad libitum*, the rate of urinary chloride excretion was found not to be significantly less than other climates (116 meq./day), while excessive amounts of salt are excreted in sweat. He also found that the average concentration of chloride in venous serum was no different from that of men in other climates (96 meq./1.), providing that the men were not dehydrated.

Recently various authors have studied the electrolyte metabolism as affected by environmental temperatures more precisely in terms of sodium, potassium, and sodium/potassium ratio in plasma, urine and sweat. Most of these studies were done on man, dogs, and rats. In ruminants, few studies were undertaken on sheep while information in this regard on cattle is scarce and unsatisfactory.

Lichton (1957) studied the rate of sweat and urine excretion in man and the sodium and potassium contents during mild exercise in heat. He showed that the concentration of sodium increased, while that of potassium decreased in sweat with increase of the rate of sweating, which was directly related to the air, skin, and rectal temperatures. The urinary sodium excretion per minute (urine sodium load), however, decreased in hyperbolic fashion with increasing sweat sodium load. Also, the urinary potassium load was negatively related to the sweat potassium load with increasing temperature. No consistent changes in serum sodium or potassium were observed with changes in thermal sweat rate.

The inverse relationship between sodium and potassium concentration in sweat with increasing the rate of sweating is not in accordance with earlier studies (Dill *et al.*, 1938) and in agreement with advancing work (Berenson and Burch, 1953). On the other hand, the inconsistency of the concentrations of blood electrolytes were confirmed by Bass *et al.* (1955). Macfarlane and Robinson (1957) studied the effect of 4-hours heat exposure (40.5°C.) in summer and winter seasons on plasma sodium and potassium in four resting humans and one sheep. They showed insignificant effect of the 4-hours exposure to heat on these concentration in plasma. In his report on heat disorders in man Ladell (1957) came to the same conclusion, except he mentioned that in heat exhaustion a fall in blood electrolytes may occur.

Urinary sodium and potassium in contrast to plasma, and similar to sweat, show consistent response to ambient temperature. Bass *et al.* (1955) studied the effect of long exposure (14 days) of heat (100-120°F.) in five young men, performing a standard amount of work daily. They came to the similar conclusion of Lichton (1957), showing that urinary excretion of sodium fell to very low levels during the first four days of heat while urinary potassium followed a pattern opposite to that of sodium in that its excretion during the first four days was relatively higher than during the rest of heat period, resulting in a negative potassium balance of about 200 percent below the pre-heat potassium retention levels. The Na/K ratio in the urine fell markedly during the first four heat days about 75 percent of the control values. Longer exposure till the end of heat period, however, changed the electrolytes trend; sodium excretion and sodium/potassium ratio increased while potassium excretion decreased, which was attri-

buted by the author to the acclimation process to heat that were shown in other measurements such as sweat composition, decrease in pulse rates, and rectal temperatures, and increase in ability to walk in heat.

Robinson and Macfarlane (1958) exposed three healthy young subjects in a psychrometric room for four hours to a hot humid atmosphere (105°F., and 30 mm. Hg) and moderate exercise. A reduction in the urinary excretion of sodium within 30 minutes of heating was observed when half the amount of sweat (250 ml.) had been lost. They found the urinary K/Na ratio increased markedly during heat exposure, which actually is in accordance with the above study, if Na/K ratio was used instead of K/Na ratio.

In dogs, although Kanter (1954a) reported that renal regulation to heat differs in man and dog, yet from his report and previous studies, Kanter (1954b) showed that urinary sodium and potassium excretion in heat behave similarly in the sweating man and the panting dogs, but they differ in magnitude between the two species. With acute exposure to heat total output and concentration of Na in the urine fell progressively in dog and less striking in man. The urinary potassium concentration in dog might exceed 500 meq./1. while in man the latter did not increase as much as in dog. While man lost 3-4 percent of his body weight upon exposure to heat of 6 hours, the dog lost 10-12 percent of its body weight.

Rats, however, are different from man and dogs in the behaviour of their electrolyte metabolism upon exposure to heat. Meffered *et al.,* (1957), and Mefferd and Hale (1958) studied the effect of 3-months exposure to simulated altitude, cold, or hot environments on adult male rats. They showed that both the urinary sodium and potassium excretion were lower in heat than in cold while the urinary Na/K ratio was as the opposite. At 5°C., 25°C., and 34°C., the values were 19.3, 12.7, and 10.4 mg./day/kg.$^{3/4}$, respectively, for sodium; 156.0, 77.5, and 62.4 mg./day/kg.$^{3/4}$, respectively, for potassium; and 0.12, 0.16, and 0.17, respectively, for urinary Na/K ratio.

Not only are rats different from man and dogs in their response to heat regarding their electrolyte metabolism, but also sheep are different. Macfarlane *et al.* (1958) studied the effect of heat (41°C.) on urinary sodium and potassium excretion and the urinary Na/K ratio in man and sheep. They found, in man, that heat caused a reduction in the rates of sodium excretion to below 20 μ eq./min., and also a fall in the Na/K in the urine, while sheep reacted differently. A 4-hour heat exposure of the latter caused a progressive increase in the sodium excretion and in the urinary Na/K ratio. The excretion of potassium in sheep declined regularly from 200 to 50 μ eq./min. during the period of heating. When sheep were later cooled with drinking water, the increased sodium excretion ceased and there was a reversion to a low ration Na/K in the urine. This is in accordance with the results of Blaxter *et al.* (1959) who showed that urinary losses of potassium fell from 21.0 to 17.8 g./24 hr. when environmental temperature increased from 15° to 38°C.

In cattle Blincoe and Brody (1951) from their work in climatic laboratories

on the effect of environmental temperature (0° to 105°F.) on the blood composition of Holstein, Jersey, Brown Swiss, and Brahman cows, found no marked difference in plasma potassium (between 5° and 50°F.) and sodium due to temperature change. Although the plasma sodium tended to decline with increasing temperature they concluded that in cattle electrolytes are not significantly disturbed by temperature change. Another study on cattle was carried out by Goberdhan (1954). He studied the effect of three levels of temperature (45°, 70°, and 80°F.) and three levels of radiation (dim., ½ full, and full light) at each of the temperature levels on the plasma and urine major inorganic constituents of Holstein, Jersey, and Brahman cows. Plasma sodium and potassium showed no consistent trend with increasing environmental temperature. The urinary sodium excretion generally declined in dim and increased in full lights with increasing temperature, while urinary potassium dropped at 70°F. and increased at 80°F. in dim and full lights.

From the aforementioned studies, because of the few observations and the interactions between radiation, heat, and lactation, a definite conclusion in cattle cannot be obtained regarding sodium and potassium metabolism. It seems, however, from work on other species that environmental temperature exerts a definite influence on electrolyte metabolism. While in man, dog, and rat, sodium excretion in the urine is decreased markedly, in sheep urinary sodium excretion is increased at high environmental temperature and vice versa at cold temperature. In heat, urinary potassium excretion, on the other hand, increases in man and dog and decreases in rat and sheep. Also at high temperatures the urinary Na/K ratio decreases in man and dog and increases in rat and sheep, while the reverse is true in cold.

In order to understand the mechanism through which environmental temperatures induce changes in electrolyte metabolism in animals, a brief mention to the factors that affect the sodium and potassium metabolism, with particular reference to hormones and diet should be given. Other factors, if they have any effect on electrolyte metabolism, are believed to achieve their influence through hormonal effects.

Hormonal Influence on Plasma and Urine Na, K, and Na/K:

Mineralocorticoids: The association of the adrenal cortex with electrolyte metabolism has been known for many years (Clinton and Thorne, 1942). Deficiency in these hormones, such as in the case of adrenalectomy or in patients with Addison's disease, increases sodium excretion and decreases sodium content in blood plasma. Potassium excretion, however, decreases and its concentration in blood plasma is increased. This imbalance in sodium and potassium metabolism is due primairly to the deficiency or absence of the mineralocorticoids (Mach and Fabre, 1955; Simpson and Tait, 1955a; Swingle *et al.,* 1959; Ross *et al.,* 1959). Of these hormones, aldosterone was shown to play a predominant part, rather than any of the mineralocorticoids or glucocorticoids, in the prevention of the symptoms of adrenal insufficiency with regard to mineral metabolism (Simpson and Tait, 1955a).

Due to the highly potent effect of mineralocorticoids on sodium and potassium metabolism bio-assay methods used for determining their activity are based on measuring their effects on the radioactive or inert sodium/potassium urinary ratio of adrenalectomized rats. Such methods have revealed, as reviewed by Simpson and Tait (1955b), that aldosterone is 120 and 100 times as potent as deoxycorticosterone in decreasing the radioactive Na^{24}/K^{42} urinary ratio and the inert Na/K urinary ratio, respectively, in adrenalectomized rats. It is also 30, 25, and 5 times as potent as deoxycorticosterone in maintaining sodium and potassium balance in Addison's disease, in inducing sodium retention in adrenalectomized rats, and in producing potassium excretion in adrenalectomized rats, respectively.

Employing the new renal artery infusion technique and collecting the urine from each kidney individually Barger *et al.* (1958) studied the effect of aldosterone infused directly in the renal artery on electrolyte excretion in normal and adrenalectomized dogs. Intrarenal infusion of aldosterone produced a unilateral kaluresis in both normal and adrenalectomized dogs. However, antinatruresis was observed in the adrenalectomized and not in the normal dogs.

Swingle *et al.* (1959) showed that witholding desoxycorticosterone acetate in adrenalectomized dogs caused a fall in plasma sodium from 146.5 to 130.0 meq./1. and an increase in plasma potassium from 3.83 to 6.24 meq./1. typical to adrenal insufficiency. Later, administration of aldosterone increased the plasma sodium and decreased plasma potassium, but they were not restored to the initial levels.

The influence of mineralocorticoids on altering the levels of sodium and postassium is not only shown in the urine and blood plasma, but it also exists in sweat, saliva, red blood cells, and carcass tissues. Robinson and Robinson (1954) have reviewed the work done by many workers on the effect of desoxycorticosterone on decreasing the sodium excretion and Na/K ratio in sweat. Simpson and Tait (1955b) reported that 20 μg. aldosterone given both orally and intravenously in man depressed the salivary Na/K ratio to about 45 percent in 2.5 hours. Jones (1958) found that in suspensions of human erythrocytes incubated with glucose and desoxycorticosterone glucoside, potassium was diminished while sodium was increased. Carcasses of rats, on the other hand, were shown to contain significantly less potassium and more sodium than normal after treatment with desoxycorticosterone acetate (Knowlton and Loeb, 1957).

Sheep are no different than man, dogs, and rats, in their response to mineralcorticoids. Goding and Denton (1957) reported that adrenalectomized sheep, with unilateral parotid fistulae were given their ordinary diet and a daily dosage of 25 mg. cortisone and 5.10 mg. DOCA, and supplementary sodium intake adequate to replace the sodium lost in saliva, withdrawal of supplementary hormone intake caused severe adrenal insufficiency in 2-4 days. The plasma Na/K fell and there was a large increase in sodium loss in the urine and the feces. There was an external deficit of 136-489 meq. In sheep with permanent unilateral parotid fistulae, the salivary Na:K ratio rose. A striking feature of adrenal suf-

ficiency in the sheep was the diuresis occurring during the first 24-48 hours. The withdrawal of DOCA alone caused changes of a magnitude similar to withdrawal of both DOCA and cortisone. DOCA was the more critical component of the hormone supplement. Administration of an increased amount of DOCA to the animal caused a rise of plasma Na:K and decrease of sodium excretion in the urine and feces. In animals with parotid fistulae, the salivary Na:K ratio fell, and the salivary secretion rate rose by 20-50 percent.

Mechanism of action of mineralocorticoids on electrolyte metabolism: The primary role of mineralocorticoids in regulating the electrolyte levels in the body, as suggested from the current reports on this subject (Bartter *et al.*, 1958), seems to be chiefly confined to their effects on the kidney.

The different thoughts concerning the mechanism by which aldosterone regulates sodium and potassium excretion in urine have been reviewed recently by Lotspeich (1958). Sodium excretion by the mammalian kidney during varied physiological circumstances is regulated by changes in the glomerular filteration rate and/or by alteration in tubular reabsorption. However, the action of aldosterone on the renal tubules that interrelate sodium and potassium transport mechanisms is well accepted nowadays. Intra-arterial injection or direct injection into one renal artery of aldosterone in nine adrenalectomized dogs off replacement therapy for 48 hours, decreased sodium and increased potassium excretion and a rise in sodium and a fall in potassium reabsorption occurred. This was associated with no consistent change in glomerular filteration rate (Ganong and Mulrow, 1958). The absence of aldosterone effect on glomerular filteration rate is also confirmed by earlier work of Cole (1957).

The results obtained by Davidson *et al.* (1958) support the interpretation that reabsorption of filtered potassium is essentially complete, and that urinary potassium is derived from secreted potassium. The dependence of potassium excretion on sodium excretion provided evidence that the secretion of potassium occurs by an exchange of potassium for sodium in the distal tubule. The data of Pitts *et al.* (1958) is also consistent with this view. They reported that a mechanism located in the distal part of the nephron exchanges cellular hydrogen and/or potassium ions for sodium ions in the tubular urine. Berger *et al.* (1958), however, finding that aldosterone caused kaluresis and no demonstrable natruresis in normal dogs, and finding that in adrenalectomized dogs, aldosterone had two to threefold greater effect on sodium excretion as compared to potassium excretion, concluded that these results did not support the hypothesis of a simple cation exchange of sodium and potassium in the distal tubule, but rather to an independence of movement.

In attempt to localize the site of renal tubules (proximal or distal) that is affected by aldosterone, Vander *et al.* (1958) showed that following adrenalectomy, the distal tubule was not able to reduce the sodium concentration ion to the low value achieved during stop-flow in normal dogs. Following aldosterone administration this distal reabsorption capacity was restored. They reported that no conclusion, however, could be made regarding the effects of aldosterone on the proximal tubule.

The question now arises as to how aldosterone could favor this cellular potassium exchange of the distal cells for sodium ions of the tubular urine. That aldosterone favors the ionization of potassium within the tubular cells (Dustan *et al.,* 1956), or alters the permeability of the cells to potassium ions (Lotspeich, 1958) has been suggested.

Studies on the effect of aldosterone on potassium ion transport in an *in vitro* preparation and its effect on the volume of distribution of K^{42} *in vivo* in the nephroectomized animals have been suggested to be most useful at this point.

Glucocorticoids: Thorne *et al.* (1955) reported that corticoids such as corticosterone and dehydrocorticosterone exhibit, as anticipated from their chemical structure, qualitatively the electrolyte-regulating activity of desoxycorticosterone in addition to the carbohydrate-regulating activity of cortisone and hydrocortisone. The sodium retaining activity of corticosterone was found equal to 4 percent of that of desoxycorticosterone. However, corticosterone was reported to have similar potassium diuresis to that of desoxycorticosterone. The similarity in effect on electrolyte metabolism between corticosterone and mineralocorticoids was, however, attributed by Settzer and Clark (1958) to the conversion of the former to the latter *in vivo.* The last author observed that during infusion of corticosterone in normal subjects the urinary excretion of aldosterone increased; the 17-OH output decreased; and Na/K ratio fell.

The effects of the other more important glucocorticoids such as cortisone on electrolyte excretion was thought in the past to be inconsistent. Ingle (1950), in his review on the biologic properties of cortisone, concluded that although cortisone and 17-hydroxycorticosterone, in most instances, increase sodium and potassium excretion, the effect of these hormones on electrolyte balance was variable and no conclusive results were obtained regarding their effects on plasma sodium and potassium. In certain types of bioassay, cortisone and hydrocortisone cause sodium retention (Thorne *et al.,* 1955), while, in others, these steroids increased sodium excretion and may reduce the response to mineralocorticoids (Singer and Venning, 1953; Johnson *et al.,* 1957; Luetscher, 1956). In an attempt to provide a definite answer to this dilemma Streeten *et al.* (1955) injected a wide range of dosages of hydrocortisone in adrenalectomized rats and determined the urinary sodium excretion of these rats. They found that small doses of hydrocortisone (1 and 10 μg. per rat) produced retention of sodium, intermediate doses (50 to 500 μg. per rat) caused increased excretion of sodium, and very large doses (1 and 25 mg. per rat) resulted in retention of sodium. They concluded, therefore, that the contradictory results reported by others might then be reconciled as being due to differences in the amounts of hydrocortisone employed in the various experiments which fitted exactly on the dose response curve of their investigation.

On the basis of the above conclusion, the effect of glucocorticoids on the plasma levels of these electrolytes should be also variable. Mills and Thomas (1957) found that intravenous injection of seven healthy subjects with cortisone

had little effect on plasma potassium. Diamant and Guggenheim (1957), conversely, showed that, in normal rats, injection of cortisone increased plasma potassium and decreased plasma sodium. After adrenalectomy or cortisone withdrawal sodium retention increased (Martin and Walker, 1957). Barger *et al.* (1958) showed that intrarenal administration of 9- -fluorohydrocortisone in normal dog produced a unilateral kaluresis, but no demonstrable natruresis. In normal human subjects, oral administration of cortisone or intravenous injection of cortisole increased output of potassium without elevation of plasma concentration, and retention of sodium (Mills and Thomas, 1958). In force-fed intact rats Eversole and Romero (1958) showed that cortisone or hydrocortisone on their acetates and delta-1 analogus had slight, if any, effect on serum sodium and potassium, but augmented urinary excretion of sodium and potassium. Ross *et al.* (1959) showed that simultaneous infusion of hydrocortisone after aldosterone infusion in three patients with Addison's disease did not modify the effect of aldosterone on sodium excretion (sodium antidiuresis), but caused an increase in potassium diuresis. Swingle *et al.* (1957) reported excessive sodium, chloride, and potassium excretion occurred in 1-dehydrocortisone-treated fasted adrenalectomized dogs. On the other hand, Swingle *et al.* (1959) showed that injection of 2-methyl-9 -fluorohydrocortisone in fasted adrenalectomized dogs exhibited similar effects on electrolyte levels in plasma and urine to that of mineralocorticoids. The plasma sodium concentration increased, while the plasma potassium level decreased. The excretion of sodium in the urine was decreased, while that of potassium was increased.

Mechanism of action of glucocorticoids on electrolyte metabolism: Although the mechanism of glucocorticoids on protein metabolism has been carefully studied, as was reviewed previously in this literature, it is not as yet clear how they produce potassium diuresis and in most cases sodium diuresis. Their site of action is clear, however, not to be the kidney, but probably in the somatic tissues. Swingle *et al.* (1957) studying the effect of gluco- and mineralocorticoids on fluid and electrolytes of fasted adrenalectomized dogs attributed the renal loss of sodium, chloride, and potassium in 1-dehydrocortisone-treated dogs, differently from mineralocorticoids' effect that caused kaluresis and antinatruresis, to an outflux of these electrolytes and water from cells including bone and collagenous tissue. Lipsett and Pearson (1958) found that continued administration of cortisone induced a negative sodium balances in adrenalectomized humans when they were on sodium-restriction diet. In many instances negative potassium balances occurred.

The glucocorticoids have a different site of action from that of mineralocorticoids is illustrated by the fact that both induce kaluresis, and yet they antagonize each other. Dasgupta and Giroud (1958) showed that after short treatment of nephritic rats with cortisone acetate, the high aldosterone secretion was reverted to normal. Swingle *et al.* (1959) from his results concluded that the adrenal glucocorticoids were assumed to be an essential part of the mechanism concerned with active transport of salt and water across cell membranes.

Reinberg and Stolkowski (1957) studied the mode of action of some corticosteroids on cellular potassium in isolated hearts and intestinal loops. They reported that the displacement of cellular potassium under the influence of corticosteroids could result from disruption of acid-base equlibrium. A competition could be set up between the hydrogen and potassium ions of the cell. Elevation of extracellular pH prevented the effect of corticosteroids on cellular potassium and caused disappearance of the changes that those hormones induced on the spontaneous activity of isolated contractile organs. If this is true *in vivo,* one expects the typical alteration in electrolyte output of respiratory alkalosis, described by Barker *et al.* (1957) or Dale and Brody (1954), would occur in the animal in spite of the assumed high glucocorticoids secretion under such circumstances.

Other hormones: Other hormones are known to have more or less influence on the electrolyte metabolism. These hormones such as thyroxine, testesterone, estrogens, and antidiuretic hormones appear unlikely to have an important role in regulating the electrolyte levels in the body as compared to the adrenalcorticoids (Strauss, 1957; Thorne, 1958; Barger *et al.,* 1958). These authors reported that antidiuretic hormones have no effect on electrolyte excretion in the urine, though due to its effect on water excretion, the concentration and not the excretion of the salts indirectly is altered.

Dietary Influence on Plasma and Urinary Na, K, and Na/K: Mineralocorticoids and particularly aldosterone are autonomous hormones. Differently from glucocorticoids they are not regulated by the pituitary adrenocorticotrophin (Simpson and Tait, 1955; Rosenfeld *et al.,* 1956; Eisenstein and Hartroft, 1957). The physiological mechanisms controlling the release of aldosterone are not completely understood (Luetscher, 1956b). Many different theories, however, have been suggested in this concern (Luetscher, 1956a; Hollander and Judson, 1957; and Sayers *et al.,* 1958). Decreasing the ratio of sodium to potassium perfused into the isolated calf adrenal glands from 42/1 to 5/1 caused a significantly augmented output of aldosterone-like material with no significant change in glucocorticoids production. Growth hormone had no effect on aldosterone production, and the stimulative effect of ACTH was unequivocally demonstrated (Rosenfeld *et al.,* 1956).

On this basis, one expects that if the animal is depleted of one or another of these electrolytes or if the ratio between sodium and potassium in the feed is changed, the aldosterone secretion, and consequently the levels of sodium and potassium in the urine and probably in the plasma, would be altered. An inverse relationship has previously been shown between sodium intake and aldosterone excretion (Luetscher and Johnson, 1954; Luetscher and Axelrad, 1954). A low level of potassium in the diet, on the other hand, was shown to decrease the aldosterone content of adrenal venous blood in rats (Singer and Stack-Dunne, 1954), while sodium or potassium loading in man is associated with decline or rise, respectively, in aldosterone secretion (Luetscher and Curtis, 1955; Miller *et al.,* 1958). The output of aldosterone was directly related to the K/Na ratio in urine and inversely related to the sodium excretion. A negative sodium bal-

ance and increase potassium balance and increase potassium retention were observed in children on a caloric-poor diets (Frischauf and Zweymuller, 1958). Potassium excretion was directly related to the amount of potassium intake in adrenalectomized patients who were on normal sodium diet and treated with cortisone (Lipsett and Pearson, 1958). Johnson *et al.* (1957) studied the effect of altering the ratio of sodium to potassium in the diet on aldosterone secretion and potassium to sodium ratio in three healthy men. They observed that when potassium intake was very low (9 meq./day) and sodium intake was high (145 meq-/day), the aldosterone output reached such low level as not to be detectable with certainty. When sodium intake was also reduced (9 meq./day) the aldosterone output rose to levels 2 to 6 times the original control values after about 7 days. Restoration of potassium to the diet (99 meq./day) during continued low sodium intake was followed by a further increase in aldosterone output in these subjects. Glucocorticoids, however, were not affected by altering the ratio of sodium to potassium in the diet. A direct relationship was apparent between the Na/K ratio in the urine with aldosterone output.

Eisenstein and Hartroft (1957) finding that aldosterone was increased by the adrenals of sodium-deficient rats, but production of corticosterone and other steroids was reduced, suggested that sodium deficiency might influence the synthetic pathways concerning with elaboration of adrenocortical hormones through factors other than ACTH.

Effect of Environmental Temperature on Water Metabolism:

Water Vaporization: It is well-known that water balance is maintained in the body by regulation of water gain and water loss. Sources of water gain are, liquid consumption, water content of food intake, and metabolic water which results from nutrients oxidation. Water loss is carried out through, skin and respiratory vaporization, urine, feces, milk, salivation, tears, and nasal tract secretion. However, fairly constant water content in the body is established by virtue of a harmoneous relationship between water intake, water vaporization (sweat, skin vaporization, and respiratory vaporization), and urinary water output which are the main variables of water balance. With increasing environmental temperature above the comfort zone the animals dissipate the greatest part of their heat load through the most efficient system that is the evaporative cooling. The importance of the evaporative cooling system in dissipating heat at high temperature is due to the high thermal capacity of water (about 1 Cal./g./°C. at 30°C.) and to its high heat of vaporization (about 580 Calories per kg.). Also, at high environmental temperature, when the temperature gradient between the body surface and the surrounding environment decreases, and the non-evaporative cooling becomes relatively inefficient, evaporative cooling renders practically the only way of ridding the animal from the heat load imposed on it. Worstell and Brody (1953) showed that at 100°F. about 90, 110, and 150 percent of the total heat production in respectively European cattle, Indian cattle, and man, are dissipated through water vaporization.

In most species, total water vaporization increases with increasing temperature. Robinson and Robinson (1954) in his review on the chemical composition of sweat showed that, in man, total vaporization increased from 0.1 to 1.0 kg. per hour upon increasing the air temperatures from 18° to 50°C. Johnson (1956) showed that in rabbits both respiratory and skin vaporization increased with rising environmental temperature in agreement with other studies on rats and rabbits. In cattle, Kibler and Brody (1950), and Kibler and Yeck (1959) obtained similar results. However, they showed that when environmental temperature increased above 80°F. the evaporation rate of Indian cattle continued to rise whereas, that of Shorthorn showed little or no change. The heat loss through respiratory vaporization may reach ⅓ of the heat loss through body surface. Cattle show a steep rise in evaporative cooling at a relatively lower environmental temperature (50°F.) than other species such as man and rabbits (85°F.).

Hayman and Nay (1958) confirmed the earlier work of Findlay and Yang (1950) and observed a seasonal variation in sweat gland volume in Jersey and Zebu x Jersey cattle. They also suggested that there was a relationship between sweat gland volume and activity and that the seasonal changes observed in the sweat glands volume were largely due to changes in ambient temperature. Recently Taneja (1959) was able to detect sweat droplets by preparing sweat prints. The distribution of sweat spots on the sweat prints was similar to that of sweat glands. However, that the main route of skin vaporization in cattle is through the sweat glands, which depends on a complicated physiological regulatory mechanism, rather than through a simple physical osmotic or diffusion mechanism (McDowell, 1958) still needs to be confirmed by more investigation.

Water Consumption: The importance of water in the management of cattle is clearly understood (French, 1956; Campbell, 1958). As many factors are known to affect water consumption of cattle, such as ambient temperature, age, body size, level of feed intake, butterfat test, and daily milk yield, Winchester and Morris (1956) worked out tables that take most of these factors into account. Ambient temperature is the most important of those facors.

Most investigators have reported that water consumption in various species increase with increasing environmental temperature above the thermoneutral zone. In man, Adolph (1947) showed that when air temperature increases from 70° to 120°F. the water requirements increases from 2 to 14 liters per day per man. Mefferd *et al.* (1957) showed that in fasting or nonfasting rats water consumption was higher in heat (34°C.) than in control (25°C.) treatments. In rabbits, Johnson (1956) reported a decline in water consumption with increasing ambient temperature. In sheep, Macfarlane *et al.* (1958) showed that water consumption *on the average* was twelve times as much as in winter. Blaxter *et al.* (1959) were in agreement with this conclusion showing water consumption in sheep at 38°C. was four times (approximately) as high as that at 8°C. Peirce (1957) reported only 50-70 percent higher water consumption in sheep in the hottest months than in the coldest months. In goats, water consumption increased from 2.3 to 4.0 liters per goat per day when ambient temperature in-

creased from 10° to 40°C. (Appleman and Delouche, 1958). In cattle, Harbin *et al.* (1958) studied the effect of natural combinations of ambient temperature and relative humidity on the water intake of lactating and non-lactating dairy cows. They showed a statistically significant positive correlation between temperature and water consumption and nonsignificant negative correlation between relative humidity and water consumption when temperature was held constant. They developed simple regression equations for lactating and nonlactating cows as, respectively, $W = 11.9 + 0.33\ T$, and $W = 11.6 + 0.29\ T$ where W is the water consumption per 100 lb. live weight and T is the average of the daily maximum and minimum temperatures. In climatic laobratory studies, Johnson *et al.* (1958) showed that the Shorthorn, Brahman, and Santa Gertrudis, which were reared at 50°F. or 80°F. increased their water consumption with increasing environmental temperatures from 65° to 105°F. This increase in water consumption occurred regardless of the consistent decline in feed consumption showing a greater influence of heat than feed on water intake. Ragsdale *et al.* (1950, 1951), on the other hand, found that with rising temperatures from 50° to 105°F. water consumption slightly increased or remained fairly constant up to 80°F. From 80° to 105°F., certain cows showed increase, particularly the dry cows, while others showed decrease in water consumption which accompanied marked drop in milk production.

The fall in environmental temperature below the comfort zone is also accompanied with increase in water consumption, but of less magnitude than that at environmental temperatures above the zone of thermoneutrality. MacDonald and Bell (1958) studying the effect of low fluctuating temperatures on water intake of lactaing Holstein-Freisian cows showed that as daily minimum air temperature decreased from 38° to 0°F. water intake increased significantly. The increases were significantly concomitant with increased foodstuffs intake. Similar conclusion was also reached in rats. In nonfasting rats, water intake was 120, 80, and 118 ml./day/kg.$^{3/4}$ at cold (5°C.), control (25°C.) and heat 34°C.), respectively. When deprived of food, water consumption decreased at cold and increased at high temperature. The values for the fasting rats were 9, 46, and 99 ml./day/kg.$^{3/4}$ at cold, control, and heat, respectively, (Mefferd *et al.*, 1958).

Mechanisms involved in thirst stimulation by environmental temperature: From the above results cold seems to stimulate thirst probably because of the high feed consumption that withdraw a great amount of body fluids for the formation of digestive juices. This is illustrated by the increase in water consumption in cold when the rats or cattle were fed *ad libitum.* When food was restricted at cold temperatures, water consumption also decreased.

Conversely, at high temperatures though food intake is depressed or even if food is deprived completely, such as in the case of fasting rats, water consumption is directly related to environmental temperature. This demonstrates a more pronounced effect of heat rather than feed deprivation effect on water intake. An interesting illustration of this conclusion is shown from the data of Blaxter *et al.* (1959). They showed that the ratio of water intake to dry matter intake (l./kg.)

at low temperatures (8° or 18°C.) was higher when feeding level was high than otherwise when the latter was low. At 18°C., the ratios were 1.83 and 1.22 on high and low feeding levels, respectively. However, at a high temperature (38°C.) quite the reverse was true. The ratio increased markedly and was much higher with low feeding level than with otherwise high feeding level. The values were 17.5 and 4.81 on low and high feeding levels, respectively. How heat could induce such an overwhelming effect is still a debate among investigators.

Thirst may be stimulated by many different factors such as body water deficit, concentration of the intracellular body fluid, sodium depletion, extracellular fluid deficiency, food ingestion, or hypertonicia of the extracellular fluid. Thirst may also be stimulated by hypothalamus injury, endocrine imbalance and nervous stimulation. Extensive studies and discussion on the subject have been given (Adolph, 1947; Andersson, 1952; Andersson and McCann, 1955; Wolf, 1956; Strauss, 1957).

It is iteresting that most of these factors, though different in nature, seem to play their role in stimulating thirst through decreasing the water content in the body especially the intracellular water. For example, increasing the osmotic pressure of both extracellular and intracellular fluids by ingestion of hypertonic urea, which diffuse in both fluids, in man did not stimulate thirst until diuresis ensued was resultant reduction in body water (Strauss, 1957). However, infusion of hypertonic solutions of sodium chloride, sulphate or acetate which remain extracellular and hence lead to the movement of water out of the cells and increase in the intracellular fluid concentration to a great extent, was found to stimulate thirst and increase water consumption much more than what occurred in urea treatment (Holmes and Gregersen, 1950). Stimulation of thirst by sodium depletion in dogs is probably due to the excretion of large volume of urine and water deficit (Holmes and Cizek, 1951; Cizek *et al.,* 1951). The large amount of water consumed in diabetes insipidus is mainly due to the great loss of body water in urine. Kamal *et al.* (1959) showed that water intake in cattle at low or high temperature was blocked when urine excretion was inhibited by ADH administration. Stimulation of thirst by food ingestion seems to be a temporary dehydration due to the withdrawal of body fluids for the formation of digestive juices. All these experiments, though of different nature, prove one thing, that is thirst and drinking is stimulated by water deficit in the body fluids.

The occurrence of osmoreceptors, thirst or drinking center in the hypothalamus is now well accepted since Andersson (1952) and Andersson and McCann (1955) showed experimentally that by electric stimulation or increasing the tonicity of a certain area in the hypothalamus of goat stimulate thirst within few seconds of the treatment.

Urine Volume and Urine Specific Gravity: At high environmental temperatures, when water loss through sweat and/or vaporization is markedly intensified, the kidneys play a big role in regulating the water content in the body. The capacity of the kidney in this concern varies to a great extent among species. Standing in the order of their relative capacities to concentrate the urine from

least to most are: man, dog, rat, kangaroo, rat or camel (Lotspeich, 1958; Schmidt-Nielsen, 1959). Cattle excrete greater amounts of urine than other mammals, mainly because they consume large volumes of water in association with their great intake of roughages, and do not sweat as profusely as other species. The horse, however, consumes about the same amount of water as the cow and yet excretes ⅓ as much urine. Dukes (1957), from different sources, reported that the urine volumes of cow, horse, pig, sheep, dog, and man are 14.2, 4.7, 4, 1, 1, and 1 liters per day, respectively, while the specific gravity for the same species are in order 1.032, 1.040, 1.012, 1.030, 1.025, and 1.020.

In cattle, although the changes in water consumption and vaporization under varying environmental temperature were studied fairly comprehensively, the part played by the kidney in water balance under such circumstances has not as yet been investigated. No information on urine specific gravity of cattle has also been reported in this regard. The only information regarding urine volume in this regard was reported in the unpublished data of Goberdhan (1956). Although different treatments of radiation, temperature, humidity, and lactation were involved in his study, one can observe from his data higher values of urine volume in heat than under control temperature (45°F.), especially in Jersey and Zebu cattle.

In sheep, the available data on urine volume changes with temperature are contradictory. Macfarlane *et al.* (1958a) in Australia reported that urine volume was lower in summer than winter months in spite of higher water consumption and lower feed consumption in the former season than in the latter. Blaxter *et al.* (1959) in Scotland, on the other hand, showed that the urine volume of sheep increased at high temperatures and in most instances exceeded the volumes of water lost as vapour especially when the sheep were in a low feeding levels. The urine volume at 38°C. was 8 1./24 hr. while that at 8°C. was only 1 1./24 hr.

In man and sheep Macfarlane *et al.* (1958b) showed that 4 hour exposure to heat (41°C.) caused marked decline in urine flow. Kellman and Weiner (1953) carried out eight experiments on two nude males exposed to heat (100° F.) for 90 minutes. They showed that during the first 45 minutes of heat exposure urine flow (10.14 ml./min.) was not much different from that of control (12.57 ml./min.). In the subsequent 45 minutes, however, the urinary flow (1.89 ml./min.) in the heat was reduced to a level much below that of control (6.08 ml./min.). The much reduced urine flow in the second half of the heat exposure was associated with a mild, but progressively increasing water deficit. The urinary flow was reduced still further (0.80 ml./min.) after leaving the hot room compared with that of the control (2.76 ml./min.).

These results were in accordance with Bader *et al.* (1952) who also showed that urine specific gravity of nude male subjects were lower at cold (60°F.) than at high (80°F.) temperatures. The increase in specific gravity of urine at high temperature in man was also confirmed by Adolph (1947). This is, however, expected since the urine metabolites evidently concentrate when water is withheld

by the kidneys in man under heat. In ruminants, urea, which is the main component that contributes to the specific gravity of urine, is utilized from the blood by the rumen microorganisms under poor feeding conditions, such as the case in hot climate, and, thus, its concentration in the urine, as well as the specific gravity of the latter, evidently drop. The ability of sheep and camels to adjust urea excretion to changing protein intake was shown by Schmidt-Nielsen (1957b). No information on urine specific gravity or urea concentration in cattle under changing climatic conditions is available to be compared with the above mentioned phenomenon in sheep or camels.

In rats both extremes of environmental temperature seem to increase urine volume. Katsh *et al.* (1954) exposing rats to cold (2-4°C.) for 28 days, showed a gradual increase in urine volume reaching 180 percent higher than control level at the 10th day. The urine volume dropped thereafter, but still was higher than control level. Meffered *et al.* (1957) showed that urine volume of rats in cold (5°C.) was slightly higher than that of rats in control temperature (25°C.), while urine volume of rats kept at 34°C. had much larger volume (46 ml./day/kg.$^{3/4}$) than that of control rats (35 ml./day/kg.$^{3/4}$). Dogs, on the other hand, decrease their urine volume at low temperature (1.8 to 4°C.) as shown by Nungesser (1955).

Mechanisms Involved in changing the urine volume and specific gravity under thermal stress: Glomerular Filteration: The regulation of urine volume is known to be under the control of glomerular filteration and renal tubular reabsorption processes. Under certain conditions both mechanism may coincide with or counteract each other and thus result in an increase or decrease in urine volume. However, it seems that water concentration in urine and thus the urine volume is mainly regulated under normal conditions by renal reabsorption rather than glomerular filteration.

Upon exposure to cold or heat, most species respond by vasoconstriction or vasodilation, respectively. In the former, less blood is shunted to the peripheral surface to conserve heat, while in the latter more blood is transferred to the outer surface of the body in the skin to dissipate heat by conduction. Consequently, in cold, more blood reaches the kidney and is thus filtered than in heat. In this concern Smith *et al.* (1952) showed that in men both the glomerular filteration and renal plasma flow were lower at high environmental temperature (50°C.) than at normal temperature (20°C.). This is in accordance with the earlier work of Radigan and Robinson (1949). However, Bader *et al.* (1952) showed that there is no important changes in effective renal plasma flow, glomerular filteration fraction when men were exposed to cold (65°F.) or warm (80°F.). However, the increase in urine volume in cold was associated with decrease in renal tubular reabsorption of water.

In sheep, McDonald and Macfarlane (1958), on the other hand, showed that glomerular filteration rate increased in summer reaching twice the rate found in winter, but there was no change in the glomerular filteration rate during the 4 hours acute heating (43-44°C.). Renal plasma flow showed no variation with

season, nor with exposure to heat. It is worth noting that, in spite of the higher glomerular filteration rate in summer than in winter in the above experiment, the same authors (Macfarlane *et al.,* 1958a, 1958b) reported that sheep had lower urine volume in summer or heat treatment than in winter or comfort temperature which was not in agreement with the work of Blaxter *et al.* (1959). It seems, thus, that sheep, particularly in this experiment, had very high tubular reabsorption capacity that reduced the urine volume in spite of the increase of glomerular filteration rate that took place in summer.

Tubular Reabsorption and Hormonal Effects: It has been mentioned before, however, that under normal conditions the rate of tubular reabsorption has a dominant effect on the regulation of water concentration in urine (urine volume) rather than on glomerular filteration. Water reabsorption in the renal tubules is mainly regulated by hormonal mechanism (Lotspeich, 1958) rather than by just autoregulation (Pappenheimer and Kinter, 1956). The following hormones are the most potent ones that are known to affect urine volume. The mechanism involved in such effect is still controversy.

(a) *Antidiuretic Hormone;* The fact that injection of antidiuretic hormone in mammals causes a decrease in urine volume is well established. This has been shown in man (Leaf *et al.,* 1953), in rats (Itoh, 1954), in dogs (Barger *et al.,* 1958), and in cattle (Kamal *et al.,* 1959).

The definite action of the hormone on urine volume was used as basis for the methods that assess antidiuretic activity in biological extracts. Chalmers and Lewis (1951) have established a quantitative direct linear relationship between the logarithm of a single intravenous doses of pitressin and the duration of response. Although it is now conclusive that the site of action of antidiuretic hormone is the renal tubules and more precisely the distal tubules, yet the mechanism by which the hormone causes antidiuresis in mammals is still controversy.

With the application of the new technique of catheterizing one renal artery and collecting urine from each kidney individually Barger *et al.* (1958) infused antidiuretic hormone in the left renal artery of the dog for 8 minutes and observed a decrease in urine flow from 40 to 60 percent only in the left kidney, while there was no effect on the other kidney. This experiment indicated that the hormone acts specifically on the renal tubules. However, there is no direct evidence for the mechanism of action of antidiuretic hormone in the mammalian nephron. That antidiuretic hormone changes the permeability of the distal tubular epithelium to water by opening pores and thus making a water permeable out of water impermeable membrance (Sawyer, 1957) is still a hypothesis based on work done on frogs and thus needs to be confirmed by work on higher mammals.

An extensive review about the physiological, biochemical characteristics of antidiuretic hormone and on its mechanism of action has been given by Strauss (1957), Lotspeich (1958) and Thorn (1958).

(b) *Glucocorticoids;* Ingle (1950), in his review on the biologic properties of cortisone, reported that the latter is very potent in stimulating diuresis. This conclusion was later confirmed by other work. In man. Luft *et al.* (1955) showed that cortisone administration in hypophysectomized patients increased the daily urine volume markedly amounting to ten liters in certain cases. The increase was parallel to an elevation of the daily urinary solute load as well as a lowering of the specific gravity from a level of 1.015 down to 1.005. Similar results of cortisone on diuresis were obtained in dogs (Montastruc, 1954) and in rats (Sala and Luetscher, 1954).

Raisz *et al.* (1957), showed that prolonged oral or intramuscular administration of large doses of cortisone or hydrocortisone increased the maximal rate of water diuresis in man. The increase consisted chiefly of an increase in free-water clearance. There was a small rise in glomerular filteration rate which did not parallel the increase in urine flow. A single intravenous infusion of hydrocortisone regularly produced an increase in glomerular filteration rate, but without a consistent increase in the diuretic response to water loading. They reported that their results indicated that the continued administration of steroid in large doses influenced the renal tubular reabsorption of water. They suggested that more free water was made available for excretion because of a redistribution of solute reabsorption between proximal and distal systems or because an alteration in the permeability of the renal tubular epithelium to water. The hydrocortisone administration thus, beside causing an abrupt increase in diuresis, also caused a change from a considerably hypertonic to a markedly hypotonic urine.

Swingle *et al.* (1957) reported from his study that 1-dehydrocortisone induced rapid revival of fasted adrenalectomized dogs from severe insufficiency. The disappearance of symptoms and return of activity and vigor were accompanied by restoration of normal values of arterial pressure and serum electrolytes. They also reported that hemodilution was evident and a profuse diuresis occurred with marked renal loss of sodium, chloride, potassium, and fluid. It was concluded that in glucocorticoid-treated dogs the fluid and electrolyte changes were presumed to be due to an outflux of sodium, chloride, potassium, and water from cells, including probably bone and collagenous tissue.

Kleeman *et al.* (1958), on the other hand, believed that diuretic effect of glucocorticoids is partially connected with renal tubular reabsorption. They compared the effect of aminophillin, which increases the glomerular filteration and alter the tubular reabsorption, with that of hydrocortisone in patients suffering from impaired water excretion. Their results indicated that the marked improvement in the excretion of water which followed the administration of hydrocortisone cannot be completely explained by improved renal hemodynamics or alteration of solute reabsorption. The data, however, supported the view that compound F-like steroids may, in the absence of antidiuretic hormone, specifically inhibit the back diffusion of water in the diluting segments of the nephron (loop of Henle and distal convulted tubule) or prevent the reabsorption or back diffusion of water in the concentrating segment.

(c) *Mineralocorticoids;* Although the mineralocorticoids, as have been mentioned before, are by far the most potent hormones in regulating the electrolyte metabolism, yet their effects upon water metabolism is variable depending on the type of hormone administered. Desoxycorticosterone acetate administration is known to induce water retention associated with hypertention in man as consequence to salt retention by the kidney (Thorn *et al.*, 1955). Aldosterone, conversely, does not cause pathological retention of water when administered, in Addisonians, or improve the water load test (Mach and Fabre, 1955). A good comparison between the effects of these two hormones on urine volume was presented by Sala and Luetscher (1954). They injected adrenalectomized rats with desoxycorticosterone acetate and with aldosterone and determined their effects on urine volume. They found that desoxycorticosterone acetate decreased the urine volume from 8.0 to 6.7 μ1./min., while aldosterone administration caused an increase of urine volume from 7.8 to 8.2 μ1./min., although it increased sodium retention markedly. Gross (1955), on the other hand, observed no effect of aldosterone on urine volume in dogs. This was confirmed by Barger *et al.* (1958) who also showed that intrarenal infusion of aldosterone in normal dogs produced no effect on water excretion.

In disagreement with the above results Cole (1957) administered aldosterone in unanesthetized rats which were infused with saline. He observed that the water loss in urine due to saline infusion was reduced after aldosterone administration. However, he attributed this observation as a secondary effect of the sodium retention.

In an adrenalectomized sheep, Goding and Denton (1957) showed that they could maintain these sheep indefinitely in good condition and with constant weight if they were given their ordinary diet and a daily dosage of 25 mg. DOCA. However, withdrawal of the supplementary hormone intake caused severe adrenal insufficiency. The striking feature of adrenal insufficiency observed in sheep was the severe diuresis that occurred during the first 24-48 hours. The withdrawal of DOCA alone caused changes of a magnitude similar to withdrawal of both DOCA and cortisone.

(d) *Other Factors;* With change in environmental temperature displacements in the mechanism of homeostasis occur in the body as described previously. These displacements including changes of blood volume, extracellular fluid, osmotic pressure may have influence on urine volume. However, it is believed that they would exert their effects through inhibiting or stimulating one or another of those hormones mentioned above. Also other hormones which have some influence on urine volume such as thyroxine, estrogens, growth hormone and adrenaline which were excluded in this review are also believed to have less importance than those which have been discussed above in regard with urine volume changes.

Effect of Environmental Temperature on Protein Metabolism:

According to Selye's Theory of Stress (1950-1956), heat and cold though

are different in nature, yet, as systemic stressors, they induce in man and in experimental animals a particular group of reactions which are the elements of the so called General Adaptation Syndrome (G-A-S). Protein catabolism results in an increase in urinary nitrogen excretion, and under severe stress leads to a negative nitrogen balance, which is one of the main manifestations of the G-A-S. The glucocorticoids which increase the nitrogen excretion are not indispensible for such protein catabolism or for the performance of stress (Selye, 1950). Protein catabolism occurs in thermal stress whether the animals are intact, thyroidectomized, or adrenalectomized (You *et al.,* 1950). In fact, glucocorticoids when secreted in optimal amounts enhance anabolism and only when secreted in toxic amounts do they favor protein catabolism.

Lathe and Peters (1949) showed that exposure of rats to a moderate degree of cold caused an increase in the excretion of urinary nitrogen and presumably increased protein catabolism, with simultaneous loss in body weight. You *et al.* (1950) studied the effect of cold exposure on the urinary nitrogen excretion of rats. They showed that cold environment (1.5°C.) caused the rats to excrete 77 percent more nitrogen in the urine than that at normal room temperature (25° C.). When the food intake in the cold was doubled the urinary nitrogen was increased further to about 2.3 times its original value. In body thyroidectomized and DCA-injected adrenalectomized rats marked increase in urinary nitrogen was observed after cold exposure. The authors thus concluded that the increased protein catabolism at cold temperature is not primarily due to the action of the thyroid or adrenal glands. The presence of the glands, however, augmented the increase and played a part in the full response seen in the normal animals. The reason for the increase in protein catabolism in adrenalectomized rats at cold temperature, was not understood.

In heat stress a similar conclusion to that mentioned above was reached by Bass *et al.* (1955). They studied the nitrogen excretion in urine and sweat of five men who were living in climatic chamber for 14 days under hot environment (100°-120°F.). The level of urinary nitrogen excretion during the heating period was markedly high, which resulted in a negative nitrogen balance of about −2.0 g./man/day that lasted throughout the heating period. The urinary 17-ketosteroids, however, decreased during the heating period indicating a decrease in glucocorticoids production in man under heat stress. No marked changes were observed in either eosinophil or total leukocyte counts.

Earlier work of Dill *et al.* (1933) showed also negative nitrogen balance in normal individuals exposed to natural heat for twenty days. The average of total nitrogen deficiency was 1.6 g. of nitrogen per day.

Conn (1949) discussing the mechanism of heat acclimatization in man reported that exposure of man to heat caused great loss of nitrogen in urine, which resulted in a severe negative nitrogen balance of about −1.5 to −3 g. per day. The period of negative nitrogen balance began with the initial day of work in the heat; and gradually declined until the fourth week of heat exposure at which time nitrogen equilibrium was restored. This negative nitrogen balance was in-

dependent of the nitrogen intake, occurring at high as well as at medium levels of protein feeding. When salt intake was restricted after nitrogen equilibrium was attained, a negative nitrogen balance again was established. The author also showed that administration of desoxycorticosterone acetate (DCA) decreased the nitrogen loss in urine and restored the nitrogen balance probably through depressing the adrenocorticotropic activity. Salts were also conserved and their excretion in urine and sweat was markedly depressed after DCA administration.

Under heat stress in man the nitrogen excretion does not only increase in urine, but also its concentration and quantity is remarkably increased in sweat. Mitchell and Hamilton (1949) showed that, under conditions of profuse as compared to minimal sweating, the concentration of nitrogen in sweat as well as the total losses of nitrogen in sweat were increased. Such losses amounted to ten times as much as that under minimal sweating. The concentration of nitrogen in sweat was not dependent on nitrogen intake. These results, however, were confirmed later by Bass *et al.* (1955) who also showed that with heat acclimation the high concentration of nitrogen in thermal sweat was decreased.

In rats, on the other hand, opposite results to those on man were obtained by Mefferd *et al.* (1957) and Mefferd and Hale (1958). These authors studied the effect of 3 months exposure to simulated altitude, cold or heat, on many metabolic characteristics of adult male rats. They showed that urea excretion in urine, which constitutes about 80 percent of the total urinary nitrogen was at a high level (1820 mg./day/kg.$^{3/4}$) in cold (5°C.). At control temperature (25°C.) the urea excretion dropped to 1130 mg./day/kg.$^{3/4}$. In heat (34°C.), the values were much lower (976 mg./day/kg.$^{3/4}$). It was observed that the decline of urea excretion at high temperatures was closely correlated with the decline in food intake.

Graham *et al.* (1959) studied the effect of ambient temperature on urine energy changes which actually represent the changes of urinary nitrogen because both are highly correlated (Elliot and Loosli, 1959) and also because urine nitrogen is almost the only source of energy in the urine. They exposed two adult wethers for 7 days at a time to each of seven thermal environments ranging from 8° to 38°C. The experiment was undertaken at high, medium, and low feeding levels. There was a statistical significant decline of about 80 percent in the urinary energy of sheep on low feeding level when the environmental temperature gradually increased from 8° to 38°C., while in the other feeding trials the decline was insignificant. The decline in urine energy with increasing temperature is, however, assumed to be associated with the decline in feed consumption in heat, though not mentioned by the author.

In cattle, no information whatsoever is available concerning the effect of environmental temperature on nitrogen excretion or retention. This fact is surprising, especially when it is realized that protein metabolism is by far the most important factor related to growth, production and reproduction in mammals and in livestock in particular. It is believed that such conflict in the aforementioned results between man and animals, under heat conditions, is mainly due to the

difference in feeding level between man and the animals (rats or sheep) under heat stress. When appetite is depressed in hot climate, animals decrease their feed consumption, while man can easily stimulate his appetite by many agents and thus maintain a fairly normal food intake. The effect of diet on nitrogen excretion will be discussed in this section.

However, it is also believed that any change in protein metabolism during heat stress would result from the effect of hormones and feeding levels rather than the catabolic effect of stress as such. It has already been mentioned in the beginning of this chapter that temperature has a definite effect on hormonal secretion as well as on feed intake in all species of mammals so far studied. In the following review, a few examples are reported showing the significance of diet as well as hormones on protein metabolism.

Dietary Effects: The effect of feed levels and composition has been early investigated by many workers. Armsby (1903) reviewed the classical work carried out by earlier investigators on different species of animals. He reported that when food is withheld from a well-nourished animal, the nitrogen excretion diminished rapidly at first and more slowly later, until within a few days it reaches minimum value which may then remain nearly unchanged for a considerable time. This was also reported by Brody (1945).

Morrison (1956) studied the effect of low feed intake on urinary nitrogen excretion and nitrogen balance in pregnant rats. He showed that with fall in food intake there was a rapid decrease in urinary nitrogen excretion. The nitrogen balance, however, remained positive even at about 65 percent fall in food intake. Mefferd and Hale (1957) found a high correlation between food intake and urea excretion in rats urine at different environmental temperatures.

Thorn *et al.* (1955) reported that in normal fasting man (12 hours) urinary nitrogen excretion declined even after hydrocortisone was continuously administered for the rest of the fasting period. This shows that the effect of nutrients depletion may overweigh the opposing effect, glucocorticoids on protein metabolism, and thus causes a decrease in nitrogen excretion. This is assumed to be true for short time fasting when the animal still has enough storage of energy sources other than protein. Upon prolonged starvation, however, when the animal is depleted from its storage of glycogen and fat, the nitrogen excretion would eventually increase. This is because body protein would be catabolized in order to provide the body with its demand of energy.

Calloway and Spector (1955) showed that restriction of 50 percent of caloric intake in young adult rats on a constant intake of 160 mg. of nitrogen resulted in negative nitrogen balance and loss in body weight. Birnbaum *et al.* (1957) showed that when nitrogen was depleted from the water-soluble diet which was fed to rats, the urinary nitrogen excretion of the animals dropped gradually until it reached a constant level. The averages were 50.4, 39.2, and 36.8 mg., total nitrogen per day on the 7th, 14th, and 20th days, respectively. As should be expected, the animals during such periods had negative nitrogen balance.

Hormonal Effects:

(a) Adrenal Cortical Hormones; The catabolic effect of glucocorticoids on body proteins either after stimulation of the adrenal cortical hormones by the ACTH or after the administration of glucocorticoids in animals is a well accepted fact. Conn (1949) showed that administration of 120 mg./day of ACTH in man causes a marked increase in urinary nitrogen excretion that resulted in a negative nitrogen balance. This was also accompanied by an increase in the glucocorticoids secretion as indicated by the elevation of urinary 17-ketosteroids excretion.

The increase of nitrogen excretion after cortisone administration has been demonstrated in man (Luft *et al.,* 1954; Doolan *et al.,* 1955), and in rats (Campbell *et al.,* 1954). The same effect on urinary nitrogen excretion was observed after cortisol administration in man (Doolan *et al.,* 1955; Pechet, 1955). Although some mineralocorticoids have slight effects on protein metabolism, the most potent mineralocorticoid hormone, aldosterone, was shown to have no effect on nitrgen balance (Sallassa *et al.,* 1957).

Mechanism of Action of Glucocorticoids on Protein Metabolism; The mechanism by which glucocorticoids increase the protein catabolism with concomitant increase in urinary nitrogen excretion and negative nitrogen balance, has been investigated by Welt *et al.* (1952), Russell (1955), Noall *et al.* (1957), and Wool and Goldstein (1958). It is well accepted among all investigators that the glucocorticoids induce their catabolic effects through enhancing the gluconeogenesis process in the liver. The amino acids delivered to the liver are deaminized to their corresponding α-ketoacids which are converted to glucose or other derivatives. However, the mechanism by which glucocorticoids enhance such process is still a debate. Russell (1955) is in favor of the theory that glucocorticoids act on the tissue protein and increase its breakdown to amino acids which are eventually deaminized by the liver. Wool and Goldstein (1958) studying the role of cortical steroids and the sympathetic amines in protein mobilization concluded that the former support some agent other than the epinephrine in promoting nitrogen mobilization.

Other studies by Noall *et al.* (1957), using the unmetabolized labelled α-aminoisobutyric acid in their study on the mechanism of action of various hormones on protein metabolism, showed that hydrocortisone injection increased by 60 percent the amino acid capture by the liver. Therefore, they concluded that the action of glucocorticoids on protein metabolism is to increase the hepatic capture of blood amino acids, thereby exposing them to accelerated destruction.

The two aforementioned theories, though different in explaining the way of furnishing amino acids to the gluconeogenesis process in the liver, are in agreement that, glucocorticoids enhance gluconeogenesis by increasing the substrate level (amino acids) in the liver. No mention, however, was given for the possible activation of enzymes which carry out the gluconeogenesis process. Recently Rosen *et al.* (1959) from their work on the glutamicpyruvic transaminase

enzyme (GPT), which is rate-limiting in gluconeogenesis, have added new information of great significance in the mechanism of action of glucocorticoids on protein metabolism. Their work indicated that a 2 to 5 fold increase in the (GPT) activity in the livers of rats occurs when cortisol, cortisone, or adrenocorticotrophic hormone, are administered subcutaneously for 4 consecutive days. This discovery evidently rejects the earlier conclusion of Astwood (1957) who reported that the site of action of the adrenal cortical secretion could not be localized at any one known chemical process or enzyme action in the body.

Another theory for the increase of urinary nitrogen excretion by glucocorticoids administration is suggested from earlier work of Ingle *et al.* (1947), and Ingle *et al.* (1953) who showed that administration of carbohydrate-active steroids to eviscerated rats caused a decrease in the rate of glucose removal from the blood stream. This was also confirmed later by Welt *et al.* (1952) who showed that the C^{14}-glucose oxidation to $C^{14}O_2$ was inhibited by cortisone administration in rats. Glucose is known to be essential for protein metabolism, as it provides the energy required for peptide synthesis (500 to 4000 calories per mole of peptide bond synthesized). Therefore, the decrease in glucose utilization due to glucocorticoids effect would result in a decrease in protein synthesis with resultant increase of dietary nitrogen loss in urine.

(b) Insulin; Evidence is accumulated from both *in vivo* and *in vitro* studies showing that protein synthesis and growth are suboptimal in diabetes and with administration of insulin in diabetic animals, nitrogen retention and growth are improved (Russell, 1955; Krahl, 1956).

Salter *et al.* (1957) showed that growth induced by exogenous insulin in hypophysectomized rats on an unrestricted diet is similar to that stimulated by somatotrophin. Insulin-induced growth was enhanced and disproportionate increase in fat was prevented by simultaneous administration of somatotrophin. A marked increase in the retention of the amount of ingested nitrogen was obtained when animals were allowed to consume glucose. However, protein sparing action of glucose was dependent upon an adequate supply of insulin.

Scow *et al.* (1958) administered 12 units of insulin daily to female rats deprived of 95 percent of their pancreas and were tube fed. This resulted in 0.4 g. gain in body weight per day, and 6 percent retention of the nitrogen fed. Krahl (1956) studied the mechanism of action of insulin on protein synthesis and used the synthesis of glutathione-C^{14} from glycine-1-C^{14} as indication of protein synthesis. It was shown that net peptide synthesis was reduced in diabetes both in liver and in diaphragm muscle. This reduction was related in large part to decreased synthesis rather than to increase breakdown of peptides in diabetic tissues. Injection of insulin into diabetic rats prior to tissue removal raised protein synthesis in excised diaphragms to a level substantally above normal. Peptide synthesis in diabetic liver slices was raised toward normal by addition *in vitro* of insulin plus glucose, but not by insulin in the absence of extracellular glucose. The author concluded that the effect of insulin on peptide synthesis is attributed, at least in part, to an action of insulin to favor glucose uptake, thereby providing

energy for peptide synthesis which varies from 500 to 4000 calories per mole of peptide bond synthesized. The mechanism of action of insulin on glucose uptake was previously discussed in details in this review.

(c) *Growth Hormone;* Earlier work demonstrated that administration of growth-promoting pituitary extract or the purified growth hormone increase increased body weight, as well as protein and water of the carcass. Recently Scow *et al.* (1958) deprived female rats of 95 percent of the pancreas and showed that administration of 0.1 mg. of growth hormone produced 1.6 g./day body weight gain and retention of 28 percent of the nitrogen. Twenty-one percent of the weight gain was protein.

Similar results were also obtained in ruminants by Struempler and Burroughs (1959) when conducting nitrogen balance experiments with lambs and growth hormone assay experiments on cattle. They concluded that nitrogen balance was favorably influenced by growth hormone administration.

Mechanism of Action of Growth Hormone on Protein Metabolism; DeBodo and Altszuler (1957) reported that the increase in nitrogen retention, and thus protein metabolism after the administration of growth hormone is possibly achieved through increasing the enzymatic activity of protein and/or amino acid synthesis; decreasing the enzymatic activity of protein and/or amino acid catabolism. Hoberman (1950) using N^{15}-glycine in normal, hypophysectomized, thyro- parathyroidectomized, and adrenalectomized rats showed that the administration of growth hormone to the hypophysectomized rats or to rats receiving ACTH caused the following: A decrease in urinary nitrogen excretion, restoration of the increased amino acid catabolism to normal, a tendency to increase the rate of utilization of amino acids for protein synthesis, and no effect on protein catabolism. This was confirmed later by Bartlett (1955) who concluded that the primary action of the growth hormone is the decrease in the rate of amino acid catabolism.

Contrary to that theory, Russell (1955) on the basis of many experiments, concluded that there was no convincing evidence for the decrease in the loss of amino acids, nitrogen of tissues, or the alteration in the accumulated blood amino acid nitrogen, due to growth hormone administration. She also reported that the anabolic effect of growth hormone was not due to the inhibition of amino acid catabolism. Russell, rather, believes that the hormone acts upon some phase of protein synthesis from amino acids. Using a radioactive unmetabolized amino acid (α-amino-isobutyric acid) Noall *et al.* (1957) showed that growth hormone exerts its anabolic action by increasing the concentrative transfer of amino acids in most tissues except the heart.

From the above discussion it is clear that no conclusion could be drawn as to what step in the protein metabolism is affected by growth hormone that accounts for the latter's anabolic action. DeBodo and Altszuler (1957) as well as Russell (1955), however, concluded that the protein anabolic effects of growth hormone are dependent on the ability of the animal to secrete adequate amounts of insulin. In such relationship the mechanism of action of growth hormone

may exist. The enhancements of body weight gain and of nitrogen retention by the growth hormone in the presence of adequate insulin have been found to exceed those of insulin alone (Salter *et al.,* 1957; Scow *et al.,* 1958).

(d) *Other Hormones;* There are many other hormones such as estrogens and androgens, which have effects on protein metabolism. However, they are of less importance than the aforementioned hormones especially in this particular experiment where the animals were young heifers and thus estrogens are not greatly involved in their protein metabolism. These two hormones and others of less importance are excluded from this review.

The Effect of Environmental Temperature on Total Plasma Protein:

The importance of plasma protein in heat stress is realized from its function in holding adequate percentage of water in the intravascular fluids and maintaining the viscosity of blood. It thus provides an efficient way of transferring the heat from the inside of the body to the outer surface in the skin for the dissipation of heat by non-evaporative processes. Plasma protein has a much lower osmotic pressure than other plasmatic substances, yet, because of its inability to diffuse through the capillaries' membrane, it provides the only osmotically active particles at the capillary membrane interface. A decrease in plasma protein to a low level produces edema, because the blood osmotic pressure would decline permitting the escape of plasma fluid to the extravascular fluid. Injecting of serum albumin in cattle suffering from the edema, which happens frequently in cows before parturition, alleviates the edema by raising the blood osmotic pressure (Larson and Hays, 1958). This, beside its other inert function, such as maintenance of acid-base equilibrium, blood viscosity, suspension stability of the erythrocytes, body protein transportation, and the mechanism of blood clotting and immunity, gives the plasma protein a profound importance in the mechanisms of heat regulation and in combating the consequences of heat stress.

The concentration of plasma protein varies among species of animals. Abritton (1952) reported the averages for horse, cow, sheep, goat, and dog are, respectively, 6.84, 8.32, 5.74, 7.27, 6.72 g. per 100 ml. plasma. Blincoe and Brody (1952) showed the mean of plasma protein in Jersey, Holstein, and Brahman, cows as 8.80, 8.97, and 9.12 percent, respectively. Larson and Touchberry (1958) showed that serum protein in various breeds of cattle is positively correlated with age and varies from 5.8 to 7.4 percent. In goats, Appleman and Delouche (1958) showed the mean of plasma protein 6.4 g. percent.

The effect of environmental temperature on plasma protein content has been investigated mostly in man. Much of this work suggests an inverse relationship between the environmental temperature and the plasma protein levels. However, when other factors such as physical exercise (DeLanne *et al.,* 1958) or dehydration (Adolph, 1947) are encountered in the temperature studies the above statement does not hold true.

Spealman *et al.* (1947) exposed two young men in psychrometric room six days at each high (91°F.) and low (69°F.) temperatures. The plasma protein con-

centration decreased in both individuals at high temperature. In one instance, the mean was 6.5 and 7.2 g./100 cc. at high and low temperatures, respectively. These results were in complete agreement with earlier work of Bazett *et al.* (1940) and Conley and Nickerson (1945). Bass *et al.* (1955) reported that although the plasma protein concentration of the subjects was elevated after one day exposure to heat, a steady decline took place thereafter to a value 12.1 percent below controls by the end of the heat period (14 days). Hyperthermia was also reported to decrease plasma protein concentration in chicks but not in rabbits (Rodbard *et al.,* 1951), whereas hypothermia increases plasma protein concentration in dogs (D'Amato and Hegnauer, 1953). It is worth mentioning that, while total plasma protein declines under heat stress, the beta-globulins which represent only 12 percent of the total protein shows definite increase due to heat injury in goats, rats, and dogs (Chanutin, 1947). Under thermal injury in rats α- and β-globulin increase, while γ-globulin and albumin fractions of plasma protein decrease (Gjessing *et al.,* 1947).

In ruminants, no adequate information to that, in man, is available in this regard. Brody (1949) studied the effect of rising temperature (70° to 100°F.) in the climatic laboratory, on the blood composition of Jersey and Holstein cows. He found no significant difference between the control cows maintained at 50°-60°F. and the experimental cows in plasma protein concentration. They concluded that ambient temperature had no significant effect on the water balance between blood and other tissues in cattle. Appleman and Delouche (1958), on the other hand, from his work on the effect of ambient temperature on various biological characteristics in goats presented with no comments some figures about plasma protein concentration at rising environmental temperature. These values at 20, 30, 35, and 40°C. were 5.5, 6.2, 6.3, and 7.6 g. percent, respectively, indicating an increase with rising temperature. However, other information is not available on ruminants to confirm these results.

Selye (1950) reported, from the work of various investigators, that most stressors which induce the General Adaptation Syndrome in organisms have a common effect on plasma protein. During the two catabolic phases of the G-A-S, plasma protein tends to fall below normal. The albumin fraction falls, while the α-globulin and sometimes fibrinogen rise. This is true in thermal injury, infection, starvation, and traumatic injury. Heat and cold are considered systemic stressors that evoke the same changes. It is not surprising, therefore, to find that plasma protein concentration decreases in man under heat stress.

Plasma protein concentration could be changed by either alteration in their rate of synthesis and catabolism or by mere physical dilution or concentration due to hemoconcentration or hemodilution effects. Such factors should be considered in any investigation that deal with changes of plasma protein concentration. The following is a brief discussion of how such factors could affect the plasma protein content and thus counteract or coincide with the temperature effect *per se,* on the plasma protein level.

Dietary Effects: The importance of diet on the plasma protein level has been understood from the work of Whipple and his associates. Madden *et al* (1943) bleeding the dogs and reinjecting the washed red cells in the animals (plasmapheresis) were able to induce a steady state of hypoproteinemia and a constant level of plasma protein production when the diet protein intake was controlled and limited. When the protein intake of such dogs was completely replaced by oral or intravenous administration of Rose growth mixture (ten amino acids essential for rat growth) plasma protein production was excellent, weight and nitrogen balance were maintained. Robischeitz-Robbins *et al.* (1943) upon feeding dogs abundant iron and protein-free or low protein diets, in accordance with the above results, were able to reduce the plasma to a very low level which was elevated later to normal after the administration of serum digests, hemoglobin digests, or amino acid mixtures either orally or intravenously.

The theory that plasma protein levels are affected by the dietary proteins is further demonstrated by the earlier work of Schoenheimer *et al.* (1942). They supplemented the casein-containing stock diet of normal adult rats for 3 days with isotopic amino acids labelled with N^{15}, and observed that the dietary nitrogen was incorporated in the plasma proteins. The rate of this process in the plasma proteins when compared with that in the kidney, liver, and intestinal tract of the same animals, was approximately the same, and all fractions of the plasma protein participated to about an equal extent. This indicted that a dynamic chemical interactions of plasma proteins occurs with body proteins and diet.

In sheep, Gorbelik (1956) showed that after keeping the rams and ewes for two months on low-protein ration, the quantity of proteins in the blood was reduced, especially the albumin and fibrinogen fractions. The globulin fractions, however, increased. Using C^{14} labelled homologus plasma protein, Yuile *et al.* (1959) noticed a slower plasma protein turnover (0.65 g./kg./day) in dogs deprived of protein than that of dogs receiving adequate dietary protein (1.0 g./kg./day).

Changes in Plasma Volume Effects: Previous studies dealing with the effect of environmental temperature on blood and plasma volume have revealed, in man and experimental animals, heat causes a gradual increase in plasma and blood volumes beginning within an hour of heat exposure followed by a progressive increase to a peak of 20-30 percent during the first week then a decline towards control values takes place thereafter. Acute cold exposure, however, has been reported to decrease the blood and plasma volume temporarily, while prolonged exposure to cold produced little change in plasma volume (Bass and Henschel, 1956).

Most studies, however, have shown that an inverse relationship between plasma volume and plasma protein concentration exists (Bazett *et al.,* 1940; Conley and Nickerson, 1945; Spealman *et al.,* 1947). Although, Rodbard *et al.* (1951) recommended the direct measurements for blood volume studies rather than plasma protein and hematocrit determinations, yet their data were in complete agreement with the above mentioned statement.

This inverse relationship between the plasma volume and plasma protein can be due to two factors. First, a mere physical dilution or concentration. For example, at high temperature when peripheral vasodilution takes place with deflection of blood to the skin to augment the thermal conductance of the surface tissue, the blood hydrostatic pressure falls below the colloidal pressure and thus the interstitial fluid shifts from the cell interspaces to plasma. Therefore, plasma protein concentration would evidently decrease as a result of being diluted by the entering fluid. In cold, as a consequence of vasocontriction, plasma protein concentration would increase in hydrostatic pressure of the blood.

This explanation, however, holds true if we assume that the fluid that moves in or out of the plasma has lower protein concentration than the plasma. Therefore, if this fluid would move to the plasma, a decrease in plasma protein concentration would result, and vice versa, if the fluid would move out. However, if this fluid that moves in and out of the plasma has a similar protein concentration to the latter as suggested by Glickman *et al.* (1941), the inverse relationship between blood volume and plasma protein would bear another meaning, i.e., shift in plasma protein metabolism at high and low temperature and presumably at high and low plasma volume. Probably less synthesis of plasma protein at high temperature than at low temperature may account accordingly for the decrease of plasma protein concentration at high temperature and plasma volume, and vice versa at low temperature. That is, in heat, where plasma volume eventually increases, a decrease in plasma protein synthesis or increase in their catabolism may take place at the same time. Such coincidence would result in the inverse relationship between plasma protein concentration and plasma volume.

Hormonal Effects: The hormones that possibly affect the plasma protein levels could be divided into two categories. Those which affect the water balance, and thus indirectly cause a dilution or concentration of plasma protein. These hormones which are mainly the mineralocorticoids and ADH, however, will be discussed later in this review in connection with water and electrolyte balance.

The other category includes those hormones that affect the protein metabolism of the body and, thus, may affect directly the plasma protein concentration. These hormones such as the growth hormone, ACTH, adrenal glucocorticoids and insulin and others were discussed in this review previously in connection with the effect of environmental temperature on urinary nitrogen and nitrogen retention. In this regard Matsuda (1956) showed that injection of each of cortisone, DOCA and ACTH intramuscularly in rabbits caused a decrease in the total serum protein. On the other hand, Bass *et al.* (1955) studied the effect of prolonged exposure to heat on plasma volume and protein content in men. They attributed the steady decline in the plasma protein content from the fifth day of exposure to heat till the end of the heat period (14 days) to a loss of fluid richer in protein than normal. The subjects had negative nitrogen balance with no increase in glucocorticoids being observed.

Effect of Environmental Temperature on Blood Glucose:

The concentration of blood glucose in adult animals is known to be constant under ordinary circumstances, and to vary within relatively narrow limits in most species of animals. The maintenance of such level, represents one of the most studied examples of that type of regulation which has been designated "homeostasis" by Cannon (1929). However, under some endogenous or exogenous factors depending upon the severity of the stimulus, the homeostatic mechanism of the animal fails to maintain the blood sugar level constant or at a "steady state". Such stimuli may be hereditary disorders in the organism, such as hormonal imbalance, liver disfunction, renal abnormalities, etc., or they may be environmentally induced.

The mean values of blood sugar in ruminants have been reviewed by Reid (1950a). They vary from 41.15 to 68.5 mg. per 100 ml. in cattle and from 32 to 64 mg. per 100 ml. in sheep. The low normal bloodglucose level in ruminants is associated with a smaller uptake of glucose by the tissues of the sheep than occurs in nonruminants, and this low uptake is accompanied by a higher uptake of acetic acid (Reid, 1950b). Blood sugar in adult ruminants is found mostly in the form of glucose, while, in the fetal blood, a considerable portion of the blood sugar is fructose. Jarrett and Potter (1952) showed that the average blood sugar level of newborn lambs was 128 mg. per 100 ml. while the average of fructose was from 40 to 80 mg. per 100 ml. Within 6 hours following birth fructose could not be detected in the blood of the lamb. The level of reducing sugar also declined until it was near that of the normal adult about the eighth week of age.

The decline in blood sugar concentration during that period parallels closely the functional development of the rumen where a great portion of the carbohydrates are fermented to volatile fatty acids by the rumen microorganism (McCandless and Dye, 1950). This suggestion was rejected by Reid (1953) who attributed the aforementioned decline to the disappearance of glucose from blood corpuscles and plasma as well as to the decrease of alimentary hyperglycemia of the ruminant animal.

The effect of cold exposure on blood sugar does not seem to be consistent in man. Although Leonhardt (1941) noted an increase in blood sugar with local application to cold, Keeton and Mitchell (1944) reported no considerable or consistent effect. Britton (1928) and Onchi (1941) showed that in cats with adrenal glands intact, which have been rendered hypoglycemic by insulin administration, a rise in blood sugar to even hyperglycemic level was observed upon their exposure to cold. Cold and hyperthermia were reported by Selye (1950) to increase blood sugar level in experimental animals especially during the resistance phase of the G-A-S.

Kanter (1959) exposed unanesthetized dogs to heat (120°F.) for 4 hours with no water available for drinking. In spite of expected hyperglycemia due to this dehydration process, a hypoglycemia developed. The author concluded that the utilization of glucose mainly by the respiratory muscles was sufficiently rapid to offset the hemoconcentration and to cause the fall in glucose levels even when

glucose was provided to the dogs by stomach tube at high temperature. In an attempt to prove this theory, the author curarized the dogs to slow the respiratory muscles activity which is used in panting on heat exposure, no fall in blood glucose was thus observed upon exposing the dogs to heat.

In cattle, however, very few studies have been carried out to investigate the effect of environmental temperature on blood glucose. Early in 1928 Fish indicated from a study on five cows that little fluctuation occurred in the blood sugar level during October, November, and December. However, there was a uniform decrease in the blood sugar during January and February and an increase again in March. The blood sugar again decreased in April, raised in May and June, and experienced a decline in July. Hodgson, *et al.* (1932), on the contrary, stated that blood sugar determinations made during July and August gave considerably higher readings than those obtained during the winter months.

Riek and Lee (1948) studying the effect of high ambient temperature in dairy cattle, milking Jersey cows to 110°F., caused a decline in blood sugar from a mean value of 55.2 to 44.5 mg. per 100 ml. blood. Brody (1949) compared nonlactating dairy cows to rising temperature 50° to 100°F. for four months to control animals maintained at 50°F. The average blood glucose for the control group was 66.0 mg. percent, while that of the experimental animals appeared to decline with increasing environmental temperature. At 70°F. the level of blood glucose was 63.0 mg. percent and at 100°F. it was 54.3 mg. percent.

The constancy of the blood sugar level apparently is due to a balance between the rate at which glucose enters the blood and the rate at which it leaves the blood. Environmental temperature could possibly alter either of these rates through different ways—especially the dietary and hormonal factors.

Dietary Effects: It has already been mentioned that the ambient temperature inversely alters the feed intake in cattle. The question which then arises is whether or not the change in feed intake in cattle is involved in the environmental temperature influence if any on blood glucose concentration in cattle.

Ruminants have shown in most instances to respond to fasting by a drop in their blood glucose concentration similarly but not of the same magnitude as monogastric animals. Hodgson *et al.* (1932) found in a study on five heifers, that during fasting over a period of nine days, the blood sugar decreased to less than 50 percent of its original value during the nine day study. Leffel and Shaw (1957) showed that restricting the feed intake for 10 to 14 days post partum in dairy cows to 35 percent of Morrisons' recommended TDN intake resulted in a statistically significant hypoglycemia and ketonemia whether the cows were previously fed a low, medium or high protein level ration. The blood glucose average (3-11 days post partum) of full-fed cows and fasted cows were respectively, 39.74 and 22.14 mg. per 100 ml. In sheep Reid (1950a) showed that a fast of 24 hours duration produced a marked hypoglycemia (8.6 mg. percent) in ewes of poor bodily condition during the last two months of gestation. In nonpregnant sheep of good condition a fast of 46 hours was insufficient to cause any pronounced fall in the blood sugar from the pre-fasting level.

Reid (1950b) showed that in sheep acetic acid, which constitutes 86-95 percent (molar basis) of the total volatile fatty acids in arterial blood, was also markedly depressed after fasting for 24 and 46 hours. Reid and Hogan (1959) showed the blood levels of glucose in ewes fed on a submaintenance diet of wheaten chaff during the last 5 weeks of pregnancy was considerably lower than these levels after feeding. Pre-feeding hypoglycemia was consistently more severe in ewes carrying twins than in ewes carrying single lambs. Terri *et al.* (1958) noted that malnutrition in male white-tailed deer fawns produced hypoglycemia with a corresponding increase in protein catabolism. In goats in late pregnancy or cows at the peak of lactation fed hay alone, Forbes (1943) found the blood sugar level fell steadily from about 50 to 30 mg. per 100 ml., rising again when full ration was given.

Fasting also decreases the utilization of blood glucose in ruminants. Reid (1958) found that the half time of injected blood glucose in sheep fed on wheaten chaff was 28 minutes, while after 96 hours fasting it was 180 minutes. The half time of the injected acetate was 7 minutes and 22 minutes in the fed and fasted sheep, respectively, which is longer than that of nonruminants who do not depend upon acetate as a source of energy.

Although fasting causes hypoglycemia in adult ruminants differently from monogastric animals, there is no appreciable rise in blood-glucose levels of adult ruminants in response to feeding. Hodgson, *et al.* (1932), Allcroft (1933), and Sampson and Boley (1940), reported earlier that the blood sugar level was not influenced by feeding in cattle and sheep. Bell and Jones (1945) noticed relatively small changes in the content of venous blood sugar of the AnkoleZebu stock of South Africa when up to 8 grams of glucose per kilogram of body weight was introduced directly into the rumen.

This was also confirmed by Schambye (1951) on sheep. Daugherty *et al.* (1956), on the other hand, showed that administration of 3.7 g. per kg. body weight by stomach tube increased the jugular blood glucose level in sheep and cattle, while 1.5 g. per kg. body weight did not cause an appreciable increase. When the steer was given access to a sugar block and ingested 3.8 lbs. of corn glucose, blood glucose level did not change appreciably. They attributed the difference in the results to the method used in ingesting the glucose.

In goats, OBara and Watase (1957) administered orally soluble starch, glucose, glycerol, citrate, acetate, propionate, and butyrate, in approximately 10 percent solution to goats, and the blood glucose level in the jugular vein was checked every hour for the first 7 hours, and once on the following day. Starch, alone caused no change in blood glucose level. Glucose and citrate induced moderate elevation when large doses were administered; glycerol induced a slight decrease at first and an increase later. Administration of acetate, propionate, and butyrate, respectively, each dose being about 3 g./kg. body weight gave rise to an increased blood glucose level, especially in the case of propionate and butyrate. Leffel and Shaw (1957) studied the effect of prepartal dietary protein level on blood glucose in cattle during prepartal and postpartal periods. They found that

the various levels of protein intake did not result in any statistically significant differences among the groups either prepartum or postpartum. Goetsch and Pritchard (1958) showed that oral administration of acetic acid to hypoglycemic fasted ewes did not significantly alter blood glucose, while propionic acid markedly increased the blood glucose level and the degree of glycosuria. Butyric acid, conversely, depressed significantly blood glucose levels.

It seems from the above studies that over feeding of carbohydrates to ruminants has no effect on blood sugar levels. This is mainly due to the breakdown of carbohydrates by rumen microorganism to volatile fatty acids which are absorbed along with the already hydrolysed portion of carbohydrates from the rumen walls to the blood stream. However, if the feed contains a large amount of free fatty acids which is not the case in ordinary ration, an increase in blood sugar concentration may insue. This, because these fatty acids and particularly the propionic acid is converted rapidly to glucose by the liver.

The rest of insignificant amounts of carbohydrates which are not attacked by the rumen microorganisms evidently pass to the rest of the digestive tract where they are poorly hydrolysed and absorbed—as compared to monogastric animals (Hale and King, 1958).

From the aforementioned discussion one can fairly say that the decline in feed consumption at high temperature contributed to a great extent to the declining effect of high ambient temperature on blood glucose. Increased feed consumption, on the other hand, at low environmental temperature does not seem to have any contribution to the effect of temperature, if any, on blood glucose in ruminants.

Hormonal Effects: Once the sugars from the rumen and the intestinal tract in ruminants reach the blood stream, they render in a dynamic state of utilization, conversion to glucose and other metabolites, oxidation to CO_2, water and energy, condensation to glycogen. The latter is stored or hydrolysed (glycogenolysis) to glucose which is released back to blood stream. All of these steps are controlled by a neurohormonal-enzymatic system. This system attempts to adjust the rates of income and outgo of the blood sugars in spite of the changing rates of their turnover in order to maintain the homeostasis of blood sugar in the body.

In this regard temperature can affect the blood glucose through altering the hormonal balance, as has been discussed early in this chapter, and, thus, increase either the glucose utilization and glycogenesis, or glycogenolysis and gluconeogenesis with consequent decrease or increase in blood glucose concentration. The mechanism through which the ambient temperature could affect the endocrine system was previously discussed in this chapter.

Hypoglycemic Hormone (Insulin); The hypoglycemic effect of insulin in cattle and sheep is not generally different from that in the man and monogastric animals (Reid, 1951; Hitchcock and Philipson, 1953). However, these species are different in their tolerance to insulin. In man, a dose of insulin of 1-2 units per kg. body weight causes characteristic hypoglycemia in a very short time (30-60 minutes) and hypoglycemic convulsions with larger doses. In ruminants hypo-

glycemia is not attained so rapidly and it extends for a long time once it is developed. In most instances, cattle do not show hypoglycemic convulsions.

Foley (1959) showed that insulin injection in cattle (0.8 units per kg. body weight) dropped the blood sugar in the normal appearing animals from 60.89 mg. percent to a minimum to 27.10 mg. percent in 2 hours. Then blood sugar returned to the normal level in 10 hours postinjection. However, no hypoglycemic convulsions were observed.

The longer period of time required in ruminants rather than nonruminants to restore the initial blood sugar level is probably due to an insufficiency of growth hormone, ACTH or any of the hyperglycemic hormones. In this concern Foley (1959) reported that injection of dwarf cattle with ACTH had improved their response to insulin, i.e., restored blood sugar level in shorter time.

Reid (1951), however, attributed the difference between ruminants and nonruminants to differences in intermediary metabolism. Black *et al.* (1957) showed that approximately ½ to ⅔ of the glucose in the cattle is catabolized through the pentose cycle rather than the Mayer Hoff pathway. The possible utilization of acetic acid by the central nervous system in the fed and fasted ruminants (Reid, 1950b) may also account for the absence of convulsions in ruminants at a low hypoglycemic level that is effective in man and monogastric animals.

Mechanism of Action of Insulin on The Regulation of Blood Sugar Level; The primary biochemical effect of insulin insufficiency as shown by various investigators is a reduction of glucose utilization for oxidation and storage. The effects on lipid and protein metabolism seem to be secondary (Renold *et al.,* 1956; Ashmore *et al.,* 1957; Shaw and Stadie, 1957; Feodor, 1955; Langdon, 1957; Krahl, 1956). The mechanism by which insulin regulated the sugar metabolism and indirectly affects the blood sugar level has been under thorough investigation by many workers. These studies have revealed some theories concerning the mechanism of action of insulin such as the hexokinase, the permeability, and the serum lipoprotein fraction theories. These theories are discussed exclusively (Stadie, 1954; deDuve, 1957; Resnick and Hechter, 1957; Levine and Goldstein, 1958).

Hyperglycemic Hormones; Although there is only one hypoglycemic hormone (insulin) secreted in the body, a vast array of hormones that increase blood sugar are well known to occur in mammals. Such hormones such as growth hormone, adrenocorticotropin, glucagon, epinephrine, adrenal cortical hormones, and others of less importance in affecting blood sugar level such as thyroxine and estrogens are adequately discussed in regard with their biological effects and mechanisms of action by Shull and Mayer (1956); Renold *et al.* (1956); DeBodo and Altszuler (1957); and Behrens and Bromer (1958).

Some of the aforementioned hormones have been administered to cattle and have shown a hyperglycemic response. These hormones are the adrenal glucocorticoids (Shaw *et al.,* 1951; Dye *et al.,* 1953; Chung, 1958),

MATERIALS AND METHODS

Animal and Management:

This study is a continuation of the previous investigation (Kamal, *et al.,* 1959). The same two groups of animals of the previous experiment were kept in their climatic chambers and served as the experimental animals in this study. At the beginning of the experiment the animals were approximately one year old heifers. Illumination, air velocity, feed, water, care and management, were the same as in the previous study. The schedule of experimental measurements is shown in appendix (a).

Treatments:

Temperature was maintained at 80°, 90°, and 52°F., respectively, for about one month in both chambers before the actual temperature series experiment started. This pretreatment was thought to thermally equalize the group of heifers that has been raised at 50°F. with that group raised at 80°F. The two groups were then exposed to rising temperature of 35°, 50°, 70°, 80°, 90°, and 95°F. for two-weeks period at each level of temperature. The schedule of temperature treatment is shown in appendix (b). The characteristics studied in this part of the investigation, were, blood glucose, plasma total nitrogen, urine total nitrogen, total digestible nitrogen, nitrogen retention, water consumpion, urine volume, urine specific graviy, plasma and urine sodium, plasma and urine potassium, plasma sodium/potassium and urine sodium/potassium.

Sampling:

Venous blood samples were collected from the jugular vein of each heifer into heparinized plastic tubes, on the ninth day of each treatment. A small fraction of the sample; 1 ml., was saved for blood glucose determination while the rest of the sample was centrifuged. Blood plasma was drawn out with a syringe and placed into 50 ml.-flask and was used for sodium, potassium and total nitrogen determinations.

Twenty-four hour urine collections were made directly after blood sampling, i.e., on the ninth day of the two weeks treatment period. The method of Hobbs *et al.* (1950) modified by Dale and Brody (1954) for urine collection was used in this study. Toluene was added to the urine as a preservative. At the end of the collection period, the volume and specific gravity of the urine were determined and urine samples were collected and stored in a deep freeze for chemical determinations.

Methods of Analysis:

Blood Glucose: The adaptation of Nelson and Somogyi methods as described by Reinhold (1953) for blood sugar determination was used in this study.

Analytical Procedure; A 9.5 ml. of 0.3 N. barium hydroxide solution is added to 1 ml. blood in 50 ml.-flask with rotation. While mixing a 9.5 ml. of 5 per-

cent zinc sulfate solution is then added and the solution is shaked vigorously then filtered. A 0.5 ml. of the barium-zinc filterate is transferred to a 15 x 125 mm. test tube and 1 ml. of alkaline copper reagent is added. After mixing by tapping, a marble is placed on top of the test tube which is then heated in vigorously boiling water for 20 minutes, and cooled directly for 1 minute in water bath at room temperature. One ml. of arsenomolybdate reagent is added to the contents of the test tube and mixed. The contents are then diluted to 10 ml. with distrilled water, and mixed again by inversion. The optical density of the contents is read at 540 mμ. in the spectrophotometer. A blank which is used to set the apparatus at zero optical density is run in the same manner except water is used instead of the barium-zince filtrate. Standard glucose solutions of 2.5, 5, 10, and 15 mg. per 100 ml. of 2 percent benzoic acid are run simultaneously with each set of samples for preparing the calibration curve. Concentration of blood glucose in the samples is read for the calibration curve and results are expressed as mg. glucose per 100 ml. blood.

Reagents;

1. 5 percent zinc sulfate.
2. 0.3 N. barium hydroxide (this solution should exactly neutralize the first solution).
3. Copper reagent, solution A; an amount of 50 g. of sodium carbonate (anhydrous), 50 g. Rochelle salt, 40 g. sodium bicarbonate and 400 g. soduim sulfate (anhydrous) were dissolved in 2 liters distilled water. The solution is filtered when precipitation occurred.
4. Copper reagent, solution B; an amount of 150 g. copper sulfate is dissolved in 1 liter water containing 0.5 ml. concentrated sulfuric acid.
5. Alkaline copper reagent is prepared on the day of sugar determination by measuring 4 ml. of solution B into a 100 ml. mixing cylinder and diluted to 100 ml. with solution A.
6. Arsenomolybdate color reagent; an amount of 100 g. ammonium molybdate is dissolved in 1800 ml. distilled water and 12 g. of disodium orthoarsenate dissolved in 100 ml. distilled water. Both the solutions are mixed together and placed in an incubator for 24-48 hours at 37°C. The solution is stable indefinitely when stored in a glass-stoppered brown bottle.
7. Standard solutions of glucose are prepared by transferring 1 g. of purest dextrose in 100 ml. volumetric flask and being dissolved and diluted to the mark with 0.2 percent benzoic acid solution. From this solution appropriate dilutions of 2.5, 5, 10, and 15 mg. glucose per 100 ml. of 0.2 percent benzoic acid are prepared.

Plasma Total Nitrogen: A micro-Kjeldahl method was used in this study for determining the plasma total nitrogen. It is realized that steam distillation prior to estimation of ammonia is recommended for all precise micro-Kjeldahl analyses. However, a simplified steam distillation device was developed by the writer (Figure 1). With this apparatus, amounts of nitrogen in the neighborhood of 1 mg. were accurately detected.

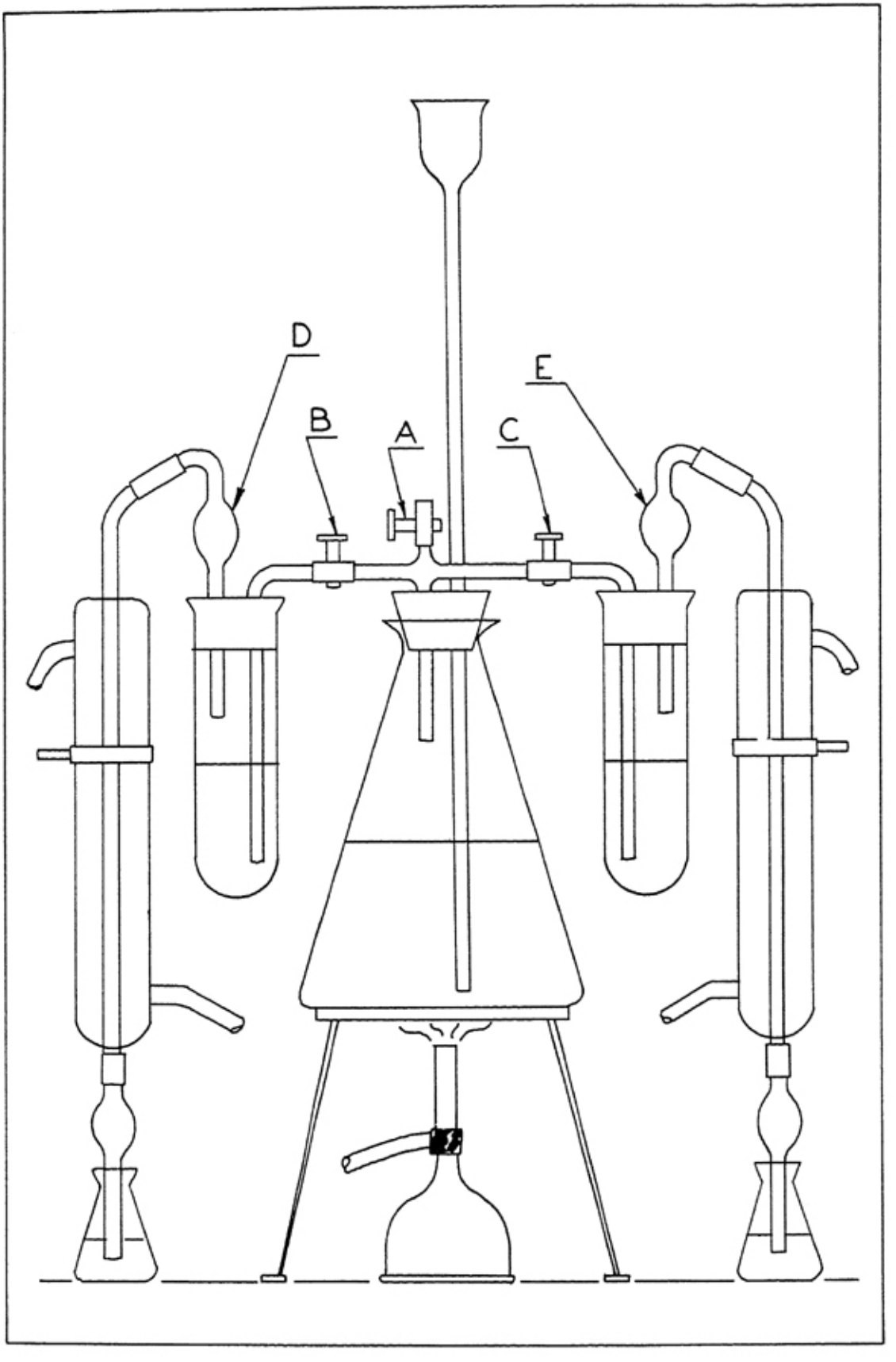

Fig. 1—Steam distillation apparatus used for plasma total nitrogen determination.

Analytical Procedure; An aliquot of 1.5 ml. of plasma is pipetted into 10 ml. volumetric flask and is made to volume with ammonia-free distilled water and then mixed. This solution contains 0.15 ml. of the original plasma per ml. An aliquot of 1 ml. of the diluted plasma is transferred to a completely dry 30 x 200 mm. pyrex test tube. An amount of 1.5 ml. concentrated sulfuric acid is added followed by 0.5 ml. of 70 percent perchloric acid and the mixture is heated over an extremely low flame of a microburner for about 5 minutes until all moisture is evaporated and the contents are charred. The flame is then increased with continuous heating for about 10 minutes until all white fumes disappear and the digestion mixture is decolorized. The tube is cooled and 25 ml. of ammonia-free distilled water are added followed by further cooling to room temperature.

For the distillation of ammonia of the digested sample, 10 ml. of 50 percent sodium hydroxide are carefully added on the inside wall of the test tube to avoid mixing with the acid solution. The test tubes are connected with the steam distillation apparatus. The steam is then allowed to pass through the samples by opening the clamp screws, B and C, enough to let the steam stir up the solution and release the ammonia. The speed of the passing steam is regulated by clamp screws A, B, and C. The ammonia and the steam that pass through the delivery tubes and condensers are trapped in receiving flasks containing few drops of methyl red indicator and 15 ml. of 0.02 N. sulfuric acid. Safety bulbs D, E, are used to prevent any alkali from reaching the receiving flasks. After collecting approximately 20 ml. distillate and flask and the bulbs are removed and washed with ammonia-free distilled water which is added to the distillate in the flask and the steam is disconnected. The apparatus is ready then for another run. The partly neutralized 0.02 N. sulfuric acid is back-titrated with 0.02 N. sodium hydroxide solution. The plasma total nitrogen is calculated and expressed as g. nitrogen per 100 ml. plasma.

Reagents;

1. Concentrated sulfuric acid.
2. A 70 percent perchloric acid solution.
3. A 50 percent sodium hydroxide solution.
4. A 0.02 sulfuric acid standard solution.
5. A 0.02 N. sodium hydroxide standard solution.
6. Methyl-red indicator.

Urine Total Nitrogen: The urine total nitrogen was determined with a macro-Kjeldahl method in this study. The method was exactly the same as the one described by Hawk *et al.* (1954). From the percent of total nitrogen in the sample and the amount of urine excreted in the 24 hours, the total nitrogen excreted in the 24-hour urine is calculated and the result was expressed as g. of urine total nitrogen per 24 hours.

Nitrogen Retention: The amount of nitrogen retained by the animals was calculated by subtracting the nitrogen excreted via urine from the digestible nitrogen of the ration which was consumed on the same day of urine collection.

Since the three heifers of each breed were fed together, only the breed averages of 50° or 80°F. group were available. The water consumption values were obtained similarly. The nitrogen retention was expressed as g. nitrogen retained per animal per 24 hours.

Plasma Sodium and Potassium: Twenty ml. of 12.5 percent trichloroacetic were mixed thoroughly with 5 ml. plasma. After about 15 minutes the mixture was filtered. The trichloroacetic acid filtrate contained 0.2 ml. of the original plasma per ml. filtrate.

Analytical Procedure; For sodium determination, a 3 ml. of the filtrate is transferred to a 50-ml. volumetric flask which is made to volume with glass-distilled water. This solution is aspirated in the flame photometer at wave length of 589 mμ. and the emission intensity is recorded as the meter deflection of apparatus. Sodium chloride standard solutions of 35, 40, 45, and 50 ppm. sodium are prepared. The emission intensity of the sample and the standard nearest to the sample are read three times alternatively. Calibration curve for sodium standard solution is prepared and the concentration of the sample solution can be read from the working curve. Correction is made for the dilution of the sample, and the result is expressed as meq. sodium per liter of plasma.

Potassium was determined by transferring 10 ml. of the trichloroacetic acid filtrate to a 150 ml. beaker. The solution was dried on a water bath to evaporate the trichloroacetic acid from the solution. The residue was then dissolved into 20 ml. glass-distilled water and is aspirated in the flame photometer at wave length of 768 mμ. Potassium nitrate standard solutions of 10, 15, 20, and 25 ppm. of potassium; each containing 300 ppm. sodium were prepared. The rest of the procedure is exactly the same as that of the sodium determination.

Reagents; Stock Standard solution of sodium chloride is prepared by dissolving 254.3 mg. sodium chloride in 1 liter of glass distilled water. This solution contains 100 ppm. sodium. Appropriate dilutions are made from this solution to obtain 35, 40, 45, and 50 ppm. sodium for working curve. Standard solutions of potassium are prepared by dissolving 300 mg. potassium nitrate in 1 liter glass-distilled water. This stock solution contained 100 ppm. potassium. Glass-distilled water containing 300 ppm. sodium is used to prepare appropriate dilutions of the stock standard solution containing 10, 15, 20, and 25 ppm. potassium.

Urine Sodium and Potassium: Ten ml. of thoroughly mixed urine sample is transferred to a 50-ml. volumetric flask. A two ml. of concentrated nitric acid is added to the aliquot in order to dissolve the suspension formed during the refrigeration of the sample. The solution is made to volume with glass-distilled water, and then filtered. The concentration of sodium or potassium in urine is known to vary in urine to a great extent; therefore, arbitrary dilutions of urine samples of 1:5, and 1:50 for sodium and potassium determinations, respectively, are made. The diluted samples are aspirated in the flame photometer similarly to plasma sodium or potassium determinations. When the readings are too high the urine samples are further diluted with glass-distilled water to 1:10 or 1:15

and 1:100 or 1:200 for sodium and potassium determinations, respectively. Standard solutions for sodium and potassium of 5-50 and 5-70 ppm., respectively, are made. The rest of the procedure is similar to that of plasma sodium or plasma potassium. The results are multiplied by the corresponding urine volume and expressed as meq. sodium or potassium per 24 hours urine collection.

Graphic and Statistical Analysis of Data:

Data were arranged for every group (50° and 80°F.) in the order of rising temperature 35°, 50°, 70°, 80°, 90°, and 95°F. (Tables 1 to 5). Semi-logarithmic paper was used for the graphic presentation of the data in this study (Figures 2-6). This type of graphic technique provides an equal comparison between the various characteristics studied in regard with their rates of change with increasing temperature.

Regression coefficients (Figures 5 and 6; Table 7) were computed for the data from 50° to 95°F. The exponential equation, $Y = a.e^{bx}$, over the linear regression equation was applied for such purpose. The advantage of this equation is that the former expresses the rates of changes percentagewise and, therefore, equal comparison between the regressions of all characteristics could be obtained. Correlation coefficient between the rising temperature (50° to 95°F.) and each of the studied characteristics as well as the statistical significance of these correlations are shown in Table 6. Test of significance between the groups' regressions (Table 9), as well as between the groups means at 35°F. (Table 8), were computed. The means of each group at 35°F. and between 50° and 95°F. are shown in Table 5. All statistical analysis of the means, regression coefficients, correlation coefficients, and "t" tests were done by the Statistical Department, University of Missouri on the electronic computer E 102.

Plotting the data on semi-logarithmic paper which has an arithmatical horizontal scale and a vertical logarithmic scale, was thought to provide a good mean for studying the rates of changes of certain characteristics with the rise in the environmental temperature. It also provides an equal comparison between the various characteristics studied in regard with their rates of change with increasing temperature. Using an exponential equation, $Y = a.e^{bx}$, rather than linear regression equation enables the investigator to express the percentage changes in certain components. For example, a slope of a particular component which has a "b" value of 0.05 means that the instantaneous rate of increase in this component with rising temperature is approximately 5 percent per every increase of 1°F. at any given temperature between 50° and 95°F.

Preliminary studies on these data showed that all the biological characteristics presented in this study tended to behave similarly, in all breeds, though with different magnitude, in either cold (35°F.) or with rising temperature (50° to 95°F.), as will be shown in Figures 2, 3, and 4. Therefore, in every characteristic the data of all breeds in each group of heifers were pooled together for the statistical analyses of correlations, regressions, and "t" tests. It was also observed in the same figures that most characteristics of both 50°F. and 80°F. groups re-

TABLE 1

AVERAGES OF PLASMA SODIUM/POTASSIUM, URINE SODIUM, URINE POTASSIUM, AND URINE SODIUM/POTASSIUM OF BROWN SWISS, HOLSTEIN, AND JERSEY HEIFERS UNDER RISING ENVIRONMENTAL TEMPERATURE (35° TO 95°F.)

REARED TEMP.	BREED	ENVIRONMENTAL TEMPERATURE, °F. 35	50	70	80	90	95	35	50	70	80	90	95
		Plasma Sodium/Potassium						Urine Potassium, meq./heifer/day					
	B.S.	36.7	35.7	34.4	32.7	34.2	36.9	1361	2882	2886	3110	1475	2508
50°F.	H.	35.3	35.2	35.4	32.9	35.1	34.3	1887	2949	3304	1884	708	1432
	J.	39.5	37.2	39.2	36.3	35.5	35.1	1136	2172	2072	1625	535	1036
	B.S.	37.3	34.7	38.6	34.3	33.2	35.9	3703	2698	2286	2069	1742	1602
80°F.	H.	41.5	36.2	38.3	34.7	35.4	40.3	4350	3229	2707	3085	1160	1375
	J.	40.7	37.6	34.4	34.3	33.7	37.6	2268	2456	2138	1564	734	1266
		Urine Sodium, meq./heifer/day						Urine Sodium/Potassium					
	B.S.	75	121	243	395	278	826	0.050	0.039	0.087	0.141	0.166	0.337
50°F.	H.	144	219	230	400	259	616	0.078	0.079	0.072	0.212	0.346	0.433
	J.	61	186	318	248	31	415	0.057	0.077	0.150	0.152	0.070	0.398
	B.S.	380	142	111	172	340	408	0.107	0.061	0.055	0.090	0.224	0.254
80°F.	H.	303	212	489	283	172	393	0.074	0.066	0.243	0.091	0.144	0.280
	J.	114	244	277	257	237	443	0.055	0.096	0.133	0.182	0.321	0.383

TABLE 2

AVERAGES OF BLOOD GLUCOSE, PLASMA TOTAL NITROGEN, PLASMA SODIUM, AND PLASMA POTASSIUM OF BROWN SWISS, HOLSTEIN, AND JERSEY HEIFERS UNDER RISING ENVIRONMENTAL TEMPERATURE (35° TO 95°F.)

REARED TEMP.	BREED	ENVIRONMENTAL TEMPERATURE, °F. 35	50	70	80	90	95	35	50	70	80	90	95
		Blood Glucose, mg./100 ml.						Plasma Sodium, meq./1.					
	B.S.	57.3	56.7	54.7	48.6	47.4	48.0	152	151	149	154	148	152
50°F.	H.	52.9	55.6	52.8	51.6	39.3	41.8	152	154	150	152	148	148
	J.	51.6	57.0	46.4	45.4	42.2	42.4	152	153	148	154	152	141
	B.S.	54.9	51.3	47.2	48.5	52.4	52.8	159	152	153	151	149	149
80°F.	H.	54.4	51.2	44.8	45.8	47.0	47.6	154	150	153	153	150	150
	J.	64.0	57.5	54.0	51.7	49.3	51.4	156	149	150	151	150	149
		Plasma Total Nitrogen, g./100 ml.						Plasma Potassium, meq./1.					
	B.S.	0.90	1.68	0.96	0.96	0.82	0.99	4.14	4.23	4.32	4.70	4.34	4.12
50°F.	H.	1.04	1.21	0.96	1.02	0.83	1.02	4.32	4.38	4.24	4.63	4.25	4.32
	J.	0.97	1.13	1.04	1.11	0.81	1.07	3.87	4.14	3.81	4.23	4.29	4.04
	B.S.	1.52	1.14	1.09	0.99	1.08	0.97	4.78	4.36	3.96	3.96	4.51	4.15
80°F.	H.	1.47	1.06	1.13	1.07	1.07	1.05	3.72	4.14	3.99	4.41	4.26	3.76
	J.	1.39	1.12	1.10	1.09	1.04	1.07	3.85	3.97	4.36	4.40	4.44	3.99

TABLE 3

AVERAGES OF WATER CONSUMPTION, URINE VOLUME, URINE SPECIFIC GRAVITY, AND TOTAL VAPORIZATION OF BROWN SWISS, HOLSTEIN, AND JERSEY HEIFERS UNDER RISING ENVIRONMENTAL TEMPERATURE (35° TO 95°F.)

REARED TEMP.	BREED	ENVIRONMENTAL TEMPERATURE, °F. 35	50	70	80	90	95	35	50	70	80	90	95
		Water Consumption, l./heifer/day						Urine Specific Gravity					
	B.S.	23.8	23.2	27.0	31.8	55.1	67.2	1.041	1.040	1.039	1.038	1.011	1.016
50°F.	H.	25.5	26.2	31.2	41.0	66.1	72.4	1.044	1.045	1.043	1.025	1.005	1.012
	J.	19.0	19.3	28.0	34.8	60.4	61.6	1.039	1.034	1.027	1.027	1.018	1.014
	B.S.	23.2	22.2	27.4	31.8	45.2	49.3	1.043	1.043	1.041	1.037	1.027	1.026
80°F.	H.	23.2	24.6	33.2	44.3	64.6	71.1	1.038	1.039	1.038	1.023	1.009	1.009
	J.	18.1	16.9	21.4	24.1	30.6	30.3	1.036	1.035	1.032	1.028	1.015	1.015
		Urine Volume, l./heifer/day						Total Vaporization, l./heifer/day					
	B.S.	3.81	7.67	8.49	8.48	20.02	22.71	4.07	6.02	6.93	13.35	12.58	18.32
50°F.	H.	4.16	7.08	8.01	15.43	22.91	26.90	4.57	7.17	10.23	14.66	11.92	18.36
	J.	3.41	6.24	14.37	15.71	12.99	24.79	2.69	3.46	5.61	7.92	7.65	12.08
	B.S.	8.65	6.54	6.23	6.31	10.60	9.41	4.28	4.67	7.69	11.41	15.46	17.15
80°F.	H.	10.62	7.60	7.62	14.35	24.80	20.26	4.27	5.07	9.21	11.47	15.09	14.55
	J.	7.50	7.66	7.33	7.22	9.80	12.00	3.12	2.90	6.70	7.94	8.99	9.36

TABLE 4

AVERAGES OF DIGESTIBLE NITROGEN CONSUMPTION, URINE TOTAL NITROGEN, NITROGEN RETENTION, AND BODY WEIGHT OF BROWN SWISS, HOLSTEIN, AND JERSEY HEIFERS UNDER RISING ENVIRONMENTAL TEMPERATURE (35° TO 95°F.)

REARED TEMP.	BREED	ENVIRONMENTAL TEMPERATURE, °F. 35	50	70	80	90	95	35	50	70	80	90	95
		Dig. Nitr. Cons., g./heifer/day						Nitrogen Retention, g./heifer/day					
50°F.	B.S.	157	148	123	132	75	106	111.0	33.4	13.9	24.6	-15.5	-1.7
	H.	196	156	157	125	65	70	133.2	35.9	41.2	39.5	11.2	-22.3
	J.	108	100	94	76	50	48	74.7	20.7	5.6	10.0	10.0	-12.7
80°F.	B.S.	163	140	127	130	77	81	39.8	42.4	29.3	48.0	- 4.6	21.7
	H.	185	160	159	164	85	89	47.3	57.7	58.0	42.5	20.0	23.3
	J.	115	101	98	79	41	33	33.0	18.6	19.4	- 2.6	2.6	-29.6
		Urine Total Nitrogen g./heifer/day						Body Weight, kg./heifer					
50°F.	B.S.	46	114	109	107	90	108	377	383	393	402	406	---
	H.	63	120	116	85	54	92	403	410	417	425	422	---
	J.	34	80	88	66	40	61	249	253	259	260	257	---
80°F.	B.S.	124	97	98	82	82	60	384	394	400	403	408	---
	H.	137	102	101	121	65	66	374	384	393	391	393	---
	J.	82	82	78	82	38	63	252	255	261	253	254	---

TABLE 5

THE MEANS OF THE SIXTEEN BIOLOGICAL CHARACTERISTICS UNDER MILD COLD (35°F.) AND HOT (50°TO 95°F.) ENVIRONMENTS IN HEIFERS REARED AT 50° AND 80°F.

ITEMS	Means at 35°F.		Means at 50° to 95°F.	
	50°F. Group	80°F. Group	50°F. Group	80°F. Group
Blood Glucose, mg./100 ml.	53.94	57.78	48.49	50.16
Plasma Total Nitrogen, g./100 ml.	0.97	1.46	1.04	1.07
Digestible Nitrogen Consumption, g./heifer/day	153.95	154.38	101.64	104.29
Urine Total Nitrogen g./heifer/day	47.64	114.34	88.73	81.19
Nitrogen Retention, g./heifer/day	106.31	40.04	12.92	23.11
Body Weight, kg./heifer	342.90	336.57	357.30	349.03
Plasma Sodium, meq./1.	152.03	156.52	150.24	150.41
Plasma Potassium, meq./1.	4.11	3.94	4.27	4.21
Plasma Sodium/Potassium	37.14	39.83	35.39	35.95
Urine Sodium, meq./heifer/day	93.24	265.63	319.17	278.78
Urine Potassium, meq./heifer/day	1461.22	3440.33	2038.56	2007.42
Urine Sodium/Potassium	0.061	0.079	0.179	0.175
Total Vaporization, 1./heifer/day	3.78	3.89	10.42	9.84
Water Consumption, 1./heifer/day	22.76	21.53	43.02	35.80
Urine Volume, 1./heifer/day	3.80	8.87	14.79	10.52
Urine Specific Gravity	1.041	1.039	1.026	1.028

TABLE 6
THE CORRELATION BETWEEN RISING TEMPERATURE (50° TO 95°F.)
AND THE VARIOUS BIOLOGICAL CHARACTERISTICS IN HEIFERS REARED AT 50° AND 80°F.

ITEMS	Correlation Coefficient "r"		D. F.	
	50°F. Group	80°F. Group	50°F. Group	80°F. Group
Blood Glucose	-0.647**	-0.210	45	45
Plasma Total Nitrogen	-0.620**	-0.410**	45	45
Digestible Nitrogen Consumption	-0.697**	-0.637**	15	15
Urine Total Nitrogen	-0.448**	-0.487**	45	45
Nitrogen Retention	-0.691**	-0.557*	15	15
Body Weight	0.074	0.038	12	12
Plasma Sodium	-0.090	-0.126	45	45
Plasma Potassium	0.028	0.032	45	45
Plasma Sodium/Potassium	-0.095	-0.031	45	45
Urine Sodium	0.432**	0.254	45	45
Urine Potassium	-0.573**	-0.651**	45	45
Urine Sodium/Potassium	0.639**	0.510**	45	45
Total Vaporization	0.704**	0.808**	45	45
Water Consumption	0.893**	0.686**	15	15
Urine Volume	0.483**	0.419**	45	45
Urine Specific Gravity	-0.707**	-0.745**	45	45

**Statistically significant at P 0.01.
*Statistically significant at P 0.05.

TABLE 7

REGRESSIONS OF THE SIXTEEN BIOLOGICAL CHARACTERISTICS ON RISING ENVIRONMENTAL TEMPERATURE (50° TO 95°F.) IN HEIFERS REARED AT 50° AND 80°F.

ITEMS	Regression Coefficient "b"		Intercept "a"	
	50°F. Group	80°F. Group	50°F. Group	80°F. Group
Blood Glucose	-0.0063	-0.0011	4.36	4.00
Plasma Total Nitrogen	-0.0073	-0.0015	0.58	0.18
Digestible Nitrogen Consumption	-0.0163	-0.0176	5.81	5.91
Urine Nitrogen	-0.0091	-0.0114	5.14	5.21
*Nitrogen Retention	-0.8380	-0.8276	77.44	86.83
Body Weight	0.0009	0.0004	5.79	5.81
Plasma Sodium	-0.0006	-0.0001	5.06	5.02
Plasma Potassium	0.0001	0.0001	1.44	1.43
Plasma Sodium/Potassium	-0.0007	-0.0002	3.62	3.60
Urine Sodium	0.0239	0.0164	3.50	4.04
Urine Potassium	-0.0200	-0.0203	9.03	9.05
*Urine Sodium/Potassium	0.0059	0.0045	-0.27	-0.18
Total Vaporization	0.0223	0.0267	0.52	0.12
Water Consumption	0.0249	0.0188	1.76	2.05
Urine Volume	0.0227	0.0126	0.68	1.23
Urine Specific Gravity	-0.0007	-0.0005	0.09	0.07

*The equation used in these characteristics was $Y = a + bx$ since the exponential equation $Y = a \cdot e^{bx}$ does not fit such low and negative values.

TABLE 8

TEST OF SIGNIFICANCE BETWEEN THE MEANS OF HEIFERS REARED AT 50° AND 80°F. WHEN EXPOSED TO MILD COLD (35°F.)

ITEMS	"t" value for testing the difference between 50° and 80°F. groups at 35°F.	D.F.
Blood Glucose	1.855*	88
Plasma Total Nitrogen	9.469***	88
Digestible Nitrogen Consumption	0.013	28
Urine Total Nitrogen	6.187***	88
Nitrogen Retention	3.776***	28
Body Weight	0.099	22
Plasma Sodium	2.869***	88
Plasma Potassium	1.091	88
Plasma Sodium/Potassium	2.225**	88
Urine Sodium	3.206***	88
Urine Potassium	4.868***	88
Urine Sodium/Potassium	0.969	88
Total Vaporization	0.292	88
Water Consumption	0.479	28
Urine Volume	4.844***	88
Urine Specific Gravity	0.687	88

***Statistically significant at P 0.01.
**Statistically significant at P 0.05.
*Statistically significant at P 0.10.

TABLE 9

TEST OF SIGNIFICANCE BETWEEN THE REGRESSION COEFFICIENTS OF THE VARIOUS BIOLOGICAL CHARACTERISTICS OBSERVED IN HEIFERS REARED AT 50° AND 80°F. WHEN EXPOSED TO RISING TEMPERATURE 50° TO 95°F.

ITEMS	"t" values for testing the difference between 50° and 80°F. groups during heat stress 50°-95°F.	D.F.
Blood Glucose	3.7036**	88
Plasma Total Nitrogen	28.3329**	88
Digestible Nitrogen Consumption	0.3289	28
Urine Total Nitrogen	0.5766	88
Nitrogen Retention	0.1031	28
Body Weight	1.6638	22
Plasma Sodium	3.4171**	88
Plasma Potassium	0.2295	88
Plasma Sodium/Potassium	3.2534**	88
Urine Sodium	8.0980**	88
Urine Potassium	0.0678	88
Urine Sodium/Potassium	5.8645**	88
Total Vaporization	1.9816*	88
Water Consumption	5.8684**	28
Urine Volume	10.9711**	88
Urine Specific Gravity	0.5635	88

**Statistically significant at P 0.01.
*Statistically significant at P 0.05.

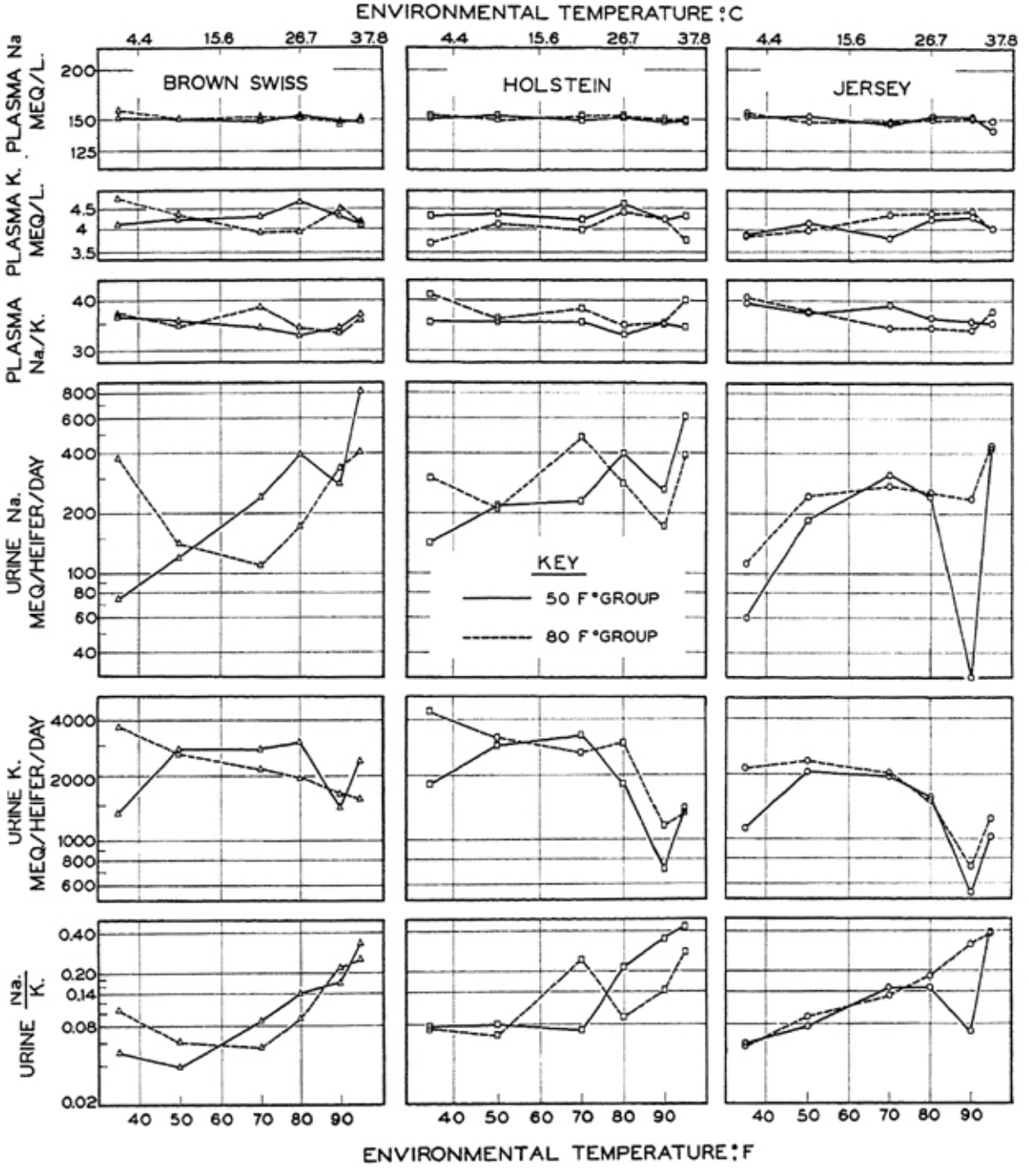

Fig. 2—Changes in plasma and urine values of sodium, potassium and sodium/potassium ratio in dairy heifers with rising environmental temperature 35°-95°F.

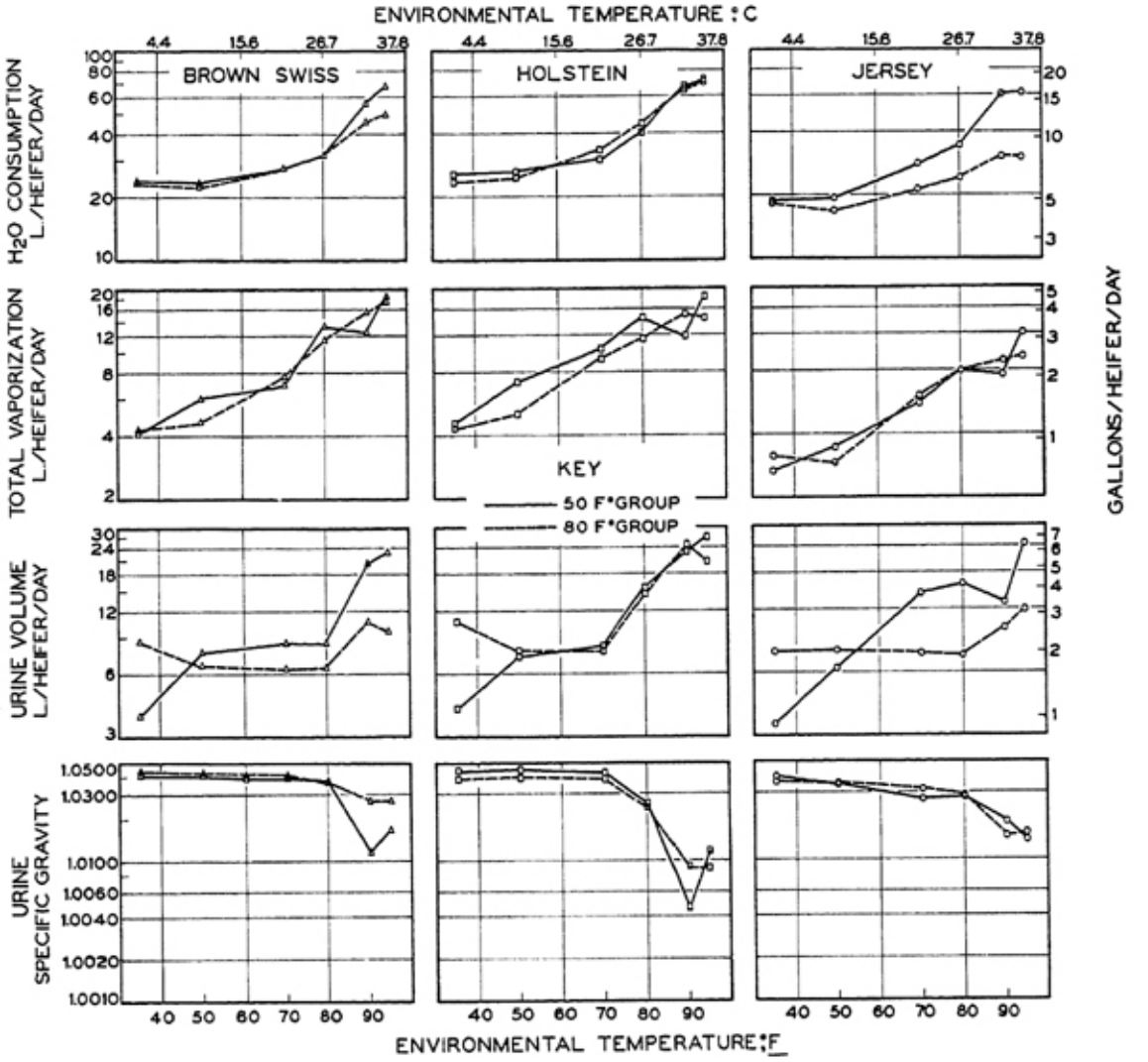

Fig. 3—Changes in water consumption, total vaporization, urine volume, and urine specific gravity in 50° and 80°F. reared heifers with rising environmental temperature 35°-95°F.

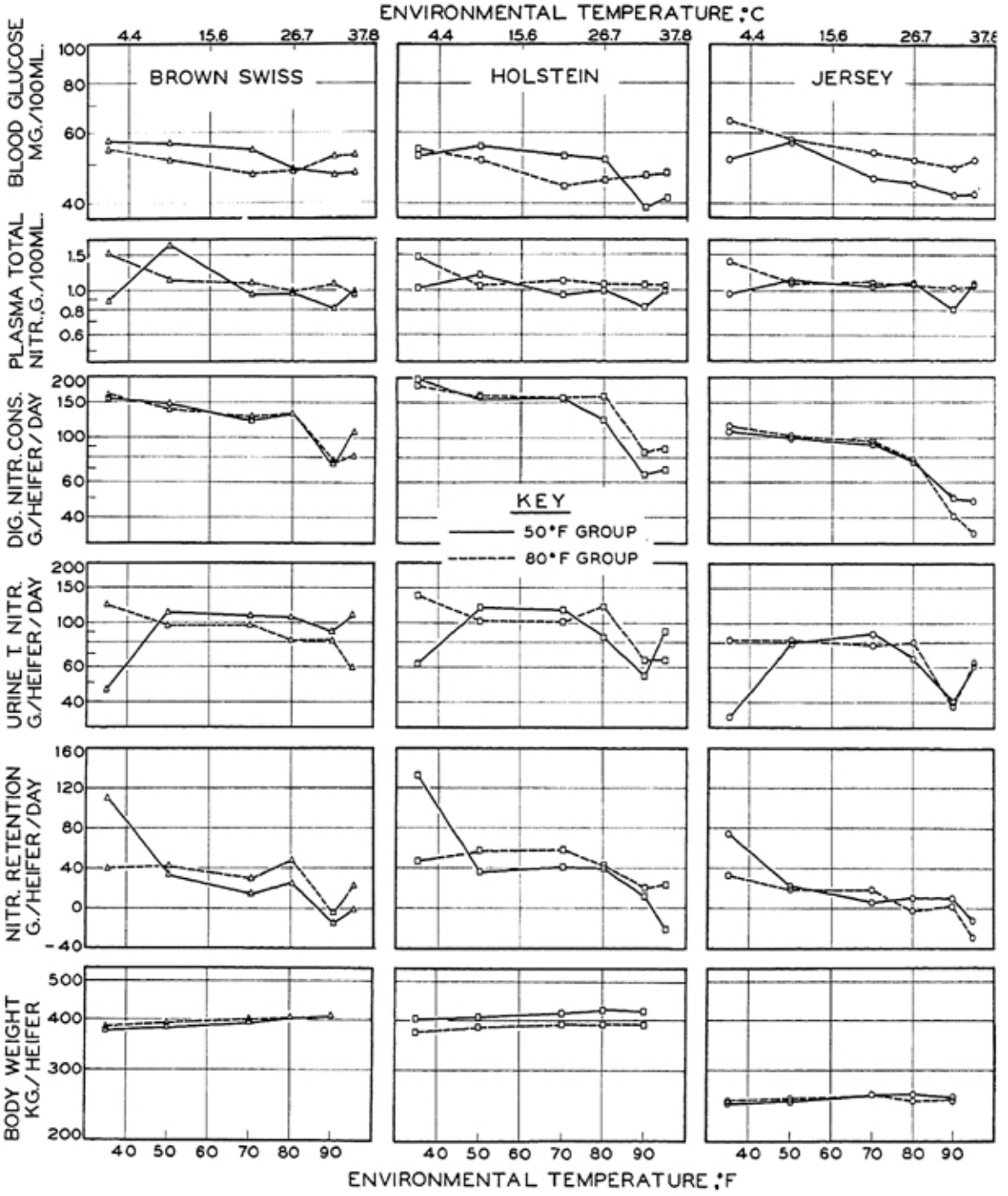

Fig. 4—Changes in blood glucose, plasma total nitrogen, digestible nitrogen consumption, urine nitrogen, nitrogen retention and body weight in 50° and 80°F. reared heifers with rising environmental temperature 35°-95°F.

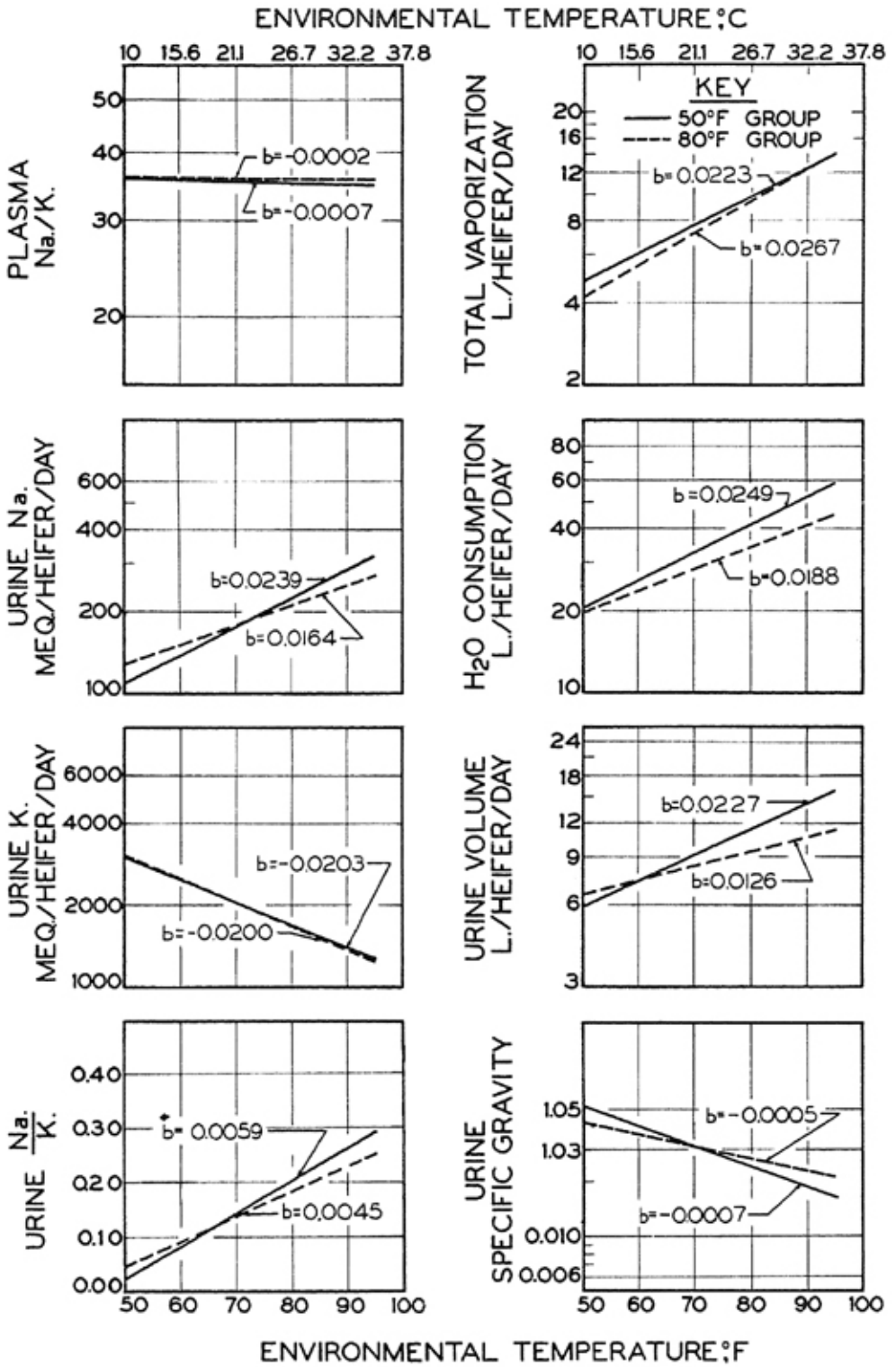

Fig. 5—The rates of changes in plasma and urine values of sodium, potassium, and sodium/potassium; total vaporization; water consumption; urine volume; and urine specific gravity of 50° and 80° reared dairy heifers with rising temperature 50°-95°F.

***Arithmic paper and linear equation Y = a + bx are used in urine Na/K.**

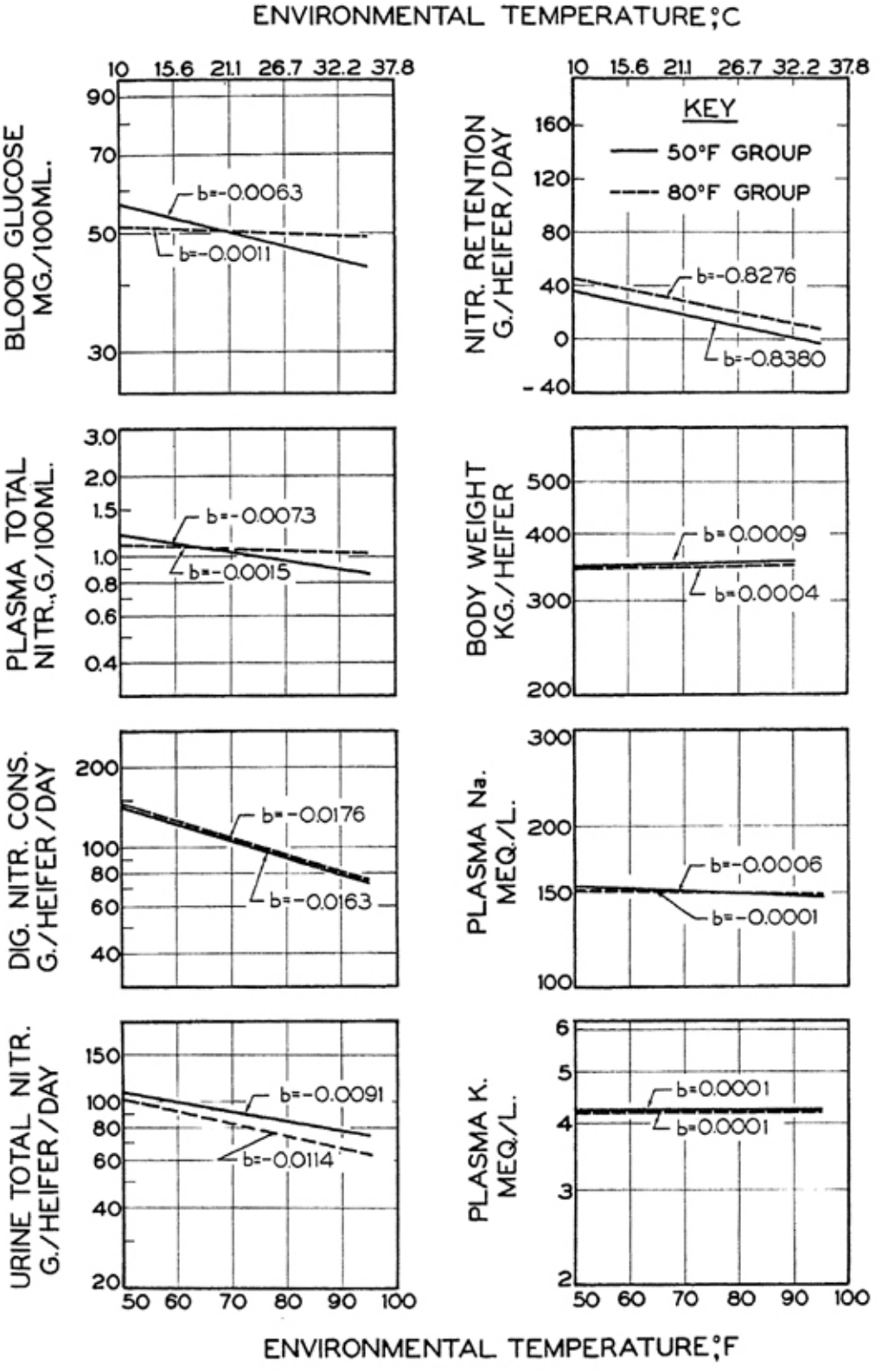

Fig. 6—The rates of changes in blood glucose, plasma total nitrogen, digestible nitrogen consumption, urine total nitrogen, nitrogen retention, body weight, plasma sodium and plasma potassium of 50° and 80°F. reared dairy heifers with rising temperature 50-95°F.

***Arithmatic paper and linear equation Y = a + bx are used in nitrogen retention.**

act differently at cold temperature (35°F.), but similarly with rising temperature (50° to 95°F.). Therefore, these two levels of temperature were handled separately in the statistical analysis.

RESULTS AND DISCUSSION

This bulletin presents the metabolic changes that occurred in the 50° and 80°F. reared groups of Brown Swiss, Holstein, and Jersey heifers during their exposure to rising environmental temperatures of 35°, 50°, 70°, 80°, 90°, and 95°F. Each temperature level was of 2 weeks duration. Sixteen biological characteristics are presented including protein, electrolyte, and water metabolism, as well as, blood glucose and plasma protein, expressed as total nitrogen.

This phase of the research has five major objectives. The first objective was to study the effect of rising temperature on these aforementioned characteristics in attempt to clarify the influence of heat and cold on carbohydrate, protein, water, and electrolyte metabolism in cattle as compared with other species of animals. In this objective it was hoped to understand some facts about the hormonal changes that are associated with these metabolic alterations under thermal stress.* An endeavor has never been undertaken before in cattle, using such as extension program, handling the data statistically, and eliminating the interference of lactation, gestation, growth, and all other environmental factors except temperature.

The second objective was to find a reliable index for heat tolerance, which is an ultimate goal of most workers in the field of bioclimatology. Studying these aforementioned sixteen biological characteristics which cover most of the major metabolic changes of the body was thought to give a clue to the characteristic of "heat tolerance"; a phenomenon which has long been awaiting clarification of bioclimatologists.

The third objective of this investigation was to answer the question which was posed in the conclusion of previous publications (Johnson and Ragsdale, 1959; Kamal *et al.*, 1959). This is, whether the heat acclimation characteristics which are thought to have been acquired by the 80°F. group of heifers would be held by the animals when they were later exposed in this investigation to cold and heat. This question will be answered in this study by comparing statistically the differences in the biological responses of cold and warm acclimated heifers to various environmental temperatures.

The fourth objective was to determine if there is any relation between what we generally call "thermal stress" in cattle (Kamal *et al.*, 1959b) and the "stress" as seen by Selye (1950). The frequent usage of the last term recently in cattle with no evidence as yet available regarding the occurrence of the General-Adap-

*Thermal stress in cattle is a physiological state manifested by various intensified or depressed biological reactions (heat production, vaporization, respiration rate, pulse rate, thyroid activity, etc.). This state may occur in cattle at temperatures which are outside the "comfort zone". The comfort zone is defined in the Mo. Env. Physiol. Series (I-LV) (1948-1959).

tation-Syndrome in cattle has led the writer to devote part of this study for investigating the possibility of the existence of "stress" in cattle under heat and cold.

The lack of information about the levels of many of these aforementioned characteristics in cattle either under comfort temperature or thermal stress has also intensified the need for this study.

The last objective of this report is to present a comprehensive literature review of the biochemical effects of environmental temperature on cattle.

Effect of Environmental Temperature and Acclimation on Electrolyte Metabolism:

Urine Sodium and Potassium Excretion: Figure 2 and Table 1 show that in cold (35°F.) the group of heifers that were raised in the thermoneutral zone (50°F.) excreted the minimum amount of sodium in urine and this was true in all breeds studied. The 80°F. reared group, however, at (35°F.) had a fairly high level of sodium excretion which was markedly higher than that of the 50°F. group. Such a difference was found highly significant, statistically (Table 8).

The urinary potassium excretion of the 50°F. group at 35°F. was similar to the sodium response. At the same low temperature the 80°F. group showed a response of potassium excretion similar to sodium excretion. Maximum level of potassium excretion in urine was, however, observed (Table 1 and Figure 2). Such difference between the two temperature groups was also found highly significant, statistically (Table 8).

It seems, however, that such significant natruresis, as well as kaluresis, in the 80°F. reared group, rather than the case in the 50°F. reared group, at cold temperature, is due to the fact that the former animals were reared at warm temperature since they were one month old. The exposure to such relatively cold temperature (35°F.), which the animals were not accustomed to, though they were thermally equalized at 50°F. for two weeks previously to this cold exposure as mentioned in Materials and Methods section, probably augmented the glucocorticoids secretion in these heifers and thus resulted in such high natruresis and kaluresis. That moderate dosages of glucocorticoids augment the sodium and potassium excretion in animals and result sometimes in negative balance in these electrolytes has been indicated by many workers (Streeten *et al.,* 1955; Swingle *et al.,* 1957; Eversole and Romero, 1958; Lipsett and Pearson, 1958; Barger *et al.,* 1958; Ross *et al.,* 1959). The site of action of these hormones on electrolyte metabolism differently from that of mineralocorticoids, is thought to be the tissues and cellular membranes (Swingle *et al.,* 1957; Swingle *et al.,* 1959).

The pathway through which the cold temperature might stimulate the oversecretion of glucocorticoids in the 80°F. reared heifers is suggested by the writer in the Review of Literature section. The Krause end-bulbs of cold receptors of the 80°F. group might have been more sensitive to a relatively higher temperature than those of the 50°F. group. Therefore, more impulses were probably passed to the hypothalamus in the 80°F. group than in the 50°F. group. That

resulted in higher secretion of ACTH from the pituitary and consequently more glucocorticoids from the adrenal cortex in the former group than in the latter. The question may rise as to how the long exposure to heat or rearing the heifers at 80°F. would make their peripheral receptors more sensitive to cold and as will be mentioned later, less sensitive to heat, is a problem that would warrant investigation. There is no doubt, however, that the aforementioned results add to the various well-known symptoms or manifestations of cold stress in cattle. Kaluresis and natruresis have not been investigated previously.

When environmental temperature was raised to 50°F. the urinary sodium and potassium of the 80°F. group decreased to a fairly intermediate level while those of the 50°F. group increased. This indicates probably an increased glucocorticoids secretion due to a mild heat stress in the 50°F. reared animals, and quite the reverse in the 80°F. group due to a relief from cold stress.

With increasing temperature from 50° to 95°F. an upward trend occurred in sodium excretion in all animals except in 50°F. reared Jerseys which showed an incidental drop at 90°F. Such increase, however, became steeper as temperature increased. The correlation between temperature and sodium excretion (Table 6), though positive in all animals, is only highly significant in the 50°F. group, but not significant in the 80°F. group. Also, a smaller regression coefficient of sodium excretion in the latter group than in the former is observed (Figure 5 and Table 7), such difference is highly significant statistically (Table 9). This indicates that the 80°F. reared group which was excreting sodium at a significantly slower rate than that of the 50°F. group with rising temperature (50°-95°F.) probably had lower glucocorticoids secretion under heat stress than the animals which were reared at cold temperature. The warm receptors (corpuscles of Ruffini) in the 80°F. reared group might have been less sensitive to heat than those of the 50°F. group, and, thus, passed less impulses to the hypothalamus. The result: lower glucocorticoids secretion.

The striking sodium depletion observed in the 50°F. group under heat stress has urged the writer to compute the sodium balance for these animals. It has been found that at 95°F. these heifers lost 14.24 g./heifer/day of sodium in urine while the total intake of sodium was 12.83 g./heifer/day, thus resulting in a negative sodium balance that amounts to -1.41 g./heifer/day, even if assumed that all the consumed sodium is absorbed by the animals.

Such negative sodium balance in cattle under heat stress is quite interesting. Man may develop a negative sodium balance under hot dry conditions due to the loss of great amounts of salts in sweat. Cattle, though, possibly do sweat, as shown recently by Taneja (1959), yet there is no information about their sweat composition. Nevertheless, cattle, like man, eliminate enormous amounts of sodium under heat stress. This elimination of salt is carried out via sweat in man and via urine in cattle. This similarty between man and cattle in sodium depletion in heat, does occur in spite of their difference in hormonal response to heat. This, however, will be discussed later in this section when comparison between species regarding the electrolyte metabolism under thermal stress is presented.

The source of the excessive urinary sodium when the heifers were already under negative sodium balance will also be discussed later in this section.

In contrast, urinary potassium remained almost constant at a high level between 50° and 70° or 80°F. in all animals and then it dropped sharply until 90°F. with a slight increase which took place afterwards in most animals at 95° F. However, the overall trend from 50° to 95°F. is represented by negative regression coefficients in both groups (Figure 5 and Table 7), and significant negative correlations between temperature and urine potassium (Table 6). There is no significant difference between the negative regressions of the 50° and 80°F. group (Tables 8 and 9).

The decrease in urinary potassium excretion under heat stress is somewhat contradictory to what has been suggested above, that high temperature might be associated with high glucocorticoid secretion in cattle, and it is well known these type of hormones increase the potassium excretion (Swingle *et al.,* 1957; Barger *et al.,* 1958; Eversole and Romero, 1958; Ross *et al.,* 1959). The reason for such confliction may be due to the change in the extracellular pH under thermal stress in cattle which modify the glucocorticoids effect on cellular potassium. It is known that heat stress in cattle is associated with hyperventilation (Kibler and Brody, 1950) which leads to a respiratory alkalosis and rise in the pH of extracellular fluid (Dale and Brody, 1954; Barker *et al.,* 1957). In this concern Reinberg and Stolkowski (1957) showed that the elevation of the extracellular pH prevented the effect of glucocorticoids on cellular potassium. This, however, may account for the sharp decline of potassium excretion in spite of the suggested high glucocorticoids activity under thermal stress in cattle, especially after 80°F. when respiratory alkalosis is expected to ensue.

Another reason for such confliction is the fact that the intake of roughages which contain about 18 times as much potassium as sodium was extremely depressed above 80°F., while the salt intake (almost sodium chloride) which was mixed with the concentrates was consumed completely before roughages were fed to the animals. Therefore, such shift in the Na/K ratio in the feed due to potassium depletion would decrease the mineralocorticoids secretion as indicated by many workers (Singer and Stack-Dunne, 1954; Rosenfeld *et al.,* 1956; Johnson *et al.,* 1957; Miller *et al.,* 1958), and thus would result in a low potassium excretion as shown in this investigation under heat stress.

Whatever the mechanism that is responsible for such changes in electrolyte metabolism in cattle, the aforementioned significant increase in sodium excretion and decrease in potassium excretion in urine at high environmental temperature are new manifestations that should be added to the many well-known reactions of heat stress in cattle.

Urine Na/K Ratio: The urinary Na/K ratio as shown in Figure 2 and Table 1 indicates that all the animals of the two groups had general upward trend in this ratio with rising temperature from 35° to 95°F. The minimum values were achieved in cold (35°F.), while the maximum values were obtained in heat (95°F.). The rate of increase in the ratio was much steeper as tempera-

ture increased. This upward trend is demonstrated by a positive and highly significant correlation between temperature (50°-90°F.) and urine Na/K ratio (Table 6).

It is well-known that the maintenance of Na/K ratio in urine is a function of mineralocorticoids through their action on the distal tubules (Vander *et al.,* 1958) probably by favoring the ionization of cellular potassium within the tubular cells (Dustan *et al.,* 1956) or by altering the cellular permeability (Lotspeich, 1958). The most reliable estimation of these hormones in various preparation is based on determination of the inert Na/K or radioactive Na^{24}/K^{42} in urine (Simpson and Tait, 1955; Hellman *et al.,* 1956). The deficiency of mineralocorticoids causes increase in sodium and decrease in potassium excretion in urine as well as an increase in Na/K ratios in urine or saliva, while quite the opposite occurs in blood plasma. This is true in sheep (Goding and Denton, 1957), in dogs (Barger *et al.,* 1958; Swingle *et al.,* 1959), and in man and rats (Mach and Fabre, 1955; Simpson and Tait, 1955a; Simpson and Tait, 1955b; Ross *et al.,* 1959).

It seems, therefore, that the significant increase in urinary Na/K ratio with rising temperature in this investigation is definitely due to a progressive decline in mineralocorticoids activity with the progressive rise in ambient temperature in cattle. This is new and important information about the thermal stress in cattle that has not been investigated before. It is worth mentioning that the 80°F. reared group as shown from Figure 5 and Table 7 had less steep increase in urinary Na/K ratio with rising temperature than the 50°F. reared animals. Such difference between the two groups is statistically significant (Table 9). This, however, indicates that the 80°F. group which was suggested previously to have significantly lower glucocorticoids secretion with rising temperature than the 50°F. group, had on the other hand, significantly higher mineralocorticoids secretion than the 50°F. group under the same mentioned climatic conditions. These results are not surprising since it is known that both types of hormones antagonize each other. Coon *et al.* (1949) prevented the great loss of urinary nitrogen that normally occur during the exposure to humid heat in man by administration of mineralocorticoids (DOCA). Dasgupta and Giroud (1958) showed that after cortisone treatment of nephritic rats, the high aldosterone secretion was depressed to normal.

It seems, therefore, that the pathway through which high temperature has depressed the mineralocorticoid secretion in the 50°F. group more than in the 80°F. group was by the higher antagonistic action of the assumed oversecreted glucocorticoids in the former group than that in the latter on mineralocorticoids. The suggested pathway of glucocorticoids stimulation under heat stress in cattle has been previously mentioned.

Plasma Sodium and Potassium Contents and Na/K Ratio: In spite of the definite changes observed above in the urinary sodium and potassium excretion as well as their ratio in urine with rising ambient temperature, the plasma levels of these electrolytes showed very slight response to cold or heat.

From Figure 2 and Tables 1 and 2 it is noticed that very slight decline in the concentration of plasma sodium took place in the three breeds which have been raised at either 50° or 80°F. The plasma content of potassium, on the other hand, showed a slight tendency to increase with rising temperature in contrast with plasma sodium. In the same Figure and Tables the plasma Na/K ratio followed a similar pattern to that of plasma sodium though of a faster rate of decline with rising temperature.

These changes in plasma electrolytes though insignificant as indicated by the correlation between rising temperature and these values (Table 6), yet they substantiate the aforementioned interpretation on urine electrolytes changes, that high environmental temperature depresses the mineralocorticoid secretion in cattle. At low temperature a significant difference between 50° and 80°F. groups occurred in plasma sodium as well as in plasma Na/K ratio (Table 8). This is probably due to a hemoconcentration in the 80°F. heifers at this low temperature as will be mentioned later. The statistical significant difference in the plasma sodium and Na/K rates of decline with rising ambient temperature, between the two groups (Table 9) also confirm the same interpretation on urine data that the 80°F. group had higher mineralocorticoids secretion than the 50°F. group under heat stress.

Comparison Between Cattle and Other Mammals in Electrolyte Metabolism Under Thermal Stress: In comparing our results with those of other workers, a lack of information and wrong interpretation in regard to electrolyte metabolism in cattle have been observed. Blincoe and Brody (1951) finding no marked change in plasma sodium and potassium contents with changing temperature, concluded that cattle maintain a fairly normal electrolyte balance at high temperature. This conclusion, however, is in complete disagreement with the results of this investigation, where statistically significant progressive increase in sodium depletion in urine occurred with rising temperature. Such depletion accounted even to a negative sodium balance in some animals at high temperature. In sheep, similar observations to this investigation was obtained by Macfarlane *et al.* (1958). They showed that heat (41°C.) caused a progressive increase in sodium excretion and Na/K ratio and a regular decline in potassium excretion in urine. Such changes were ceased when sheep were cooled by watering. In accordance with these results Blaxter *et al.* (1959) also showed that when environmental temperature increased from 15° to 38°C. urinary loss of potassium fell from 21.0 to 17.8 g./24 hr.

On the other hand, when comparing our results on cattle or the aforementioned results on sheep with those of monogastric animals, a complete difference between the ruminants and the latters in electrolyte metabolism under thermal stress can be observed. In man (Bass *et al.,* 1955; Lichton, 1957; Robinson and Macfarlane, 1958; Macfarlane *et al.,* 1958) and in dogs (Kanter, 1954a; Kanter, 1954b) showed that in contrast to ruminants heat decreases sodium excretion and Na/K ratio and increase potassium excretion in urine. Rats, however, are different from cattle in some aspects and similar in another regarding

electrolyte metabolism under thermal stress. Mefferd *et al.* (1957) and Mefferd and Hale (1958) showed that urinary sodium and potassium excretion were lower in heat than in cold while urinary Na/K ratio was higher in heat than in cold.

Although such discrepancy between ruminants and monogastric animals were observed by Macfarlane *et al.* (1958) from their work on man and sheep, yet no explanation in this regard has been as yet provided. The glucocorticoids and mineralocorticoids are important in electrolyte metabolism of sheep, man, and other different species of animals as was discussed previously in the Review of Literature section. It would thus seem that such discrepancy in electrolyte metabolism between ruminants and monogastric animals under heat stress is due to difference in their adrenal cortex response to such a stressor. In man, heat causes a decrease in glucocorticoid secretion (Bass *et al.,* 1955; Hallman *et al.,* 1956; Watanabe and Yoshida, 1956; Macfarlane and Robinson, 1957; Robinson and Macfarlane, 1958), while mineralocorticoids were reported to increase in man upon exposure to heat (Hellman *et al.,* 1956). However, in ruminants, the available observations on sheep (Macfarlane *et al.,* 1958; Blaxter *et al.,* 1959), and on cattle, from the present study suggest a decreased mineralocorticoids and probably increased glucocorticoids upon exposure to heat. Such difference in the most potent factors that regulate electrolyte metabolism between ruminants and man is probably the main reason for the discrepancy in the reaction of both species to heat in regard with urinary excretion of electrolytes. It is rather interesting, however, that these two species have similar response in their thyroid glands activity under thermal stress as mentioned in the Review of Literature section.

The insignificant changes in plasma levels of electrolytes under thermal stress which are demonstrated in this investigation on cattle, are on the other hand, in accordance with the previous results on cattle (Blincoe and Brody, 1951), and on man (Bass *et al.,* 1955; Lichton, 1957; Macfarlane and Robinson, 1957). Such results are not surprising because of the fact that most mammals have various homeostatic mechanisms which by virtue of their close cooperation and integrity tend to maintain most of blood metabolites at fairly constant levels in face of various external forces. Therefore, the effects of these external forces on the organism are largely reflected in urine composition rather than in blood. Only when one or more of the various homeostatic mechanisms such as the excretory system or peripheral utilization, etc. are disturbed that significant changes in blood levels of certain metabolites are appeared.

It seems, however, from the results of this investigation, that cattle are as efficient as man in maintaining their plasma levels of electrolytes with minimum changes under thermal stress. Bass *et al.* (1955) showed that a negative potassium balance of –40 meq. was obtained in man under heat stress with no marked change in plasma electrolyte contents. They also observed a severe negative sodium balance during heating period, with no concomitant change in plasma content of sodium.

In cattle, in this investigation, a negative sodium balance of about -70 meq. per animals per day was obtained at 95°F. without significant change in plasma sodium concentration. To find such a similarity between man and cattle in having a negative sodium balance under heat stress, though they have different response in regard with adrenal cortical hormones, is of great interest. The explanation for such confliction is that, at high temperatures, cattle eliminate great amounts of sodium in their urine because of deficiency in mineralocorticoids, as indicated by the elevated urine Na/K ratio. While the hypersecretion of mineralocorticoids in man under thermal stress prevents the loss of sodium in urine, these hormones have slow effect on sweat glands to conserve sodium, thus, negative sodium balance would ensue before sweat glands start to respond to mineralocorticoids and conserve sodium. The effect of mineralocorticoids on sodium excretion by sweat glands was demonstrated by Conn (1949) while the ability of sweat glands to conserve sodium loss after long exposure to heat was shown by Bass *et al.* (1955) and the slow response of sweat glands to mineralocorticoids was suggested by Ladell (1957).

Another question is how could the cattle, as well as man, maintain their plasma electrolytes levels fairly unchanged in face of the negative sodium balance under heat stress. This probably is achieved by mobilizing sodium from possibly a stored-up salt in the body fluids, as has been suggested in man (Joesephson, 1957), through the action of glucocorticoids. Swingle (1957), however, suggested that the severe kaluresis and naturesis induced by glucocorticoids administration is due to an outflux of these electrolytes and water from cells including bone and collagenous tissue. Whatever the source of sodium that could replete the sodium deficit under thermal stress, the maintenance of a fairly constant level of electrolytes in blood plasma is achieved under such condition in cattle as it is in man.

Comparison Between Cattle and Other Mammals in Their Averages of Plasma and Urine Electrolytes: The means of sodium and potassium in this investigation (Table 5) are somewhat higher than those values of Blincoe and Brody (1951) and lower than those of Dale *et al.* (1954) on dairy cows. The bovine plasma sodium and potassium contents are generally similar to those of man (Gamble, 1954; Hawk *et al.,* 1954), dogs (Davis and Ball, 1958), and rats (Hannon *et al.,* 1958). However, the urine excretion of these electrolytes as well as the ratio between sodium to potassium is expected to vary between cattle and other species such as man (Bass *et al.,* 1955), rats (Eversole and Romero, 1958) or chicken (Brow *et al.,* 1958), whose values are previously mentioned in the Review of Literature section. The urinary sodium excretion of heifers in this study is lower than sodium excretion in man's sweat of 10 liters (Robinson and Robinson, 1954).

The average value of urinary Na/K ratio of heifers in this study is from 0.06 to 0.18 (Table 5), while that of man is about 3.5 (Bass *et al.,* 1955) and in rats about 0.3 (Simpson and Tait, 1955b). The reason for the low urinary Na/K ratio in cattle as compared to other species is probably due to a higher mineralo-

corticoids activity in cattle than in other animals. Cattle feed consists of plant material which may contain 18 times as much potassium as sodium. Rosenfeld *et al.* (1956) showed that perfusion of the calf adrenals by a high potassium and low sodium perfusion medium had significantly augmented output of aldosterone-like material. Therefore, such high potassium as compared to sodium content in cattle feed may be responsible for the lower urinary Na/K ratio or higher mineralocorticoids in cattle rather than in omnivores or carnivores.

Effect of Environmental Temperature and Thermal Acclimation on Water Metabolism:

In this section, the main pathways of water income and outgo are discussed together, yet separately from nitrogen retention which will be discussed later. A water retention or water balance study could not be computed in this investigation, because the determinations of total vaporization, as well as water consumption, were not obtained on the same days of urine collections.

Urine Volume and Urine Specific Gravity: Figure 3 and Table 3 show that at a low temperature (35°F.) the 50°F. reared group excreted the minimum volume of urine and this was true for all breeds. The 80°F. group, however, excreted at the same temperature a fairly high amount of urine which was twice as much as that excreted by the 50°F. group as shown in Table 5. Such difference is highly significant statistically (Table 8).

The reason for such difference between the two groups seems to be the same one mentioned before which caused significantly high natruresis and kaluresis in the 80°F. reared group when they were exposed to cold (35°F.). That is, such cold temperature caused a hypersecretion of glucocorticoids in these animals (80°F. group) which were not adjusted to cold, and therefore, resulted in their relatively higher urine volume of a lower though insignificant specific gravity (Table 8) than in the 50°F. reared group.

Such diuresis of the 80°F. groups resembles the "cold diuresis" described by Bader *et al.* (1952) which is associated with low specific and high urinary solute load in the same time. The high solute load of the 80°F. group at 35°F. has already been mentioned before in the previous part. That glucocorticoids administration causes marked diuresis, due to their action on renal tubular reabsorption (Kleeman *et al.,* 1958), or to an outflux of water and electrolytes from cells (Swingle *et al.,* 1959), has been indicated in man (Ingle, 1950; Luft *et al.,* 1955; Raisz *et al.,* 1957; Kleeman *et al.,* 1958), rats (Sala and Luetscher, 1954), and dogs (Montastruc, 1954; Swingle *et al.,* 1957). Such "cold diuresis" in warm acclimated (80°F.) cattle has not been demonstrated before and could be added to the other symptoms of cold stress in cattle.

When temperature was raised to 50°F., the urine volume of the 80°F. group, with exception of Jersey heifers, dropped, while that of the 50°F. group increased exactly the same as occurred in sodium and potassium excretion. This indicates, probably, a disappearance of cold stress from the 80°F. group and existence of a mild heat stress in the 50°F. group.

With rising ambient temperature from 50° to 95°F. all animals of both groups showed an upward trend in urine volume and a downward trend in urine specific gravity reaching a maximum and minimum levels for both, respectively, at 95°F. in most animals. The rate of increase of urine volume and the rate of decrease of urine specific gravity were highly pronounced as temperature increased as shown in Figure 3. The correlation between rising temperature (50°-95°F.) and urine volume of specific gravity is highly significant statistically (Table 6). This significant increase in urine volume and decrease in urine specific gravity along with the previously reported increase in sodium excretion with rising temperature from 50° to 95°F. confirm our belief that glucocorticoids secretion increases in cattle under heat stress. Such diuresis, however, that occurs at high environmental temperature is a new knowledge about thermal stress in cattle.

The decrease in mineralocorticoid secretion with rising environmental temperature as indicated before by the significant rise in urinary Na/K ratio might have contributed also to such diuresis. The occurrence of severe diuresis due to withdrawal of DOCA in sheep was shown by Goding and Denton (1957). The importance of desoxycorticoids rather than aldosterone in regulation of urine volume has been indicated (Sala and Luetscher, 1954; Thorn *et al.,* 1955; Mach and Fabre, 1955; Barger *et al.,* 1958). The insufficiency of mineralocorticoids in this experiment was probably brought about by the antagonistic effect of glucocorticoids. Such antagonism was indicated by Dasgupta and Giroud (1958).

Another explanation of such significant increase in urine volume and decrease in urine specific gravity under thermal stress is that glucocorticoids secretion, which is suggested to be elevated at high environmental temperature in cattle might prevent the release of antidiuretic hormone, which is known to depress urine excretion in cattle (Kamal *et al.,* 1959), as well as in other species (Leaf *et al.,* 1953; Itoh, 1954; Barger *et al.,* 1958), through its action on the renal distal tubules (Barger *et al.,* 1958).

The significant decrease in specific gravity with rising temperature is due to many factors. First, the depression in potassium excretion which reached about 70 percent lower at 95°F. than that at 50°F.; secondly, the dilution of urine constituents through the excretion of great amount of water in urine caused by the action of the aforementioned hormonal disturbance; and, finally, the possible utilization of urea from blood by rumen microorganism as a result of the marked decline in feed intake under thermal stress similar to camels and sheep under such food deprivation (Schmidt-Nielsen, *et al.,* 1957; Schmidt-Nielsen, 1957b).

It should also be added that the 80°F. group had a slower rate of increase in urine excretion and, consequently, had slower rate of decrease in urine specific gravity with rising temperature than the 50°F. group as indicated from the regression coefficients (Figure 5, and Table 7). Such difference between the two groups is statistically significant for urine volume and insignificant in urine specific gravity (Table 9). This, however, indicates that the 80°F. group, as mentioned before in discussion of electrolytes metabolism, had lower gluco-

corticoids and higher mineralocorticoids and probably higher antidiuretic hormone secretion under thermal stress than the 50°F. group.

It was surprising to find no available data on the water metabolism and particularly urine volume and urine specific gravity of cattle under thermal stress for the writer to make comparative analysis. In sheep, however, in two reports, Macfarlane *et al.* (1958a), and Macfarlane *et al.,* (1958b) showed that urine volume decreased markedly in summer or under artificial heating, while Blaxter *et al.* (1959) showed that urine volume increased about 8 folds and, in most instances, exceeded the water lost as vapor—in complete agreement with these results on cattle at 95°F. where the urine volume was 6-8 times higher than that at 35°F. and exceeded the total vaporized moisture at 95°F. in all breeds. The confliction in the data obtained on sheep, however, is probably due to the difficulty encountered in urine collection reported in one report of the former investigators.

In man, on the other hand, the change in urine volume under thermal stress (heat or cold) is quite the reverse of the present study on cattle or that of Blaxter *et al.* (1959) on sheep. In cold, urine volume is much higher than that at high environmental temperature in man (Adolph, 1947; Bader *et al.,* 1952; Kellman and Weiner, 1953; Macfarlane *et al.,* 1958b). Rats have high urine volume at both extremes of ambient temperature (Katsh *et al.,* 1954; Mefferd *et al.,* 1957).

This difference between ruminants and man in urine excretion under thermal stress may be due to their difference in hormonal response particularly the adrenal cortical hormones to thermal stress as mentioned before. That is, the significant diuresis of man in cold and of ruminants at high temperatures may be due to the high glucocorticoid, low mineralocorticoid and low antidiuretic, hormone secretion in the former (Itoh and Kimura, 1953; Kellman and Weiner, 1953; Thorn *et al.,* 1955; Bass *et al.,* 1955; Hellman *et al.,* 1956; Watanabe and Yoshida, 1956; Macfarlane and Robinson, 1957; Robinson and Macfarlane, 1958; Macfarlane *et al.,* 1958b), and probably to the opposite response of these aforementioned hormones to thermal stress is cattle. Such possibility was confirmed in sheep in regard with mineralocorticoids activity from the results of Macfarlane *et al.* (1958) on sheep and man though the authors had different interpretations. They, however, showed that urinary Na/K ratio decreased in man and increased in sheep during 4-hours of heat exposure which indicate according to the main function of mineralocorticoids, as mentioned before, respectively, high and low mineralocorticoids secretion in man and sheep under heat stress.

Total Vaporization: It is observed from Figure 3 and Table 3 that in all breeds whether they were reared at 50° or 80°F. a stepwise increase in total vaporization took place with increasing temperature from 35°F. to 95°F. This correlation coefficients which were computed only for the values between 50° and 95°F. indicate a highly significant effect of temperature on total vaporization (Table 6). These results are in accordance with previous work on cattle (Kibler and Brody, 1950; Kibler and Yeck, 1959), on man (Robinson and Robinson, 1954), and on rabbits (Johnson, 1956).

There are no differences between the two groups of heifers in the total amounts of moisture vaporized at 35°F. (Table 8). However, the rates of increase in total vaporization with rising temperature (50°-95°F.) as shown by the regressions coefficients in Figure 5, and Table 7 is significantly higher, only at the 5 percent level, in the warm (80°F.) reared group, than in the 50°F. reared heifers (Table 9).

This suggests that the 80°F. reared group had probably acquired a functional improvement in their evaporative cooling system when they were reared at young age under a constant warm (80°F.) temperature for one year. Such improvement has been shown in the data of Kibler and Yeck (1959) on the same animals during their growth period. However, it seems that this acquired characteristic is responsible for the significantly higher percentage rate of increase in total vaporization of the 80°F. than that of the 50°F. reared groups under heat stress.

It is interesting, however, that in spite of this high rate of vaporization of the 80°F. group under heat stress, the absolute vaporization average of this latter group is not higher than that of the 50°F. group during rising temperature (Table 5). This is somewhat similar to the responses of the heat tolerant Zebu cattle which have lower total vaporization than the heat intolerant Shorthorn at high temperature and, yet, they have a more rapid increase in vaporization rate with rising environmental temperature (Kibler and Yeck, 1959).

It seems that rearing the 80°F. group at constant warm temperature for one year period did not only relatively reduce their glucocorticoids and relatively increase their mineralocorticoid and antidiuretic hormone secretion as suggested previously through changes in the sensitivity of the peripheral thermal receptors, but also it improved the functions of the evaporative cooling system of these animals. This improvement, probably, took place in the skin vaporization system. That accounts for most of the total vaporization. Whether this was achieved by improving the function of the sweat glands or the permeability of skin to water diffusion system, is an open question that warrants investigation. The existence of sweat glands in cattle has long been known, but their function was questioned. Hayman and May (1958) observed seasonal variation in sweat gland volume and attributed that to seasonal changes in the sweat glands activity. Recently, Taneja (1959) collected droplets of sweat from the sweat glands themselves of cattle, and thus demonstrated the functioning of sweat glands in cattle.

Therefore, the significantly higher rate of increasing vaporization in the 80°F. group than in that of the 50°F. group under heat stress may be due to a better efficiency of the sweating glands of the former rather than to the latter under heat stress. However, such interpretation should be accepted with precaution since the difference itself between the two groups in this concern was barely significant (Table 9).

Water Consumption: From Figure 3, and Table 3 it is shown that Brown Swiss, Holstein, and Jersey, heifers of the two reared temperatures 50° and 80°F. had progressive increase in water consumption as temperature increased from 35°

to 95°F. The rate of increase was somewhat faster at the high ambient temperatures. Statistical analyses of the data showed that such influence of temperature on water intake was highly significant in both groups (Table 6). These results are in accordance with other work done on cattle (Johnson *et al.,* 1958; Harbin *et al.,* 1958), sheep (Macfarlane *et al.,* 1958; Blaxter *et al.,* 1959), goats (Appleman and Delouche, 1958), rats (Mefferd *et al.,* 1957), man (Adolph, 1947), and not on rabbits (Johnson, 1956).

It is quite surprising to find that some animals, particularly the Jersey heifer, No. 633, had quite an abnormal water metabolism identical to the well-known diabetes insipidus disease in man. At high temperatures this heifer consumed water and excreted urine 6 and 8 times greater than the other Jersey's. The urine specific gravity was also abnormally low. When ADH was administered to this heifer a marked depression in urine and water consumption insued which was, however, of shorter duration than in the other normal treated heifer (Kamal *et al.,* 1959a). It seemed desirable to compute the heat dissipation of such an animal that had abnormal water metabolism if cold drinking water of 50°F. was provided under hot climate of 95°F. Such animal was found to be able to dissipate about 20 percent of its total heat production, as compared to only 7 percent in normal heifers, through thermal conduction between water, body, and urine. The significance of such characteristic will be discussed later in connection with heat tolerance.

There was no difference between the two groups in water consumption at low temperature 35°F. However, with increasing temperature from 50° to 95°F., the 80°F. group had slower rate of increase than the 50°F. group (Figure 5, and Table 7). Such difference is highly significant statistically (Table 9).

The slower rate of increase in water consumption of the 80°F. group, than of the 50°F. group, is not surprising since it has already been mentioned that the former group had slower rate of increase in urine volume than the latter group with rising ambient temperature. Kamal *et al.* (1959a), in this regard, showed that when urine excretion was inhibited in cattle by ADH injection, at high or comfort temperature, water consumption was stopped in normal heifers for a period of 10 hours.

Thirst stimulation has been discussed by many workers (Adolph, 1947; Andersson, 1952; Andersson and McCann, 1955; Wolf, 1956; Strauss, 1957). These workers and others mentioned previously have shown that thirst could be stimulated by many factors such as food ingestion, sodium depletion, extracellular fluid deficiency, concentration of the intracellular body fluid, body water deficit, and the hypertonicity of the extracellular fluid. However, most of these factors though different in nature still prove that thirst is stimulated through one main factor, which is the decrease in water concentration in the body. This has also been recently confirmed by writer's experiment (Kamal *et al.,* 1959a), which suggests the following mechanism by which water intake of the heifers was increased with rising environmental temperature (35°-95°F.).

Increasing ambient temperature causes significant increase in water loss

through urine and vaporization in cattle as previously mentioned. Such water loss causes a temporary body water deficit with resultant concentration of body fluids (extracellular and intracellular) including the fluids of the thirst center of the hypothalamus. The latter, thus, passes nerve impulses to the higher brain centers producing the sensation of thirst and active drinking response. Such response was demonstrated experimentally in goats either by inducing a hypertonic condition in the thirst center of the hypothalmus or by electric stimulation of the latter (Andersson, 1952; Andersson and McCann, 1955). If water is available, the animal repletes his water loss by drinking enough water to cause the increase in blood volume, which is demonstrated in cattle under heat stress by Dale *et al.* (1956). With continuous exposure to heat and with increasing the environmental temperature, the process of water depletion and repletion occur frequently in short or long intervals depending on the magnitude of heat and humidity. The high frequency of this process at high environmental temperature with a rise in the latter is suggested to account for the increase of water consumption of heifers under such temperature conditions.

Effect of Environmental Temperature and Thermal Acclimation on Plasma Protein Content:

The data of plasma protein content of the 50° and 80°F. reared heifers are presented in Figure 4 and Table 2 and expressed as plasma total nitrogen. It is shown from this Figure and Table that, in cold, the 80°F. reared heifers had about 50 percent higher plasma protein content than that of the 50°F. animals. This was true for all breeds and such difference was found to be highly significant statistically (Table 8).

The inverse relationship between plasma volume and plasma protein content is a well known fact (Bazett *et al.,* 1940; Conley and Nickerson, 1945; Spealman *et al.,* 1947; Rodbard *et al.,* 1951; Bass and Henschel, 1956). The explanation for such phenomenon can be understood from the following example. Under high environmental temperature when vasodilation occurs with deflection of blood to skin to augment the thermal conductance via body surface, the hydrostatic pressure in the blood vessels falls below the colloidal pressure. Therefore, the interstitial fluid is shifted from the cell interspaces to the plasma due to pulling force of the plasma protein, i.e., colloidal pressure. This results in an increase in plasma volume and, consequently, a dilution of the plasma protein.

From the previous water balance chart (Figure 3) it is observed that although both 50° and 80°F. groups had almost the same value in either water consumption or total vaporization, the 80°F. group had significantly higher water loss via urine than the 50°F. heifers. This was true in all breeds. It is thus expected that the 80°F. animals had lower plasma volume and therefore, higher plasma protein content than the 50°F. group. This is because both groups gained the same amount of water and yet 80°F. group lost twice or more as much water as the 50°F. group.

With increasing temperature from 35° to 50°F. quite the reverse occurred.

The 80°F. group dropped, while the other group increased. These opposite changes in plasma protein content in the two groups of heifers were quite identical to the corresponding changes in water loss in 80°F. and 50°F. heifers (Figure 3).

Raising the temperature from 50° to 95°F. the plasma protein content decreased progressively in all breeds of both groups. Such decline is presented by significant negative correlations between plasma protein content and environmental temperature (Table 6), and also negative regression coefficients (Table 7). The decline in plasma protein content at high environmental temperature and its increase in cold has been demonstrated in man (Bazett *et al.*, 1940; Conley and Nickerson, 1945; Spealman *et al.*, 1947; Bass *et al.*, 1955).

In cattle, the only available information is the data of Brody (1949) where no appreciable difference between the control cows (50°-60°F.), and the experimental cows (70°-100°F.) was found in plasma protein concentration and therefore, it was reported that water balance in cattle unlike man could be maintained fairly normal at high temperatures (Blincoe and Brody, 1951). In goats, Appleman and Delouche (1958) reported some values of plasma protein at different temperatures. Although the authors did not discuss this particular data it seems that there was a tendency in plasma protein to increase with rising temperature (20° to 40°C.).

The significant decline in plasma protein with rising temperature in the present investigation, though, seems to be due primarily to an increase in plasma volume, which increased in cattle at high temperature (Dale *et al.*, 1956); yet, other factors may also be involved. The effect of nitrogen intake on plasma protein concentration has been indicated in sheep (Gorbelik, 1956), in rats (Schoenheimer *et al.*, 1942), and in dogs (Madden *et al.*, 1943; Robischeitz-Robbins *et al.*, 1943). These workers indicated that when the protein intake is reduced the plasma protein concentration decreases. In this study, the nitrogen intake as mentioned before was significantly depressed with rising temperature (Table 6). Such decline, which reached barely the maintenance level at high temperatures, is expected to contribute to the decrease in plasma protein concentration with rising environmental temperature.

The data of this investigation on water, electrolyte, and protein, metabolism which will be discussed later, suggest an increase in the catabolic glucocorticoids hormones with rising temperature in cattle. The injection of these hormones in rabbits causes a decrease in the total serum protein (Matsuda, 1956). Therefore, it is also possible that the decline in plasma protein concentration with rising environmental temperature in this study is partially due to the suggested high glucocorticoids secretion in cattle at high temperatures.

When comparing the two groups it is shown from Figure 6 and Table 7 that the heifers which were raised at 80°F. demonstrated again the same milder response to heat, true too, of the previously mentioned characteristics. They had a significantly slower decline in plasma protein concentration with rising temperature than the 50°F. group (Table 9). This difference, however, cannot be

due to difference between the two groups in nitrogen consumption since no significant difference in the latter is indicated (Table 9). The possibility that this difference is due to a lower secretion of glucocorticoids in the 80°F. group than in the others under thermal stress has been suggested previously in connection with water and electrolyte metabolism, and later in protein metabolism. On the basis of the inverse relationship that exist between plasma volume and plasma protein content (Bazett *et al.,* 1940; Conley and Nickerson, 1945; Spealman *et al.,* 1947; Rodbard *et al.,* 1951; Bass and Henschel, 1956), one can also suggest that the slower decline in the 80°F. group in plasma protein is due to a slower increase in plasma volume in the 80°F. reared heifers with rising temperature. This means a milder response to heat in the 80° group than in the 50°F. group. This mild response to rising environmental temperature in the heat acclimated heifers has been indicated in urine sodium, urine Na/K, water metabolism, and now in plasma protein, and later on protein metabolism and blood glucose. Such consistency in the results in all breeds indicates the significance of the factor that is responsible for such a milder response to heat in the 80°F. reared heifers than in the 50°F. group. Whether this factor is a lesser sensitivity to heat in the receptors of the periphera, or of the hypothalamus, or whether it is the action of some other mechanisms, to be discussed later, is a problem that warrants investigation.

Effect of Environmental Temperature and Acclimation on Protein Metabolism:

Figure 4 and Table 4 show that the curves of urine total nitrogen for the breeds that were reared at 50° or 80°F. are quite identical to their corresponding curves of urine potassium (Figure 2). At low temperature (35°F.) the 80°F. reared group of Brown Swiss, Holstein, and Jersey, heifers indicated the same response to cold as mentioned before in regard to urine volume as in regard to sodium and potassium excretions. They had a maximum urinary nitrogen excretion, which is significantly higher (0.01) than that of the 50°F. group (Table 8). In contrast, the 50°F. group had a fairly low level of urine nitrogen.

The difference in urine total nitrogen is not due to significant difference in the digestible nitrogen consumption between the two groups as shown in Table 8. This resembles the previous observation on water metabolism in that the 80° F. group had a higher urine volume than the 50°F. group at the same low temperature (35°F.), and this was not due to any significant difference in water consumption.

A higher urinary nitrogen excretion in the 80°F. reared group than in the 50°F. animals, with a similar digestible nitrogen consumption at 35°F., resulted in a significantly lower nitrogen retention in the former group than in the latter (Table 8). Though the difference between the two groups in body weight was not significant, the 80°F. group gained about 22 percent less weight than the other group during this period of cold exposure (35°F.). The averages of body weight at the beginning of the experiment were 322.1 and 320.3 kg./heifer for

the 50° and 80°F. groups, respectively. When these averages are compared to those at 35°F. (Table 5) the resultant gain in body is 20.8 and 16.3 kg./heifer for the 50° and 80°F. groups, respectively. This lower gain in body weight at low temperature in the 80°F. reared group than in the other group is a reflection of the corresponding changes in nitrogen excretion and retention.

To find that the temperature level at which an animal is raised influences its reaction at cold to such a significant extreme provides valuable information on acclimation. Heifers that were reared at 50°F. demonstrated more comfort at temperatures lower than at which they were raised as shown by high nitrogen retention and body weight gain at 35°F. It seems that thermal acclimation could move the comfort zone of the animals back and forth according to the temperature of acclimation as was suggested earlier by Brody (1948). Other workers have shown that rearing animals under warm climate renders them sensitive to cold, as shown by low nitrogen retention and body weight gain at 35°F. In this regard, Mefferd and Hale (1958) studying cross adaptation in rats came to the conclusion that cold exposure of heat-acclimated rats constituted the most severe stress they had employed. Treichler and Mitchell (1941) found that prior environmental temperature exerts a greater influence on excretion of endogenous nitrogen than does the prior plane of nutrition.

It seems that this significantly higher nitrogen excretion and less nitrogen retention as well as lower gain in body weight of the 80°F. group than in the 50°F. group, which occurred during the cold exposure (35°F.), is due to the same reason that caused higher urine volume as well as higher sodium and potassium excretion in the former group than in the 50°F. group at the same low temperature. It has already been mentioned in the Review of Literature section, that the maintenance of protein metabolism is mainly controlled by diet and hormones. At this low temperature (35°F.) the two groups were consuming almost the same high level of adequate amounts of a balanced ration. The digestible nitrogen consumption as shown in Table 5 was about 150 g./heifer/day in both groups. Therefore, the difference between the two groups in nitrogen excretion and retention cannot be due to difference in feeding levels.

The hormones that affect protein metabolism in these young heifers, as mentioned in the review, are glucocorticoids, insulin, and growth hormones. The glucocorticoids are catabolic, while the other two are anabolic. Glucocorticoids enhance the protein mobilization and its breakdown to amino acids (Russell, 1955; Wool and Goldstein, 1958). These hormones also enhance further hepatic capture of the amino acids from blood after having been released from tissue protein (Noall *et al.,* 1957). Moreover, the glucocorticoids act specifically on the enzyme glutamic-pyruvic transaminase which is rate-limiting in gluconeogenesis (Rosen *et al.,* 1959). They also inhibit the glucose uptake by tissues (Ingle *et al.,* 1947; Welt *et al.,* 1952; Ingle *et al.,* 1953), and, thus, depress protein synthesis by not providing the energy required for such synthesis. The inhibition of glucocorticoids on the anabolic mechanism of insulin by forming an inhibitor factor in the serum lipoprotein fraction was indicated by Bornstein and

Park (1953). The anabolic effects of growth hormone are, however, dependent on insulin sufficiency (Russell, 1955; DeBodo and Altszuler, 1957).

The specific catabolic effects of glucocorticoids on protein metabolism mentioned above as well as their inhibitory effects on insulin and consequently on growth hormone anabolic actions result in the appearance of the excessive nitrogen excretion, low nitrogen retention and sometimes loss in body weight observed by many workers (Campbell *et al.,* 1954; Luft *et al.,* 1954; Doolan *et al.,* 1955; Pechet, 1955). This evidence, therefore, enhances the belief that the higher nitrogen excretion, lower nitrogen retention and lower body weight gain as well as previously mentioned higher kaluresis, natruresis and urine excretion in the 80°F. group than in the 50°F. group at cold exposure is due mainly to a high glucocorticoids secretion in former group upon exposure to low ambient temperature (35°F.).

Another explanation for the aforementioned differences between the two groups upon their exposure to cold is suggested from the fact that "stress", as such, is associated with protein catabolism, even in the absence of the adrenal glands (Selye, 1950; You *et al.,* 1950). The last authors observing marked increase in urinary nitrogen excretion in the DCA-injected adrenalectomized rats upon their exposure to cold, concluded that, although the presence of adrenal glands is important in the full response to cold and augmentation of nitrogen excretion, the increased protein catabolism at cold temperature was not primarily due to the action of adrenal glands. However, the question rises as to whether the "stress" as seen by Selye and others exists in cattle. No information whatsoever is known about the occurrence of the G-A-S in cattle which is the manifestation of "stress". This subject, will be dealt with later in the discussion. However, it is very possible that the higher nitrogen excretion, and lower nitrogen retention and body weight gain in the 80°F. heifers than in the others in this investigation could be due to both "stress" along with augmentation of glucocorticoids secretion as a function of "stress" in the former group. The reason for such higher sensitivity to cold in the 80°F. group rather than in the 50° F. reared heifers was discussed in this discussion.

When environmental temperature increased to 50°F. a sharp rise in nitrogen excretion accompanied by a marked decline in nitrogen retention occurred in all breeds which were reared at cold temperature. In the 80°F. group a decline in urinary nitrogen excretion of the Brown Swiss, and Holstein, and little change in that of Jersey accompanied by slight opposite changes in nitrogen retention took place with the same rise in temperature to 50°F. This indicates probably an increased glucocorticoids secretion due to a mild "warm" effect in the 50°F. group, and decrease in these hormones in the 80°F. group, with exception of the Jersey heifers, due to a relief of cold stress. The abnormal response of the 80°F. Jersey heifers at 50°F. in nitrogen excretion and nitrogen retention was also demonstrated in urinary excretion of sodium and potassium (Figure 2). It seems that these heifers did not gain from their acclimation to heat as compared to the other breeds, as will be shown later with rising environmental temperature.

With rising environmental temperature above 50°F. the nitrogen excretion stayed at a fairly high and constant level till 70° or 80°F. in most of the animals and it declined, thereafter, until 90°F. with a slight rise in most animals at 95°F. However, the overall trend from 50° to 95°F. in nitrogen excretion is represented by negative regression coefficients in both groups (Figure 6, and Table 7), and highly significant negative correlations between rising temperature (50°-95°F.), and urine nitrogen (Table 6). The nitrogen retention in 50° and 80°F. groups also declined progressively with rising temperature from 50° to 95°F. The effect of temperature is shown statistically significant as shown by the correlation coefficients (Table 6).

The decrease in urinary nitrogen with rising temperature is quite identical to that of potassium excretion which was previously discussed. Urinary excretion under thermal stress is affected by two opposing factors, the suggested increase in glucocorticoids that tend to elevate the nitrogen excretion and the deprivation of food or nitrogen source which is shown to be significantly depressed with rising temperature (Table 6). That the decline in food intake is associated with a decline in nitrogen excretion was indicated earlier by Armsby (1903), and more recently by Calloway and Spector (1955), Morrison (1956), and Birnbaum *et al.* (1957). This is true at low, medium and high temperatures (Mefferd and Hale, 1957b). In man, food deprivation was shown by decrease urinary nitrogen excretion even during continuous administration of hydrocortisone (Thorn *et al.,* 1955).

Under such circumstances, however, where the decline in nitrogen intake masks the effect of any catabolic factor that may increase the nitrogen excretion, the best way to know whether or not there is a catabolic reaction (for example, enhanced gluconeogenesis by glucocorticoids oversecretion) taking place when nitrogen excretion is reduced by low nitrogen intake, is to study the change in nitrogen retention. If no induced catabolic reaction is envolved, nitrogen retention should not change, regardless of the change in nitrogen intake, unless the animal is on a submaintenance feeding level or on a nitrogen free diet, which were not the case in the present study. On the other hand, if there is an induced catabolic reaction the nitrogen retention should change at any feeding level.

Nitrogen retention was computed, however, by subtracting urine nitrogen from digestible nitrogen consumption. One constant digestible coefficient at all temperature levels was used assuming that temperature has, if any, very slight affect on digestibility that would be of negligible effect on the computed nitrogen retention. This assumption is true as indicated by Graham *et al.* (1959) on sheep and recently by Davis (1960) on cattle, who showed that increasing temperature from 50° to 90°F. caused only 5 percent increase in digestibility. With rising temperature (50° to 95°F.) all heifers in this investigation showed a gradual decline of nitrogen retention of about 100 percent. This amounted in most animals to a negative nitrogen balance at 90° and 95°F. This decline is represented by negative regression coefficients (Figure 6, and Table 7), and was found

highly significant, statistically (Table 6). This indicates that rising environmental temperature is associated with progressive increased protein catabolism. Again, whether this increased protein catabolism is due to "stress", increased glucocorticoids secretion, or to both is a problem that warrants investigation. In cattle, however, such a vital measurement, i.e., nitrogen retention has never before been investigated under thermal stress. The writer believes that nitrogen retention, as will be discussed later, is the basis on which selection for heat tolerance in cattle should be made.

Excessive urinary and sweat, nitrogen excretion, and negative nitrogen balance, during heat exposure were indicated in man (Dill *et al.,* 1933; Mitchell and Hamilton, 1949; Conn, 1949; Bass *et al.,* 1955), in rats (Mefferd *et al.,* 1957; and Mefferd and Hale, 1958), and in sheep (Graham *et al.,* 1959). This excess occurs in spite of a low glucocorticoids production in man at high temperatures (Bass *et al.,* 1955; Hellman *et al.,* 1956; Watanabe and Yoshida, 1956; Macfarlane and Robinson, 1957; Robinson and Macfarlane, 1958). From this, it is apparent that protein catabolism cannot be due primarily to glucocorticoids secretion, as also indicated by the work of You *et al.* (1950) which is reported in the review of this study. It seems that the stressor heat, as such, may increase protein catabolism through its direct action on gluconeogenesis without the mediation of the glucocorticoids. However, if, in some way, the secretion of these catabolic hormones is stimulated by high environmental temperature, such as the case in cattle and not in man, as suggested from the present results, the increased protein catabolism that occurs in heat would eventually augment. In this investigation the previous results on water and electrolyte metabolism suggest that the decrease in nitrogen retention with rising temperature is due to an increase in glucocorticoids secretion under thermal stress is cattle. However, the fact that "stress" may occur in cattle and thus may contribute to the decrease in nitrogen retention, as well as to the other results shown in this investigation, is very possible. That "stress" may occur in cattle during exposure to heat, however, will be discussed later. It is interesting to find that the Jersey heifers, which have long been known to be heat tolerant, showed the same rate of decline, if not faster, in nitrogen retention with rising temperature from 50° to 95°F. similar to the other breeds. This indicates that some animals within a heat tolerant breed could be exceptionally intolerant to heat, assuming that the nitrogen retention is a good index of heat tolerance, as will be discussed in Chapter V.

Comparison of the 50° with the 80°F. groups in their change in protein metabolism with rising temperature (50°-95°F.) showed that the 80°F. reared heifers retained 23.11 as compared to 12.92 g./heifer/day in the 50°F. group, while there was no appreciable difference between the two groups in the digestible nitrogen consumption (Table 5). The rate of decline in nitrogen retention, as shown in Figure 4, is slower in the 80°F. reared Brown Swiss and Holstein heifers than in 50°F. animals of the same breeds. The 80°F. reared Jersey heifers, however, which had higher and faster increase in sodium excretion (Figure 2) than the 50°F. Jerseys, also showed lower level and faster decline in nitrogen

retention than the 50°F. reared Jerseys (Figure 4). The percentages of decline in nitrogen retention at 95°F. as compared to 50°F. in 50°F. reared Brown Swiss, Holstein, and Jersey heifers were 105, 162, and 161 percent, respectively, while the values for the 80°F. reared animals in the same order were only 49, 60, and 259 percent, respectively. Such abnormal response of the 80°F. Jerseys indicates that they did not benefit from their earlier acclimation to heat. When the data of all breeds, including those of the Jersey heifers, were pooled for computing the regression coefficients of nitrogen retention, eventually no significant difference was obtained between the 50° and 80°F. reared groups in the rates of decline of nitrogen retention with rising temperature (50°-95°F.), as shown in Table 9.

It is rather interesting to find that, in spite of the significant decline in nitrogen retention with rising temperature, there was no significant change in body weight with rising temperature from 50° to 95°F. (Table 6). This is not surprising because the animals, though they had low nitrogen retention at high temperatures, were still on a positive nitrogen balance during most of the heating period and, thus, were expected to be gaining weight, but at a declining rate. It should also be emphasized that these animals were not completely mature and, thus, were gaining some weight, though a small amount, regardless of the heat stress. This accounts for the difference between these results and others on mature cows. Ragsdale *et al.* (1951) showed that high temperature reduces the body weight of cows. The possibility that the animals were retaining water under heat stress also cannot be excluded, as indicated by higher blood and plasma volume in cows under heat stress (Dale *et al.*, 1956), and, as has been shown earlier in this discussion of plasma proteins, by the fact that the heifers were progressively hydrated with rising temperature from 50° to 95°F.

Effect of Environmental Temperature and Thermal Acclimation on Blood Glucose Content:

The concentration of blood glucose in adult animals is known to be constant under ordinary circumstances, and to vary within relatively narrow limits in most species of animals. The maintenance of such levels represents one of the most typical examples of that kind of regulation which has been designated "homeostasis" by Cannon (1929). However, because of to some endogenous or exogenous factors, depending on the severity of the stimulus, the homeostatic mechanism of the animal fails to maintain the blood sugar level constant or at a "steady state". Such stimulus could be provided by hereditary disorders, such as hormonal imbalance (for example, low insulin secretion with resultant diabetes mellitus), liver disfunction, renal abnormalities, etc., or it could be environmentally induced. The objective of this phase of the study was to find if and how the environmental factors, and particularly the ambient temperature, could alter the blood glucose level in cattle.

It is observed from Figure 4, and Table 2 that when the dairy heifers in this study were exposed to cold (35°F.) the Jersey heifers that were raised at 80°F.

had higher blood glucose level than that of the 50°F. animals. The other two breeds, though, had almost similar values to those of the 50°F. reared animals, yet their values seem to be somewhat more elevated than their general average. The overall mean, however, of all breeds of the 80°F. group is shown to be higher than that of 50°F. group (Table 5). Such difference is statistically significant, but only close to 5 percent level (Table 8).

It has been mentioned before that blood glucose level could be markedly altered by hormonal and dietary affects. The feeding level, however, was not significantly different between the two groups (Table 8), while the glucocorticoids secretion of the 80°F. group, according to the present results on water, electrolyte, and protein metabolism, are suggested to be higher in the 80°F. than in the 50°F. groups. The hyperglycemic action of glucocorticoids has been demonstrated on cattle (Shaw *et al.,* 1951; Dye *et al.,* 1953; Chung, 1958) as well as in man and other species (Renold *et al.,* 1956; Shull and Mayer, 1956). These hormones increase blood glucose level mainly by enhancing gluconeogenesis through activating the enzyme glutamic-pyruvic transaminase (Rosen *et al.,* 1959), and enhancing the hepatic capture of amino acids (Noall *et al.,* 1957). They also contribute to the high level of blood glucose by inhibiting the peripheral utilization of the latter (Ingle *et al.,* 1947; Welt *et al.,* 1952; Ingle *et al.,* 1953), or by inhibiting the hypoglycemic action of insulin (Bornstein and Park, 1953).

It is possible that other hyperglycemic hormones such as epinephrine were secreted at a greater rate in the 80°F. than in 50°F. group during cold exposure. That epinephrine injection causes hyperglycemia in cattle has recently been demonstrated (Schultz, 1959).

With increasing temperature to 50°F., the blood glucose content of the 50° F. reared Holstein and Jersey heifers increased, while that of the 80°F. reared heifers decreased. Similar but slight changes, however, took place in the Brown Swiss heifers. As temperature increased from 50° to 95°F. a gradual decline in blood glucose concentration in both groups took place. Such decline is highly significant statistically, as shown from the correlation coefficients (Table 6). These results are in accordance with those of Riek and Lee (1948) and Brody (1949) on cattle and of Kanter (1954) on dogs.

The decrease of blood glucose level with rising temperature is a result of two opposing factors. The significant diuresis, natruresis, decrease in plasma protein content, in nitrogen retention as well as in the mineralocorticoids (increase urine Na/K) observed in this investigation under heat stress suggested an increase in the glucocorticoids secretion. These hormones, as mentioned above, have hyperglycemic action in cattle as well as in other species, and therefore, an increase rather than a decrease should be expected to occur in the blood glucose level at high temperature. The significant decline in feed consumption with rising tempearture, however, as observed in this study (Table 6) is well known to cause a decrease in blood glucose concentration in cattle (Hodgson *et al.,* 1932; Leffel and Shaw, 1957), in sheep (Reid and Hogan, 1959), and in goats and

deer (Forbes, 1943; Teeri *et al.*, 1958). A third factor involved in the decline of blood glucose content in the rising temperature is the marked increase in respiratory activity under heat stress which was indicated in this study as well as in previous work on cattle (Kibler and Brody, 1949). This increase in respiration rate causes a rapid utilization of blood glucose by the respiratory muscles and thus results in the decrease in blood glucose content under heat stress. In this regard, Kanter (1954) showed that when dogs were panting severely under heating condition (120°F.) neither dehydration nor ingestion of glucose at a rate of 1 percent of body weight per hour could prevent the decline in blood glucose.

It seems, therefore, that the highly significant decline in blood glucose in these dairy heifers was mainly due to a high respiratory activity of these animals as well as to the significant decline in feed consumption which occurred with rising environmental temperature. These factors were of such magnitude that they overweighed the hyperglycemic effect of the suggested glucocorticoids hypersecretion under heat stress.

The regression coefficients (Figure 6, and Table 7) show that the blood glucose content of the 80°F. group was declining with rising temperature at a slower rate than that of the 50°F. reared heifers. The difference between these two rates of decline is highly significant, statistically (Table 9). This, however, is not due to a difference between the two groups in their rates of decline in feed consumption (Table 9). No difference also seemed to occur between the two groups in the rate of increase in respiration rate (unpublished data). The glucocorticoids secretion were suggested previously to be lower in the 80°F. group than in the other group with rising environmental temperature and, thus, could not account for the slow decline in blood glucose content of the 80°F. group.

It is possible, however, that this difference is caused by that heat acclimation might have increased the metabolic efficiency of the 80°F. reared heifers. This would enable the animals to utilize less amount of glucose than the 50°F. group and to produce the same amount of energy used for the respiratory muscular activity under heat stress. In this case the 80°F. group would have had less energy expenditure and higher net energy than the 50°F. group under heat stress. This, however, is confirmed by the fact that 80°F. group, with exception of the Jersey heifers, had higher nitrogen retention than the 50°F. group under heat stress (Figure 4, and Table 4). The mechanisms involved in such metabolic adaptation caused by acclimation of the animal to certain environment has been discussed by Potter (1958).

GENERAL DISCUSSION

The Effect of Mild Cold (35°-50°F.) and Heat (50°-95°F.) on Protein, Carbohydrate, Electrolyte, and Water Metabolism:

The dairy heifers that were raised at constant temperature (50° and 80°F.) for one year were exposed to rising environmental temperature (35° to 95°F.),

after they had been thermally equalized at 80°, 90°, and 52°F., for about one month so that a comparison of the reactions of warm acclimated heifers to those of the heifers that were acclimated to cold, under various environmental temperatures, could be made.

In cold (35°-50°F.), the direction and magnitude of the change in biological reactions studied thus far were largely dependent on the rearing temperature. The responses of the 80°F. group to cold were opposite, and significantly different from those of the 50°F. group in most characteristics. However, higher temperatures (50°-95°F.), the temperature at which the heifers were raised, had effect on the magnitude of responses of the animals, but had no effect on the general trend of the responses. This was true in all breeds studied.

It is observed that the group of heifers that was reared in mild cold (50°F.) showed favorable responses to temperatures lower than that temperature at which it was raised. At 35°F. there was very low or almost minimum excretion of nitrogen, sodium, potassium, and water in urine. Nitrogen retention was remarkably high. At the same low temperature the 80°F. reared heifers were demonstrating catabolic reactions. The animals had marked diuresis, kaluresis, and natruresis, as well as elevated blood glucose concentration. The urinary excretion of nitrogen was at a maximum level, and nitrogen retention was fairly low. Plasma volume seemed to be reduced due to the diuresis and to the very low level of water consumption. This was indicated by an elevated plasma protein concentration. The differences between 50° and 80°F. groups in all these reactions in cold was found to be statistically significant (Table 8). Furthermore, the 80°F. group had about 22 percent less body weight gain than the 50°F. group during the period of cold exposure.

It is well-known that glucocorticoids, when administered in moderate dosages in man, dogs, or rats, cause augmentation of urinary excretion of sodium and potassium (Streeten *et al.,* 1955; Swingle *et al.,* 1957; Eversole and Romero, 1958; Lipsett and Pearson, 1958; Barger *et al.,* 1958; Ross *et al.,* 1959; Swingle *et al.,* 1959). They also cause marked diuresis in these animals (Ingle, 1950; Luetscher, 1954; Montastruc, 1954; Luft *et al.,* 1955; Raisz *et al.,* 1957; Swingle *et al.,* 1957; Kleeman *et al.,* 1958). Glucocorticoids also enhance the breakdown of protein to amino acids as well as the destruction of the amino acids by gluconeogenesis with resultant increase in urinary nitrogen excretion and decrease in nitrogen retention (Campbell *et al.,* 1954; Luft *et al.,* 1954; Russell, 1955; Doolan *et al.,* 1955; Pecht, 1955; Noall *et al.,* 1957; Wool and Goldstein, 1958; Rosen *et al.,* 1959). From this evidence, it is probable that the natruresis, kaluresis, diuresis, the elevated blood glucose concentration, the excessive nitrogen excretion, and low nitrogen retention, observed in the 80°F. reared group at cold temperature (30°F.) is caused by an elevation of glucocorticoids secretion. It seems that the Krause endbulbs or cold receptors of the periphera in the 80° F. reared heifers were sensitive to such mild cold (35°F.), thus, they were passing many nervous impulses at this temperature to the hypothalamus, causing, thereby, high ACTH and glucocorticoids secretion and, consequently, the afore-

mentioned reactions. This mechanism, however, is explained in detail in the Review of Literature section.

Cold is a "systemic stressor" that evokes in the body a group of metabolic reactions, such as diuresis, kaluresis, excessive loss of chlorides in urine, and high blood glucose concentration (Selye, 1950). These reactions are similar to those that occurred in the 80°F. reared heifers during cold exposure. It is possible, therefore, that the exposure of the 80°F. reared heifers to cold has induced "stress" in the animals, as indicated by the occurrence of the aforementioned reactions. These results, however, as well as the possible occurrence of "stress" in cattle at cold climate, as will be discussed later, have not been previously investigated.

When temperatures were raised to 50°F., both groups demonstrated opposite changes to each other in most of the studied characteristics. The 50°F. reared heifers started to show unfavorable reactions which were catabolic in nature. The 80°F. group, on the other hand, with the exception of the Jersey heifers, showed some indication of relief of cold stress. Diuresis, kaluresis, and natruresis, were ameliorated to a great extent in the 80°F. group. Plasma volume also seemed to be restored as shown by the depression in plasma protein which is known to have an inverse relationship with plasma volume (Bazett *et al.,* 1940; Conley and Nickerson, 1945; Spealman *et al.,* 1947; Rodbard *et al.,* 1951; Bass and Henschel, 1956).

With rising temperatures (50° to 95°F.), all the heifers that were raised at either 50° or 80°F. showed similar directional changes in practically all characteristics so far studied, though the magnitude of response was significantly different between the two groups. There were significant increases in nitrogen loss (i.e., decrease in nitrogen retention), in urinary sodium excretion, and in urinary Na/K ratio, with even negative sodium and nitrogen balance at high temperatures. However, the urinary nitrogen and potassium excretion as well as the blood glucose and plasma protein concentrations were significantly decreased. Opposite changes, but insignificant, to those of urine electrolytes took place in plasma electrolytes. Water consumption, total vaporization and urine volume of a low specific gravity were significantly increased while digestible nitrogen consumption was significantly decreased. No significant change in body weight, however, was observed. The statistical significance of all these results was indicated in Table 6.

The changes in electrolyte metabolism, as well as the marked diuresis, observed in the heifers of this study with rising temperature are typical of mineralocorticoids insufficiency where potassium excretion decreases and sodium and urine excretion, as well as the urinary Na/K ratio, increase (Simpson and Tait, 1955a; Simpson and Tait, 1955b; Mach and Fabre, 1955; Goding and Denton, 1957; Barger *et al.,* 1958; Swingle *et al.,* 1959; Ross *et al.,* 1959). The increase in sodium excretion, urine volume, and decrease in nitrogen retention, suggest an increase in glucocorticoids secretion as mentioned before. Such increase in glucocorticoids might have prevented the release of the ADH and, thus, con-

tributed to the diuresis and low specific gravity of urine at high temperature. That ADH inhibits urination in cattle has been indicated (Kamal *et al.,* 1959). The mechanism by which heat could stimulate the glucocorticoids secretion has been discussed earlier. The significant diuresis as well as high total vaporization, that occurred in these heifers with rising temperature, however, do not seem to cause dehydration or negative water balance in the animals. This, because there was a significant decline in plasma protein concentration with rising temperature which may indicate a hydration rather than dehydration. The decrease in mineralocorticoids secretion at high temperatures in cattle might be brought about by the increased sodium to potassium ratio in the feed caused by significant depression in hay consumption, or by the suggested increase in glucocorticoids secretion. That mineralocorticoids secretion is regulated by the levels of sodium and potassium intake has been demonstrated (Singer and Stack-Dunne, 1954; Rosenfeld *et al.,* 1956; Johnson *et al.,* 1957; Miller *et al.,* 1958).

The inverse relationship between glucocorticoids and mineralocorticoids suggested in the heifers at high temperature is not surprising. Dasgupta and Giroud (1958) showed that after short treatment of nephritic rats with cortisone acetate the high aldosterone secretion was reverted to normal.

The significant decline in potassium, urinary nitrogen excretion, and in blood glucose concentration with rising temperature, however, are somewhat contradictory to what is expected to occur with high glucocorticoids secretion. The decline in the concentration of these elements, however, may be a result of the significant decline in feed consumption with rising temperature which probably overweighed the expected effect of glucocorticoids on these constituents. The decrease in urinary potassium and nitrogen excretion and in blood glucose concentration due to the deficiency of these elements in diet has been indicated for potassium (Johnson *et al.,* 1957), for nitrogen (Thorn *et al.,* 1955; Calloway and Spector, 1955; Morrison, 1956; Birnbaum *et al.,* 1957), and for blood glucose (Reid, 1950; Leffel and Shaw, 1957; Goelsch and Pritchard, 1958; Reid and Hogan, 1959). It is also possible that the respiratory muscular activity, which increases in cattle in heat (Kibler and Brody, 1949), was utilizing a greater amount of blood glucose with increasing temperature, as shown from the work of Kanter (1954), and, thus, contributed to the gradual decline in blood glucose with rising temperature. The respiratory alkalosis and the elevation of blood pH, that accompanied such high respiratory rates in heat (Dale and Brody, 1954), might also have prevented the normal effect of glucocorticoids on cellular potassium, as shown by Reinberg and Stolkowski (1957), and, thus, resulted in the decrease, instead of an increase, in urine potassium in these heifers at high environmental temperatures. These aforementioned reactions, i.e., diuresis, natruresis, low nitrogen retention, are, however, typical to those of "stress" which are reported by Selye (1950).

The comparison between these results on cattle, and those on man and other species of animals, has been extensively discussed in the Results and Discussion section. While cattle and sheep show increase in urine volume, urinary

sodium and urinary Na/K ratio in heat, man shows the opposite of these reactions in heat. This is probably because of the difference between ruminants and man in their hormonal response to heat. It has been shown, in man, that the mineralocorticoids are decreased in heat. The present results on cattle, however, suggest quite the opposite. Such a difference between these two species is not understood. Probably the warm receptors in cattle are more sensitive to heat than they are in man whose skin temperature is cooled to a great extent by profuse sweating.

In this study, the interference of the effect of low feed intake with that of heat *per se* in altering the metabolic responses of animals to rising environmental temperature has been demonstrated by the unexpected decline in blood glucose content and in urinary nitrogen and potassium excretions as explained above. Such interference could be eliminated by feeding the animals a constant amount of ration at all temperatures by introducing the refused feed at high temperatures into the rumen through a fistula.

The Possibility of the Occurrence of "Stress" in Cattle During Their Exposure to Heat and Cold:

"Stress" as defined by Selye (1956a) is a state manifested by specific syndrome which consist of all the nonspecifically induced changes within a biologic system. In this sense, "stress" has its own characteristic form, i.e., G-A-S and L-A-S, but no particular cause. It can be induced by various stressors such as cold, heat, injury, etc., and it has been characterized by definite measurable organ lesions and biochemical alterations reported by Selye (1950-1956a). The fundamental reactions of the G-A-S are adrenal hyperactivity, nitrogen loss, hyperkalemia, hypochloremia, thymo-lymphatic involution, low lymphocyte count, and gastrointestinal disturbances or ulcers. Therefore, in order to investigate the occurrence of "stress" in cattle, these animals should perform the typical G-A-S whenever they are exposed to various types of stressors, similar to rats, mice, and man. No such information, however, is available on cattle. Therefore, it is important that the term "stress" whenever used in animal husbandry, should be defined in order to avoid the confusion with the G-A-S reactions. The writer has defined thermal stress in cattle at the beginning of the last chapter. It is a state elicited in cattle upon their exposure to temperatures which are above or below the comfort zone. Such state is manifested by an array of biological reactions showed by, among others, Brody *et al.,* (1948-1957), Findlay (1950), Johnson *et al.* (1957, 1958), MacDonald and Bell (1958), and Lee (1959).

Nevertheless, there are some observations in the present study, as well as in others, which indicate that "stress" may occur in cattle. In this investigation, two stressors were applied on cattle, i.e., heat and cold. Both stressors induced similar changes in certain characteristics. Diuresis, natruresis, and low nitrogen retention, were observed either in cold, such as in 80°F. reared heifers, or in heat, such as in 50° and 80°F. reared heifers. The occurrence of high glucose content in cold in this study also is an indication of "stress". The adrenal corti-

cal hyperactivity in artificial heating or in summer season has been indicated in cattle by the decrease in blood cholestrol and ascorbic acid (Brody, 1949; Blincoe and Brody, 1951) or by the increase in reducing corticoids (Holcombe, 1957). The occurrence of abomasal ulcers in cattle, due to non-specific factors, has been recently demonstrated (Tasker *et al.,* 1958).

This data indicates that a definite group of physiological and biochemical alterations which are similar to those of the G-A-S could be induced in cattle by no one specific stimulus, such as cold or heat in this particular study. It can be concluded, then, that "stress", as seen by Selye (1950), may occur in cattle during what is generally called thermal stress in cattle.

Indication of Thermal Acclimation in Dairy Cattle:

It has been previously mentioned in this discussion that the 80°F. group was acclimated to heat, as indicated by certain physiological, hormonal, and enzymatic changes, after they have been exposed to high temperature for a one-year period. Whether this acclimation would be held, or not, after exposing the heifers to both extremes of temperature was then questioned. In the second part of this study, both 50° and 80°F. reared heifers were exposed to rising temperature (35°-95°F.) after they have been thermally equalized for one month at 80°, 90°, and 52°F.

From the discussion, it is obvious that in cold (35°-50°F.) the rearing temperature had significant influence not only on the levels of most of the characteristics studied (Table 8), but also on the changes of these characteristics with rising temperature, as shown in Figures 2-4. The responses of the 80°F. group to cold were exactly the opposite of, and significantly different from, those of the 50°F. group. This was true in regards to urine volume; urine excretion of nitrogen, sodium and potassium; nitrogen retention; blood glucose and plasma protein concentrations.

In heat (50°-95°F.), however, the rearing temperature exerted no effect on the directional changes in the aforementioned characteristics, but it had significant influence on the magnitude of responses. The 80°F. reared group showed significantly milder heat stress than the 50°F. group. They had significantly slower rates of increase in water consumption, urine volume, sodium excretion, urine Na/K ratio, and significantly slower decrease in plasma protein, and blood glucose concentrations, with rising temperature (Table 9). The nitrogen retention in the 80°F. group was about 80 percent higher than that of the 50°F. group during the heating period (Table 5). However, because of the irregularity and the abnormal heat intolerance of these particular 80°F. Jersey animals, as indicated earlier in this investigation, by their higher and faster rate of increase of sodium excretion, a statistically significant difference between the 50° and 80°F. reared groups in their rates of decline in nitrogen retention with rising temperature could not be obtained.

These results, however, indicate that raising dairy calves at warm temperature (80°F.) improves their performance at high environmental temperatures, i.e.,

they were more heat tolerant than those raised at a comfortable temperature (50°F.), as shown by milder stress reactions in the former treatment than in the latter during heat exposure. Such acquired characteristics also are not lost by exposing the acclimated animals to adverse environments. Raising the calves at low temperature (50°F.), which is the comfortable temperature for cattle, enables the animals to perform much better in colder, or even in freezing, temperature than at the rearing temperature. However, it is apparent that the acclimation of calves to warmth renders them very sensitive to low tempearture and they show the symptoms of "stress" in cold (35°F.), which is usual in European evolved cattle. Some animals, however, such as the 80°F. Jerseys in this experiment, did not gain from heat acclimation. They even had a lower nitrogen retention than the 50°F. Jerseys during rising temperature (50°-95°F.).

The thermoneutrality zone, which is known to be between 40° and 60°F. (Ragsdale *et al.,* 1949; Kibler and Brody, 1949), seems to be shifted towards the freezing temperature in the cold acclimated heifers and towards 70° or 80°F. in the warm acclimated group. The question arises as to what caused the 80°. reared heifers to have higher catabolic reactions in cold and less in heat than the 50°F. group. The sensation of cold or heat is known to be a function of sensory receptors in the skin (Krause end-bulbs for cold, and corpuscles of Ruffini, for heat) which have been identified physiologically and histologically (Bazett, 1951; Hensel *et al.,* 1951; Dodt, 1952; Ham, 1957; Blight, 1957). These receptors when stimulated by cold or heat pass nervous impulses to the hypothalamus, which, as suggested by Sayers *et al.* (1958), passes through the portal venous system a neurohumor from the median eminence to the adenohypophysis that regulates the ACTH secretion. The latter, in turn, stimulates the glucocorticoids secretion which enhance the catabolic reactions as discussed before.

It seems, however, that exposing the calves for one year at a constantly high temperature (80°F.) rendered their warm receptors less sensitive to heat and their cold receptors more sensitive to cold, while opposite reactions might have taken place in the 50°F. group. Therefore, when the 80°F. reared heifers, after the acclimation process, were exposed to temperature far below the rearing temperature, i.e. 35°F., their sensitive cold receptors passed many nervous impulses to the hypothalamus that resulted in high secretion of ACTH and glucocorticoids that accounted for the natruresis, kaluresis, diuresis, high nitrogen excretion, and low nitrogen retention, or "stress". The same steps might have taken place in both the warm and cold acclimated heifers when they were exposed to heat, with a resultant of the same symptoms mentioned above. However, it seems that the nervous impulses were more frequent and at earlier temperature (50°F.) in the cold acclimated heifers than in the warm acclimated heifers. Scholander (1958) reported from his observation on the acclimation of man to cold that acclimation could be attained by insensitivity to body cooling. On a cold night, while the white man shivered and could not sleep, the native aborignals slept motionless with no shivering though their skin temperature was much lower than that of the white man.

It is noticed that, while the blood glucose concentration in the 50°F. heifers was significantly declining with rising temperature, the 80°F. group showed insignificant changes in their blood glucose during heating period (Table 6) This difference between the two groups in blood glucose changes under heat stress was not accompanied by significant differences in feed consumption or respiratory activities. Therefore, it is possible that metabolic acclimation also took place in the 80°F. heifers and that they were able to perform the same respiratory activity as the 50°F. group with less utilization of blood glucose. Such metabolic acclimation might be brought about by the induction of new enzymes (adaptive enzymes), as suggested by Potter (1958). Such adaptive enzymes might be induced by the maintenance of high glucocorticoids level in the warm acclimated heifers during their earlier long exposure to heat (80°F.). The induction of such enzymes in mammals by cortisone administration has been demonstrated (Horton and Franz, 1959; Sereni *et al.,* 1959). Studying the acclimatization of cattle to heat, Bianca (1959) suggested that the acclimated calves had low metabolic cost of breathing, since the heart rate for a given respiratory rate became smaller from the first to the last exposure to heat.

A Biochemical Index of Heat Tolerance in Cattle:

Physiologists have used different parameters for determining the heat tolerance in cattle. Most of these parameters are based on measuring the changes in body temperature, respiration rate, cardiac function, vaporization rate, or heat production. The changes in these characteristics though indicate the generally called "thermal stress" in cattle, yet they do not necessarily pertain to the degree of heat tolerance. Under hot conditions, the body temperature may not increase appreciably, because of high respiratory vaporization. The animal may, thus, be considered heat tolerant, on the basis of the Iberia heat tolerance test; yet, it is probably suffering from a respiratory alkalosis brought about by the increased respiration rate. Moreover, the increase in body temperature, does not necessarily indicate a poor tolerance to heat. When cattle are acclimated to heat they gain normal weight under hot conditions even with an elevated body temperature, as shown earlier in this study.

During the last two months of thermal acclimation the 80°F. reared calves were gaining more weight than the 50°F. reared calves though the rectal temperature of the former group was about 0.5°F. higher than that of the latter group (Kibler, 1960). This has also been demonstrated by Bianca (1959) who showed that heat acclimated dairy calves were gaining normal weight though their body temperature was elevated. Schmidt-Nielsen et al. (1957) showed that, in hot conditions, the camel lets his body temperature increase up to 6°F. more than normal without showing symptoms of distress or increase in respiration rate. Cardiorespiratory activities are considered to be an unreliable method for heat tolerance (McDowell *et al.,* 1953; Beakley and Findlay, 1955; Findlay, 1957; Kibler, 1957). The evaporative cooling, *per se,* was found by Kibler and Yeck (1959) not to be a determining factor in heat tolerance at temperatures up to

100°F. The Shorthorn cattle showed higher respiratory and total vaporization per animal, per unit weight, and per unit surface area at low and high temperatures up to 100°F. than the heat tolerant Brahman cattle. Kibler (1957) showed that heat production per unit area or per unit weight is a misleading index for heat tolerance in cattle if they are under heat decline in plasma volume, such as in camels and donkeys (Schmidt-Nielsen, 1959), does not seem reasonable because cattle maintain high plasma volume in heat (Dale *et al.*, 1956), yet they are intolerant to heat.

From the previous discussion, it seems that the characteristics of heat tolerance are elusive and vague. They depend on the integrity of various systems such as the respiratory, circulatory, excretory, nervous, endocrine, and enzymatic systems. The coordination of all these systems under thermal stress is different, not only between species, but also between breeds and even between individuals within the breed. Some animals may combat heat most efficiently through depressing their energy metabolism to a greater extent than other animals. Others may attain the same goal by an efficient evaporative or nonevaporative cooling system. Other animals may have an enzymatic system that is capable of reacting normally at a relatively high body temperature. Heat tolerance may also be achieved in animals by low sensitivity of their warm receptors to heat, as has been suggested in the warm acclimated heifers in this study. Such variation between animals in methods of combating heat stress makes unsatisfactory the attempts to attribute heat tolerance in cattle to one particular mechanism.

In this study, a more practical approach to finding an index for heat tolerance was undertaken. The concept is based on the fact that a heat tolerant animal must have the least depression in the net energy (ΔF) during heat exposure. Therefore, the determination of the depression in the net energy at a specific high temperature is thought to provide a quantitative estimation of heat tolerance in cattle. The methods of determining the net energy have many limitations as shown by Flatt (1959), and particularly if such determinations are carried out under specific controlled temperature. The nitrogen retention, which is used in this study in comparing the 50° with 80°F. reared heifers in their heat tolerance, is much simpler in determination than any methods of energy retention, and yet, it is directly related to the changes in net energy. The nitrogen intake at the comfort temperature (50°F.), and at 95°F., is computed and multiplied by the digestion coefficient of the ration, which does not change markedly with change in temperature—only about 5 percent, either in cattle (Davis, 1960) or sheep, (Graham *et al.*, 1959). The urinary nitrogen at 50° and 95°F. is then subtracted from the corresponding values of digestible nitrogen consumption, to obtain the nitrogen retention at both temperature levels. The heat tolerant animal should, however, have a low percentage of decline in nitrogen retention at 95°F.

In this investigation when environmental temperature was raised from 50° to 95°F. the nitrogen retention, as shown in Table 4, decreased 105, 162, and 161 percent in the 50°F. reared Brown Swiss, Holstein, and Jersey heifers, respectively. The 80°F. Brown Swiss, and Holstein heifers had only 49 and 60 per-

cent, respectively, depression in nitrogen retention. The 80°F. reared Jersey heifers, which did not show any advantage of the heat acclimation, showed a decline in nitrogen retention of 259 percent. With the exception of the 80°F. Jersey heifers, these results indicate that the 80°F. reared heifers were remarkably more heat tolerant than the 50°F. reared animals.

The nitrogen retention method as shown in this investigation can be used as a simple and valid index of heat tolerance for further application in selecting heat tolerant animals and in establishing a heat tolerant breed of cattle. The animals could be scanned in a climatic chamber where nitrogen retention is determined at 50° and 95°F. and, thus, the percentage of depression in nitrogen retention is computed and expressed as a coefficient of heat tolerance.

SUMMARY AND CONCLUSION

At approximately one year of age, both groups were thermally equalized for one month at 80°, 90°, and 52°F., respectively, and then exposed to rising temperature 35°, 50°, 70°, 80°, 90°, and 95°F. for a two-weeks period at each temperature level.

(1) In cold (35°F.), diuresis, kaluresis, natruresis, *higher* blood glucose concentration, *higher* plasma protein concentration, *higher* nitrogen excretion, and *lower* nitrogen retention, were observed in the warm acclimated heifers than in cold acclimated animals. These differences between the two groups were found to be statistically significant.

(2) These results indicated the occurrence of "stress" in cattle at cold exposure (35°F.) if they were previously raised in warm climate. They also indicated that the performance (high anabolism) of cattle near freezing temperature is markedly improved if the cattle were previously raised at mild cold temperature (50°F.).

(3) Practical significance of such results is understood in the management of dairy cattle in cold climate regions where the environmental temperature often falls below freezing.

(4) Increasing the temperature to 50°F. caused a relief of the cold stress in the warm acclimated heifers and effects such as caused by a mild heat stress in the cold acclimated group as shown by the opposite reactions in both groups in most of their characteristics studied.

(5) Further progressive increase in environmental temperature (70°, 80°, 90°, and 95°F.), in both groups demonstrated statistically significant decreases in nitrogen retention, increases in sodium excretion and Na/K ratio in urine, and negative nitrogen and sodium balance at the higher temperatures.

(6) The urinary nitrogen and potassium excretion, as well as the blood glucose and plasma protein concentrations were significantly decreased.

(7) Plasma electrolytes changed inversely to urine electrolytes, though they were statistically insignificant.

(8) Water consumption, total vaporization, and urine volume, increased. Urine specific gravity decreased. These changes were statistically significant.

(9) The digestible nitrogen consumption significantly decreased, while no significant change in body weight was observed with rising environmental temperature.

(10) These results indicated an increase in glucocorticoids secretion, a decrease in mineralocorticoids secretion, probably an inhibition of the antidiuretic hormone release, and an increase in plasma volume with rising temperature from 50° to 95°F. They also indicated the occurrence of "stress" in cattle during exposure to heat regardless of the rearing temperature.

(11) It was observed that the warm acclimated group of heifers, with the exception of the Jersey animals, had a significantly milder heat stress than the cold acclimated heifers. This was demonstrated by slower increases in urine volume, water consumption, sodium excretion, urine Na/K ratio, and more rapid increase in total vaporization, as well as slower decreases in blood glucose, plasma protein, and nitrogen retention, in the warm acclimated heifers than in the cold acclimated group. When data of all breeds were pooled together, these differences between the two groups in the degree of response to heat were found statistically significant, except in nitrogen retention, because of the abnormally severe negative nitrogen balance and the inconsistency of the 80°F. reared Jersey heifers.

(12) These results, however, indicated the stability of the acquired acclimation characteristic even after the exposure to adverse temperatures. Heat stress is significantly lessened if the cattle are previously reared at warm climate (80°F.). They become more heat tolerant and have less catabolic reactions during the heat exposure. These results have practical significance in the management of European evolved dairy cattle in tropic or subtropic regions.

(13) These results further indicated that some animals are incapable of acclimation to heat, as in the instance of the 80°F. reared Jersey animals in this study. They had about 250 percent depression in nitrogen retention when temperature was raised from 50° to 95°F., while the other 80°F. reared Brown Swiss and Holstein had only about 55 percent depression as compared to about 140 percent depression in the 50°F. reared group, as computed from Table 4. Whether this incapability of heat acclimation is a genetic characteristic of the Jersey breed or of these particular individuals of the 80°F. Jerseys must be investigated before encouraging the importation of Jersey cattle in hot climatic regions of the world.

In this investigation, the mechanisms involved in the manifestation of the thermal stress reactions, in the thermal acclimation, as well as the occurrence of the G-A-S in cattle under cold and heat, have been discussed.

The application of the nitrogen retention method as a reliable index of heat tolerance, explained earlier, seems to be a most fruitful mean of establishing a heat tolerant breed of cattle.

With the rapid progress in the application of radioisotopes technique in agricultural science, heat tolerance could also be determined by the use of the "whole animal counter" for determining the percentage decline of total body

K^{40}, which is directly related to the tissues or body protein of the animals, when they are exposed at 50° then at 95°F. in climatic chambers.

BIBLIOGRAPHY

Adolph, E. F., *Physiology of Man in the Desert.* New York: Interscience Publishers, Inc., 1947.

Adolph, E. F., Tolerance to Heat and Dehydration in Several Species of Mammals. *Am. J. Physiol.,* 151:564, 1947.

Albert, A., Tenny, A., and Ford, E., The Effect of Cortisone and Corticotropin on the Biological Decay of Thyroidal Radio-Iodine. *Endocrinoloy,* 50:324, 1952.

Albritton, E. C., *Standard Values in Blood.* Philadelphia, 1952.

Allcroft, W. M., Diurnal Variations in the Blood Sugar Level of the Lactating Cow. *Biochem. J.,* 27:1820, 1933.

Andersson, B., Polydepsia Caused by Intrahypothalamic Injections of Hypertonic NaCl-Solutions. *Experientia,* 8:157, 1952.

Andersson, B., and McCann, Hypothalamic Control of Water Intake. *J. Physiol.,* 129: 44 P, 1955.

Appleman, R. D., and Delouche, J. C., Behavioral, Physiological, and Biochemical Responses of Goats to Temperature 0 to 40°C. *J. Animal Sci.* 17:326, 1958.

Armsby, H. P., *The Principles of Animal Nutrition.* 1st ed., New York: John Wiley & Sons, 1903.

Ashmore, J., Cahill, G. F., Jr., Hastings, A. B., and Zotter, S., Studies on Carbohydrate Metabolism in Rat Liver Slices. VIII. Effect of Ions on Hormones and Pathways of Glucose-6-Phosphate Metabolism. *J. Biol. Chem.,* 224:225, 1957.

Atswood, E. B., In *"The Hormonal Regulation of Energy Metabolism".* Springfield, Ill.: Charles C. Thomas Publisher, 1957.

Bader, R. A., Eliot, J. W., and Bass, D. E., Hormonal and Renal Mechanism of Cold Diuresis. *J. Appl. Physiol.,* 4:649, 1952.

Barger, A. C., Berlin, R. C., and Tulenko, J. F., Infusion of Aldosterone 9- α-Fluorohydrocortisone, and Antidiuretic Hormone into the Renal Artery of Normal and Adrenalectomized Unanesthetized Dogs: Effect on Electrolyte and Water Excretion. *Endocrinology,* 62:804, 1958.

Barker, E. S., Singer, R. B., Elkinton, J. R., and Clark, J. K., The Renal Response in Man to Acute Experimental Respiratory Alkalosis and Acidosis. *J. Clin. Invest.,* 36:514, 1957.

Barlow, G., Agersborg, H. P., Jr., and Keys, H. E., Blood Levels of 17-Hydroxycorticosteroids in Hyperthermic Dogs. *Proc. Soc. Exp. Biol. and Med.,* 93:280, 1956.

Bartlett, P. D., In *"Hypophyseal Growth Hormone, Nature and Actions".* New York: McGraw-Hill, 1955.

Bartter, F. C., Biglieri, E. G., Pronove, P., and Delea, C. S., *Aldosterone, and International Symposium.* London: Churchill, 1958.

Bass, D. E., and Henschel, A., Responses of Body Fluid Compartments to Heat and Cold. *Physiol. Revs.,* 36:128, 1956.

Bass, D. E., Kleeman, C. R., Quinn, M., Henschel, A., and Hegnauer, A. H., Mechanisms of Acclimatization to Heat in Man. *Medicine,* 34:323, 1955.

Bazett, H. C., A Theory of Reflex Control of Temperature at Rest and During Exercise. *Fed. Proc.,* 10:152, 1951.

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

Beakley, W. R., and Findlay, J. D., The Effect of Environmental Temperature and Humidity on the Frequency of the Heat Beat of Ayrshire Calves. *J. Agr. Sci.,* 45:461, 1955.

Beck, R. N., The Effect of Cortisone and Corticotropin on the Release and Peripheral Metabolism of Thyroid Hormone. *Endocrinology,* 62.9, 1958.

Behrens, O. K., and Bromer, W. W., Glucagon. *Vitamins and Hormones,* 16:264, 1958.
Bell, F. R., and Jones, E. R., Glucose Tolerance in the Bovine. *J. Comp. Path. Ther.,* 55:117, 1945.
Berenson, G. S., and Burch, G. E., A Study of the Sodium, Potassium and Chloride Contents of Thermal Sweat of Man Collected From Small Isolated Areas of Skin. *J. Lab. & Clin.* Med., 42:58, 1953.
Bianca, W., Acclimatization of Calves to a Hot Humid Environment. *J. Agr. Sci.,* 52:305, 1959.
Bing, H. Z., Skouby, A. P., Sensitization of Cold Receptors by Substances with Acetylcholine Effect. *Acta Physiol. Scan.,* 21:286, 1950.
Birnbaum, S. M., Greenstein, J. P., and Wintz, M., Nutritional Studies with Water-Soluble, Chemically Defined Diets. II. Nitrogen Balance and Metabolism. *Arch. Biochem. and Biophys.,* 72:417, 1957.
Black, A. L., Kleiber, M., Butterworth, E. M., Brubacher, G. B., and Kaneko, J. J., The Pentose Cycle as a Pathway for Glucose Metabolism in Intact Lactating Dairy Cow. *J. Biol. Chem.,* 227:537, 1957.
Black, D. A. K., *Essentials of Fluid Balance.* Springfield, Ill.: Charles C. Thomas Publishers, 1957.
Blaxter, K. L., Graham, N., McC., Wainman, F. W., and Armstrong, D. G., Environmental Temperature, Energy Metabolism and Heat Regulation in Sheep. II. The Partition of Heat Losses in Closely Clipped Sheep. *J. Agr. Sci.,* 52:25, 1959.
Bligh, J., The Initial of Thermal Polypnoea in the Calf. *J. Physiol.,* 136:413, 1957.
Blincoe, C. R., The Influence of Climatic Factors on Thyroid Activity and Radioiodine Metabolism of Dairy Cattle. Ph.D. Thesis, University of Missouri, 1955.
Blincoe, C., The Influence of Constant Ambient Temperature on the Thyroid Activity and Iodine Metabolism of Shorthorn, Santa Gertrudis, and Brahman Calves During Growth. *Mo. Agr. Exp. Sta. Res. Bul.,* 649, 1958.
Blincoe, C., and Brody, S., The Influence of Diurnally Variable Temperatures on the Thyroid Activity and Iodide Metabolism of Jersey and Holstein Cows. *Mo. Agr. Exp. Sta. Res. Bul.,* 579, 1955a.
Blincoe, C., and Brody, S., The Influence of Temperature on Blood Composition of Cattle. *Mo. Agr. Exp. Sta. Res. Bul.,* 488, 1951.
Bonsma, J. C., Breeding Cattle for Increased Adaptability to Tropical and Subtropical Environments. *J. Agr. Sci.,* 39:204, 1949.
Bornstein, J., and Park, C. R., Inhibition of Glucose Uptake by the Serum of Diabetic Rats. *J. Biol. Chem.,* 205:503, 1953.
Britton, S. W., Studies of the Conditions of Activity in Endocrine Glands XXII. Adrenin Secretion on Exposure to Cold, Together with a Possible Explanation of Hibernation. *Am. J. Physiol.,* 84:119, 1928.
Brobeck, John R., Food and Temperature. *Recent Progress in Hormone Research* XVI., 439-466, 1960.
Brody, S., *Bioenergetics and Growth.* New York: Reinold Publishing Corporation, 1945.
Brody, S., Influence of Ambient Temperature, 50° to 100°F., on the Blood Composition of Jersey and Holstein Cows. *Mo. Agr. Exp. Sta. Res. Bul.,* 433, 1949.
Brody, S., Kibler, H. H., Ragsdale, A. C., Blincoe, C., Thompson, H. J. Worstel, D. M., *et al., Mo. Agr. Exp. Sta. Res. Bul. Series* (Environmental Physiology and Shelter Engineering with Special Reference to Domestic Animals), 1948-1957.
Brody, S., Physiological Backgrounds. *Mo. Agr. Exp. Sta. Res. Bul.,* 423, 1948.
Brown, K. I., Brown, D. J., and Meyer, R. K., Effect of Surgical Trauma, ACTH, and Adrenal Cortical Hormones on Electrolytes, Water Balance and Gluconeogenesis in Male Chickens. *Am. J. Physiol.,* 192:43, 1958.
Brown-Grant, K., The Effect of ACTH and Adrenal Steroids on Thyroid Activity

With Observations on the Adrenal-Thyroid Relationship. *J. Physiol.,* 131:58, 1956.
Calloway, D. H., and Specotr, H., Nitrogen Utilization During Caloric Restriction. III. The Effect of Preceeding Diet. *J. Nutrition,* 57:73, 1955.
Campbell, I. L., Water Requirements of Dairy Cows. *Massey Agr. Coll. Sheep Farming Ann.,* 1958:53, 1958.
Campbell, R. M., Sharp, G., Boyne, A. W., and Cuthbertson, D. P., Cortisone and the Metabolic Response to Injury. *Brit. J. Exp. Path.,* 35:566, 1954.
Cannon, W. B., Organization and Physiological Homeostasis. *Physiol. Rev.,* 9:397, 1929.
Chanutin, A., Plasma Protein in Normal and Injured Dogs, Goats, and Rats. *Am. J. Med.,* 3:507, 1947.
Chung, A. C., Some Physiological Effects of Growth Hormone, Glucocorticoids, Adrenocortocotropic Hormone and Insulin on Dairy Cows. *Dissertation Abst.,* 19:32, 1958.
Cizek, L. J., Semple, R. E., Huang; K. C., and Gregersen, M. I., Effect of Extracellular Electrolyte Depletion on Water Intake in Dogs. *Am. J. Physiol.,* 164:415, 1951.
Clark, I., The Effect of Cortisone Upon Protein Synthesis. *J. Biol. Chem.,* 200:69, 1953.
Clark, R. T., Jr., Crisculo, D., Guy, G., and Hoelscher, B., Metabolism of Rats Chronically and Acutely Exposed to Various Environmental Conditions. *Fed Proc.,* 14: 28, 1955.
Clinton, M., Jr., Thorn, G. W., Eisenberg, H., and Stein, K. E., Effect of Synthesis on Desoxycorticosterone Acetate Therapy on Plasma Volume and Electrolyte Balance in Normal Dogs. *Endocrinology,* 31:578, 1942.
Cole, D. F., Effect of Aldosterone on Renal Excretion of Intravenously Administered Saline. *Endocrinology,* 60: 562, 1957.
Conley, C. L., and Nickerson, J. L., Effects of Temperature Change on the Water Balance in Man. Am. J. Physiol, 143:373, 1945.
Conn, J. W., The Mechanism of Acclimatization to Heat. *Adv. Int. Med.,* 3:373, 1949.
Cortell, R., Rawson, R. W., The Effect of Thyroxine on the Response of the Thyroid Gland to Thyrotropic Hormone. *Endocrinology,* 35:488, 1944.
Dale, H. E., and Brody, S., Thermal Stress and Acid-Base Balance in Dairy Cattle. *Mo. Agr. Exp. Sta. Res. Bul.,* 562, 1954.
Dale, H. E., Brody, S., and Burge, G. J., Effect of Environmental Temperature Rhythms on Blood and Serum Volumes and Body Water in Dairy Cattle. *Fed Proc.,* 15:43, 1956.
Dale, H. E., Gloria, J., Burge, and Brody, S., Environmental Temperature and Blood Volume. *Mo. Agr. Exp. Sta. Res. Bul.,* 608, 1956.
Dale, H. E., Goberdhan, and Brody, S., A Comparison of the Effect of Starvation and Thermal Stress on the Acid-Base Balance of Dairy Cattle. *Am. J. Vet. Res.,* 15:197, 1954.
D'Amato, H. E., and Hegnauer, A. H., Blood Volume in the Hypothermic Dog. *Am. J. Physiol.,* 173:100, 1953.
DasGupta, D., and Giroud, C. J. P., Experimental Amino Nucleoside Nephrosis. I. Action of Cortisone on Aldosterone and Corticosterone Production. *Proc. Soc. Exptl. Biol. and Med.,* 98:334, 1958.
Davidson, D. C., Levinsky, N. C., and Berliner, R. W., Maintenance of Potassium Excretion Despite Reduction of Glomrular Fibration During Sodium Diuresis. *J. Clin. Invest.,* 37:548, 1958.
Davis, A. V., Effect of Constant Environmental Temperatures and Relative Humidities on Feed Digestion by Lactating Holstein Cows. Ph.D. Thesis, University of Missouri, 1960.
Davis, J. O., and Ball, W. C., Jr., Effects of a Body Cast on Aldosterone and Sodium

Excretion in Dogs with Experimental Ascites. *Am. J. Physiol.,* 192:538, 1958.
DeBodo, R. C., and Altszuler, The Metabolic Effects of Growth Hormone and Their Physiological Significance. *Vitamins and Hormones,* 15:205, 1957.
deDuve, C., and Hers, H. G., Carbohydrate Metabolism. *Ann. Rev. Biochem.,* 26:151, 1957.
DeLanne, R., Barnes, J. R., and Brouha, L., Changes in Concentration of Plasma Protein Fractions During Muscular Work and Recovery. *J. Appl. Physiol.,* 13:97, 1958.
Diamant, E. J., and Guggenheim, K., Electrolyte Metabolism in Pyridoxine, Riboflavin, and Pantothenic Acid-Deficient Rats. *Am. J. Physiol.,* 191:108, 1957.
Dill, D. B., Hall, F. G., and Edwards, H. T., Changes in Composition of Sweat During Acclimatization to Heat. *Am. J. Physiol.,* 123:412, 1938.
Dill, D. B., Jones, B. F., Edwards, H. T., and Oberg, S. A., Salt Economy in Extreme Dry Heat. *J. Biol. Chem.,* 100:755, 1933.
Dodt, E., The Behaviour of Thermoreceptors at Low and High Temperatures with Special Reference to Ebbecke's Temperature Phenomena. *Acta Physiol., Scand.,* 27:295, 1952.
Doolan, P. D., Welham, W. C., and Kyle, L. H., Studies on the Effect of ACTH and Certain Adrenal Steroids on Total Body Fat. *Metabl. Clin. and Exp.,* 4:39, 1955.
Dougherty, R. W., Klavano, C. S., Dickson, W. M., and Klavano, P. A., Blood Sugar Levels in Cattle and Sheep Fed Various Amounts of Glucose. *Cornell Veterinarian,* 47:397, 1956.
Dukes, H. H., *The Physiology of Domestic Animals.* 7th Ed., Ithaca, New York: Publishing Associates, 1957.
Dustan, H. P., Corcoran, A. C., and Page, I. H., Renal Function in Primary Aldosteronism. *J. Clin. Inv.,* 35:1357, 1956.
Dye, J. A., Roberts, S. J., Blampied, N. Fincher, M. G., The use of Cortisone in the Treatment of Ketosis in Dairy Cows. *Cornell Vet.,* 43:128, 1953.
Eisenstein, A. B., and Hartroft, P. N., Alterations in the Rat Adrenal Cortex Induced by Sodium Deficiency. Steroid Hormone Secretion. *Endocrinology,* 60:634, 1957.
Elliot, J. M., and Loosli, J. K., Effect of the Urinary Ratio of Hay to Concentrate on Milk Production, Ration Digestibility, and Urinary Energy Losses. *J. Dairy Sci.,* 42:836, 1959.
Epstein, D., Cantarow, A., Friedler, G., and Paschkins, K. E., Inhibition of Thyroid Function by Cortisone and ACTH in Hypophysectomized Rats. *Proc. Soc. Exp. Biol. Med.,* 82:50, 1953.
Evans, E. S., Contopoulos, A. N., and Simpson, M. E., Hormonal Factors Influencing Calorigenesis. *Endocrinology,* 60:403, 1957.
Eversole, W. J., and Romero, B. E., Effects of 11-Oxycorticoids on Renal Excretion of Water and Electrolytes in Force-fed Intact Rats. *Proc. Soc. Exp. Biol. & Med.,* 99:282, 1958.
Feodor, L., Lipid Metabolism. *Ann. Rev. Biochem.,* 24:675, 1955.
Findlay, J. D., The Effects of Temperature, Humidity, Air Movement, and Solar Radiation on the Behaviour and Physiology of Cattle and Other Farm Animals. *The Hannah Dairy Res. Inst. Bul.,* 9, 1950.
Findlay, J. D., The Respiratory Activity of Calves Subjected to Thermal Stress. *J. Physiol.,* 136:300, 1957.
Findlay, J. D., and Yang, S. H., The Sweat Glands of Ayrshire Cattle. *J. Agr. Sci.,* 40:126, 1950.
Flatt, W. P., Methods of Determining the Energy Value of Feeds. Symposium on the Energy Value of Feeds. *J. Dairy Sci.,* 42:752, 1959.
Flock, E. V., Bollman, J. L., Grindlay, J. H., and Orvis, A. L., Metabolism of l-Thyroxine and l-Triiodothyronine in the Absence of the Liver. *Am. J. Physiol.,* 187: 407, 1956.

Foley, C. W., Variations in the Physiological Response to Stress in Dwarf and Normal Beef Animals. Ph.D. Thesis, University of Missouri, 1959.

Forbes, R. M., Ketosis in Ruminants. *Cornell Vet.*, 33:27, 1943.

Freedman, H. H., and Gordon, A. S., Endocrine Factors in the Response of the Hypothyroid Rats to Cold. *Endocrinology*, 56:135, 1955.

French, M. H., The Importance of Water in the Management of Cattle. *East African Agr. J.*, 21:171, 1956.

Frischauf, H., and Zweymuller, E., Investigations on the Emaciation of Fatty Children. II. Communication: The Excretion of Electrolytes and Nitrogen. *Arch. Interntl. Pharmacodyn.*, 114:127, 1958.

Gamble, J. L., *Chemical Anatomy, Physiology, and Pathology of Extracellular Fluid.* 6th Ed.: Cambridge, Mass., 1954.

Ganoug, W. F., and Mulrow, P. J., Rate of Change in Sodium and Potassium Excretion After Injection of Aldosterone Into the Aorta and Renal Artery of the Dog. *Am. J. Physiol.*, 195:337, 1958.

Gjessing, E. C., Chanutin, A., and Floyd, C. S., An Electroforetic Study of Plasma and Plasma Fractions of Normal and Injured Rats. *J. Biol. Chem.*, 169:657, 1947.

Glickman, N., Hick, F. K., Keeton, R. W., and Montgomery, M. M., Blood Volume Changes in Men Exposed to Hot Environmental Conditions for a Few Hours. *Am. J. Physiol.*, 134:165, 1941.

Goberdhan, C. K., A Study of the Inorganic Constituents in the Blood and Urine of Dairy Cows Under Thermal Stress and Radiation. Ph.D. Thesis, University of Missouri, 1954.

Goding, J. R., and Denton, D. A., The Effects of Adrenal Insufficiency and Overdosage with DOCA in Bilaterally Adrenalectomized Sheep. *Australian J. Exptl. Biol. & Med. Sci.*, 35:301, 1957.

Goetsch, G. C., and Prtichard, Effects of Oral Administration of Short-Chain Fatty Acids on Certain Blood and Urine Constituents of Fasted, Phlorhizin-Treated Ewes. *Am. J. Vet. Res.*, 19:637, 1958.

Gorbelick, R. V., The Problem of Protein and Its Fractions, and Urea Contents in Sheep Blood Under Reduction Protein Intake. *Trudy Saratovsk. Zootekh. Vet. Inst.*, 1956:172, 1956 *(J. Biol. Abst.* 33:2085, 1959).

Graham, N., McC., Wainman, F. W., Blaxter, K. L., and Armstrong, D. G., Environmental Temperature, Energy Metabolism and Heat Regulation in Sheep. I. Energy Metabolism in Closely Clipped Sheep. *J. Agr. Sci.*, 52:13, 1959.

Greer, M. A., Studies on the Influence of the Central Nervous System on Anterior Pituitary Function. *Rec. Prog. Hor. Res.*, 13:67, 1957.

Greer, M. A., and Erwin, H. L., Evidence of Separate Hypothalamic Centers Controlling Corticotropin and Thyrotopin Secretion by the Pituitary. *Endocrinology*, 58: 665, 1956.

Hale, H. B., Sayers, G., Syndor, K. L., Sweat, M. L., and Fossan, D. D. V., Blood Adrenocorticotrophic Hormone and Plasma Corticosteroids in Men Exposed to Adverse Environmental Conditions. *J. Clin. Invest.*, 36:1642, 1957.

Hale, W. H., and King, R. P., Glucose Tolerance in Lambs as Affected by Type of Ration. *Proc. Iowa Acad. Sci.*, 65:224, 1958.

Ham, A. W., *Histology.* 3rd Ed. Philadelphia: J. B. Lippincott Co., 1957.

Hannon, J. P., Larson, A. M., and Young, D. W., Effect of Cold Acclimatization on Plasma Electrolyte Levels. *J. Appl. Physiol.*, 13:239, 1958.

Harbin, R., Harbough, F. S., Neeley, K. L., Fine, N. C., Effect of Natural Combinations of Ambient Temperature and Relative Humidity on the Water Intake of Lactating and Nonlactating Dairy Cows. *J. Dairy Sci.*, 41:1621, 1958.

Hardy, J. D., and Oppel, T. W., Stimulation of the Cold End Organs of the Human Skin by Radiation. *Am. J. Physiol.* (Proc.), 123:89, 1938.

Harris, G. W., and Woods, J. W., Hypothalamus-Pituitary Thyroid Relationships. *Ciba Foundation Colloquia on Endocrinology,* 10:3, 1957.

Hawk, P. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry.* 13th Ed. New York: The Blakiston Company, 1954.

Hayman, R. H., and May, T., Sweat Glands in Zebu (Bos Indicus L.) and European (B. Tanrus L.) Cattle. II. Effects of Season and Exercise on Sweat Gland Volume. *Australian J. Agr. Res.,* 9:385, 1958.

Hechter, O., and Pincus, G., Genesis of Adrenocortical Secretion. *Physiol. Rev.,* 34: 459, 1954.

Heitman, H., Jr., Kelly, C. F., and Bond, T. E., Ambient Air Temperature and Weight Gain in Swine. *J. Animal Sci.,* 17:62, 1958.

Hellman, K., Collins, K. J., Gray, C. H., Jones, R. M., Lunnon, J. B., and Weiner, J. S., The Excretion of Urinary Adrenocortical Steroids During Heat Stress. *J. Endocrinol.,* 14:209, 1956.

Hensel, H., Storm, L., and Zotterman, Y., Electrophysiological Measurements of Depth of Thermoreceptors. *J. Neurophysiol.,* 14:423, 1951.

Hensel, H., and Zotterman, Y., The Response of the Cold Receptors to Constant Cooling. *ACTA Physiol. Scand.,* 22:96, 1951.

Heroux, O., and Hart, J. S., Comparison of Four Indices of Adrenal Activity in Rats Acclimated to 30°, 15°, 1°C. *Am. J. Physiol.* 178:445, 1954.

Hitchcock, M. W. S., and Philipson, A. T., The Tolerance of Sheep to Low Concentration of Blood Sugar. *J. Physiol.,* 105:42, 1953.

Hobbs, C. S., Hansard, S. L., and Barrick, E. R., Simplified Methods and Equipment Used in Separation of Urine From Feces Eliminated by Heifers and by Steers. *J. Animal Sci.,* 9:565, 1950.

Hoberman, H. D., Endocrine Regulation of Amino Acid and Protein Metabolism During Fasting. *Yale J. Biol. Med.,* 22:341, 1950.

Hodgson, R. E., Riddell, W. H., Huges, N. C., Factors Influencing the Blood Sugar Level of Dairy Cattle. *J. Agr. Res.,* 44:357, 1932.

Holcombe, R. B., Investigation on the Urinary Excretion of "Reducing Corticoids" in Cattle and Sheep. *Acta Endocrinol.,* 34 (Suppl.):1, 1957.

Hollander, W., and Judson, W. E., Electrolyte and Water Excretion in Arterial Hypertention. I. Studies in Non-Medically Treated Subjects with Essential Hypertention. *J. Clin. Invest.,* 36:1460, 1957.

Holmes, J. H., and Cizek, L. J., Observations on Sodium Chloride Depletion in The Dog. *Am. J. Physiol.,* 164:407, 1951.

Holmes, J. H., and Gregersen, M. I., Observations on the Drinking Induced by Hypertonic Solutions. *Am. J. Physiol.,* 162:326, 1950.

Horton, H. R., and Franz, J. M., Effect of Ethonine on the Cortisone-evoked Stimulative of Tryptophan Peroxidase-Oxidase Activity. *Endocrinology,* 64:258, 1959.

Ingle, D. J., The Biologic Properties of Cortisone. A Review, *J. Clin. Endocrinol.,* 10: 1312, 1950.

Ingle, D. J., Nezamis, J. E., and Humphrey, L. M., Effect of Adrenal Cortical Extract and Steroids Upon Glucose Tolerance of Eviscerated Rats. *Proc. Soc. Exp. Biol. & Med.,* 84:45, 1953.

Ingle, D. J., Prestrud, M. C., Nezamis, J. E., and Kuizenga, M. H., Effect of Adrenal Cortex Extract Upon the Tolerance of the Eviscerated Rat for Intravenously Administered Glucose. *Am. J. Physiol.,* 150:423, 1947.

Intoccia, A., and Middlesworth, L. V., Thyroxine Excretion Increase by Cold Exposure. *Endocrinology,* 64:462, 1959.

Isler, H., LeBlond, C. P., and Alexander, A. A., Mechanism of the Thyroid Stimulation Produced by Sodium Chloride in the Mouse. *Endocrinology,* 62:159, 1958.

Itoh, S., The Release of Antidiuretic Hormone from the Posterior Pituitary Body on Exposure to Heat. *Jap. J. Physiol.*, 4:185, 1954.

Itoh, S., and Kimura, M., Occurrence of Antidiuretic Substance in Human Urine After Heavy Sweating. *Jap. J. Physiol.*, 3:277, 1953.

Jarrett, I. G., and Potter, B. J., Carbohydrate Metabolism in the Young Lamb. *The Aust. J. of Exp. Biol. and Med. Sci.*, 30:207, 1952.

Johnson, B. B., Lieberman, A. H., and Mulrow, P. J., Aldosterone Excretion in Normal Subjects Depleted of Sodium and Potassium. *J. Clin. Invest.*, 36:757, 1957.

Johnson, H. D., Comparison of the Effect of Environmental Temperature on Rabbits and Cattle. Ph.D. Thesis, University of Missouri, 1956.

Johnson, H. D., and Ragsdale, A. C., Changes in Thyroid I^{131} Release Rate During Growth in Holstein, Brown Swiss, and Jersey Calves at Constant Environmental Temperatures (50° and 80°F.). *J. Dairy Sci.*, 42:182, 1959.

Johnson, H. D., and Ragsdale, A. C., Effects of Constant Environmental Temperatures of 50° and 80°F. on the Growth Responses of Holstein, Brown Swiss, and Jersey Calves. *Mo. Agr. Exp. Sta. Res. Bul.* (In Press).

Johnson, H. D. and Ragsdale, A. C., The Effect of Rising Environmental Temperatures (35°-95°F.) On Thyroid I^{131} Release Rate of Holstein, Brown Swiss and Jersey Heifers. *J. of Agri. Sci.* 54:3, 1960.

Johnson, H. D., Ragsdale, A. C., and Cheng, C. S., Comparison of the Effects of Environmental Temperatures on Rabbits and Cattle. Part I. Influence of Constant Environmental Temperatures (50° and 80°F.) on the Growth Responses and Physiological Reactions of Rabbits and Cattle. *Mo. Agr. Exp. Sta. Res. Bul.*, 640, 1957.

Johnson, H. D., Ragsdale, A. C., and Cheng, C. S., Comparison of the Effect of Environmental Temperature on Rabbits and Cattle. Part II. Influence of Rising Environmental Temperature on the Physiological Reactions of Rabbits and Cattle. *Mo. Agr. Exp. Sta. Res. Bul.*, 648, 1958.

Johnson, H. D., Ragsdale, A. C., and Yeck, R. G., Effects of Constant Environmental Temperatures of 50° and 80°F. on the Feed and Water Consumption of Brahman, Santa Gertrudis, and Shorthorn Calves During Growth. *Mo. Agr. Exp. Sta. Res. Bul.*, 683, 1958.

Johnston, J. E., Hindery, G. A., Burnett, W. T., and Guidry, A., Comparison of Methods of Measuring Thyroid Function of Dairy Cattle. *J. Dairy Sci.*, 42:927, 1959.

Jones, E. S., An In Vitro Action of Desoxycorticosterone (DOC) on Red Cell Electrolytes. *Experienta*, 14:72, 1958.

Kalant, N., McIntyre, W. C., and Wilansky, D. L., Thyroid Function in Experimental Nephrotic Syndrome. *Endocrinology*, 64:333, 1959.

Kamal, T. H., Johnson, H. D., and Ragsdale, A. C., Effect of Heat Acclimation on Physiological and Biochemical Responses of Dairy Heifers to Various Temperatures (35°F.-95°F.). *J. Animal Sci.*, 17:1227, 1958b.

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

Kamal, T. H., Johnson, H. D., and Ragsdale, A. C., Effects of Temperature and Age on Growth Rate, Blood GSH and PBI^{131} Levels. *Fed. Proc. 17*:993, 1958a.

Kamal, T. H., Johnson, H. D., and Ragsdale, A. C., Water Consumption in Dairy Cattle as Influenced by Environmental Temperatures and Urine Excretion. *J. Dairy Sci.*, 42:926, 1959a.

Kanter, G. S., Effect of Heat on Regulation of Body Fluids, and Electrolytes in Dogs. *Am. J. Physiol.*, 178:259, 1954.

Kanter, G. S., Excretion During Heat Stress in Dog and Man. *Am. J. Physiol.,* 179: 649, 1954a.

Katsh, S., Katsh, G. F., and Osher, P., Adrenal, Pituitary, and Urinary Ascorbic Acid Levels in Rats Subjected to Hypothermic Environment. *Am. J. Physiol.,* 178:457, 1954.

Keeton, R. W., and Mitchell, The Effect of Excessive Intakes of Certain Water-Soluble Vitamins on Man's Resistance to Cold. OEMcmv-234 *Final Report to Com. Med. Res. O.S.R.D.,* July, pp. 75, 1944.

Kellman, K., and Weiner, J. S., Antidiuretic Substance in Urine Following Exposure to High Temperatures. *J. Appl. Physiol.,* 6:194, 1953.

Kibler, H. H., Energy Metabolism and Cardiorespiratory Activities in Shorthorn, Santa Gertrudis, and Brahman Heifers During Growth at 50 and 80°F. Temperatures. *Mo. Agr. Exp. Sta. Res. Bul.,* 643, 1957.

Kibler, H. H., Energy Metabolism and Related Thermoregulatory Reactions in Brown Swiss, Holstein, and Jersey Calves During Growth at 50° and 80°F. Temperatures. *Mo. Agr. Exp. Sta. Res. Bul.* 743, 1960.

Kibler, H. H., and Brody, S., Effects of Temperature, 50° to 105°F. and 50° to 9°F. on Heat Production and Cardiorespiratory Activities in Brahman, Jersey, and Holstein Cows, *Mo. Agr. Sta. Res. Bul.,* 464, 1950.

Kibler, H. H., and Brody, S., Influence of Temperature, 5° to 95°F. on Evaporative Cooling From the Respiratory and Exterior Body Surfaces in Jersey and Holstein Cows. *Mo. Agr. Exp. Sta. Res. Bul.,* 461, 1950.

Kibler, H. H., and Brody, S., Influence of Temperature, 50° to 5°F. and 50° to 95°F. on Heat Production and Cardiorespiratory Activities of Dairy Cattle. *Mo. Agr. Exp. Sta. Res. Bul.,* 450, 1949.

Kibler, H. H., and Yeck, R. G., Vaporization Rates and Heat Tolerance in Growing Shorthorn, Brahman, and Santa Gertrudis Calves Raised at Constant 50° and 80° F. Temperatures. *Mo. Agr. Exp. Sta. Res. Bul.,* 701, 1959.

Kimura, M., Influence of Antidiuretic Hormone on Adrenal Cortical Activity in Rats. *Jap. J. Physiol.,* 4:24, 1954.

Kleeman, C. R., Maxwell, M. H., and Rockney, R. E., Mechanism of Impaired Water Excretion in Adrenal and Pituitary Insufficiency. I. The Role of Altered Glomerular Filtration Rate and Solute Excretion. *J. Clin. Invest.,* 37:1799, 1958.

Kleiber, M., Energy Metabolism. *Ann. Rev. Physiol.,* 18:35, 1956.

Knowlton, A. I., and Loeb, E. N., Depletion of Carcass Potassium in Rats Made Hypertensive with Desoxycorticosterone Acetate (DCA) and With Cortisone. *J. Clin. Invest.,* 36:1295, 1957.

Kot, P. A., and Klitgaard, H. M., Elimination of C^{14}-Labelled Thyroxine in the Bile, Urine, and Expired Air of Rats in Altered Thyroid States. *Endocrinology,* 64:319, 1959.

Krahl, M. E., Functions of Insulin and Other Regulatory Factors in Peptide Formation by Animal Cells. *Rec. Progr. Hormone Res.,* 12:199, 1956.

Ladell, W. S. S., Disorders Due to Heat. *Trans. Roy. Soc. Trop. Med. and Hyg.,* 51: 189, 1957.

Langdon, R. G., The Biosynthesis of Fatty Acids in Rat Liver. *J. Biol. Chem.,* 226:615, 1957.

Langley, L. L., and Kilgore, W. G., Carbon Dioxide as a Protecting and Stressing Agent. *Am. J. Physiol.,* 180:277, 1955.

Larson, B. L., and Hays, R. L., An Explanation for Bovine Parturition Edema and Treatment with Blood Protein Relacements. *J. Dairy Sci.,* 41:995, 1958.

Larson, B. L., and Touchberry, R. W., Blood Serum Protein Level as a Function of Age. *J. Animal Sci.,* 18:983, 1959.

Lathe, G. H., and Peters, R. A., Some Observations on the Comparative Effects of Cold and Burns on Protein Metabolism in Rats. *Quart. J. Exp. Physiol.,* 35:55, 1949.

Leaf, A., Bartter, F. C., Santos, R. F., and Wrong, O., Evidence in Man that Urinary Electrolyte Loss Induced by Pitressin is a Function of Water Retention. *J. Clin. Invest.,* 32:868, 1953.

Lee, D. H. K., The Status of Animal Climatology with Special Reference to Hot Conditions. *Animal Breed Abstr.,* 27:1, 1959.

Leffel, E. C., and Shaw, J. C., Studies on Ketosis in Dairy Cattle. XXI. The Effect of Different Levels of Protein and Energy Intake. *J. Dairy Sci.,* 40:981, 1957.

Leonhardt, H., Uber den Einfluss Thermischer Hautreize auf den Blutzucker Spiegel. *Deutsches Arch. F. Klin. Med.,* 187:132, 1941.

Levin, L., The Effects of Several Varieties of Stress on the Cholesterol Content of the Adrenal Glands and The Serum of Rats. *Endocrinology,* 37:34, 1945.

Levine, R., and Goldstein, M. S., The Action of Insulin. *Scientific American,* 198:99, 1958.

Lichton, I. J., Competition Between Sweat Glands and Kidneys for Salt and Water in Man. *J. Appl. Physiol.,* 11:223, 1957.

Lipsett, M. B., and Pearson, O. H., Sodium Depletion in Adrenalectomized Humans, *J. Clin. Invest.,* 37:1394, 1958.

Lotspeich, W. C., Kidney, Water and Electrolyte Metabolism. *Ann. Rev. Physiol.,* 20: 339, 1958.

Louisiana Progress Report 1958, Breeding Better Dairy Cattle for the South. *Louisiana Agr. Exp. Sta. Dairy Dept. Publ.,* 11, 1959.

Luetscher, J. A., Jr., *Aldosterone, Advances in Internal Medicine.* W. Dock and I Anapper Eds. Chicago: The Year Book Publishers, Inc., 8:155, 1956b.

Luetscher, J. A., Jr., Studies on Aldosterone in Relation to Water and Electrolyte Balance in Man. *Rec. Prog. Hormone Res.,* 12:175, 1956.

Luetscher, J. A., Jr., and Axelrad, B. J., Increased Aldosterone Output During Sodium Deprivation in Normal Man. *Proc. Soc. Exper. Biol. & Med.,* 87:650, 1954.

Luetscher, J. A., Jr., and Curtis, R. H., Aldosterone: Observations on the Regulation of Sodium and Potassium Balance, *Ann. Int. Med.,* 43:658, 1955.

Luetscher, J. A., Jr., and Johnson, B. B., Observations on the Sodium-Retaining Corticoid (Aldosterone) in Urine of Children and Adults in Relation to Sodium Balance and Edema. *J. Clin. Invest.,* 33:1441, 1954.

Luft, R., Olivecrona, H., Sjogren, B., Ikkos, D., and Ljunggren, H. Therapeutic Results of Hypophysectomy in Metastatic Carcinoma of the Breast and In Severe Diabetes Mellitus; Adrenocortical Function After Hypophysectomy. *Ciba Found. Coll. on Endocron.,* 8:438, 1955.

Luft, R., Sjogren, B., Ikkos, D., Ljunggren, H., and Tarukoski, H. Clinical Studies on Electrolyte and Fluid Metabolism Effect of ACTH, Desoxycorticosterone Acetate and Cortisone; Electrolyte and Fluid Changes in Acromegaly. In: *Rec. Prog. Hor. Res.,* 10:425, 1954.

Macchi, I. A., Hechter, O., Studies of ACTH Action Upon Perfused Bovine Adrenals: Minimal ACTH Concentration Requisite for Maximal Glandular Response. *Endocrinology,* 55:426, 1954.

Macfarlane, W. V., Morris, R. J. H., and Howard, B., Heat and Water in Tropical Merino Sheep. *Australian J. Agr. Res.,* 9:217, 1958.

Macfarlane, W. V., and Robinson, K. W., Decrease in Human Excretion of 17-Katogenic Steroids and 17-Ketosteroids with Increasing Ambient Temperature. *J. Appl. Physiol.,* 11:199, 1957.

Macfarlane, W. V., and Robinson, K. W., Seasonal Changes in Plasma Antidiuretic Activity Produced by a Standard Heat Stimulus. *J. Physiol.,* 135:1, 1957.

Macfarlane, W. V., Robinson, K., Howard, B., and Kinne, R., Heat, Salt and Hormones in Panting and Sweating Animals. *Nature,* 182:672, 1958.

Mach, R. S., and Fabre, J., Clinical and Metabolic Effects of Aldosterone. *Ciba Foundation Colliquia on Endocrinology,* 8:361, 1955.

Madden, S. C., Carter, J. R., Kattus, A. A., Jr., Miller, L. L., and Whipple, G. H., Ten Amino Acids Essential for Plasma Protein Production Effective Orally or Intravenously, *J. Exp. Med.,* 77:277, 1943.

Man, E. B., Kydd, D. M., and Peters, J. P., Butanol-Extractable Iodine of Serum. *J. Clin. Inv.,* 30:531, 1951.

Martin, M. M., and Walker, G., Studies with Na^{22}-An Assessment of Sodium Balance and Distribution. *Metabolism,* 6:466, 1957.

Matsuda, N., Effect of Cortisone, DOCA, and ACTH Upon Calcification of Rabbit Dentine and Amount of Serum Calcium, Inorganic Phosphate and Total Protein. *Gumma, J. Med. Sci.,* 5:289, 1956. (B.A., 33:13599, 1959).

McCandless, E. L., and Dye, J. A., Physiological Changes in Intermediary Metabolism of Various Species of Ruminants Incident to Functional Development of Rumen. *Am. J. Physiol.,* 162:434, 1950.

McDonald, J., and Macfarlane, W. V., Renal Function of Sheep in Hot Environments. *Aust. J. Agr. Res.,* 9:680, 1958.

MacDonald, M. A., and Bell, J. M., Effects of Low Fluctuating Temperatures on Farm Animals. II. Influence of Ambient Temperature on Water Intake of Lactating Holstein-Freisian Cows. *Canad. J. Animal Sci.,* 38:23, 1958.

MacDonald, M. A., and Bell, J. M., Effects of Low Fluctuating Temperatures on Farm Animals. IV. Influence of Ambient Air Temperature on Feed Intake of Lactating Holstein-Friesian Cows. *Canadian J. Animal Sci.,* 38:148, 1958.

McDowell, R. E., Physiological Approaches to Animal Climatology. *J. Hereditary,* 49: 52, 1958.

McDowell, R. E., Lee, D. H. K., Fohrman, M. H., and Anderson, R. S., Respiratory Activity as an Index of Heat Tolerance in Jersey and Sindhi x Jersey (F_1) Crossbred Cows. *J. Amer. Sci.,* 12:573.

Mefferd, R. B., Jr., and Hale, H. B., Effects of Altitude, Cold, and Heat on Metabolic Interrelationship in Rats. *School of Aviation Medicine,* U.S.A.F., Report No. 57-141, 1957.

Mefferd, R. B., and Hale, H. B., Studies on Cross-Adaptation. *School of Aviation Med.,* U.S.A.F., Reports No. 58-106, 58-107, 58-108, 1958.

Mefferd, R. B., Hale, H. B., Martens, H. H., Nitrogen and Electrolyte Excretion of Rats Chronically Exposed to Adverse Environments. *School of Aviation Med.,* U.S.A.F., Report No. 57-66, 1957.

Miller, T. R., II., Fallon, W. W., and Lloyd, C. W., Divergence in Magnesium, Sodium, and Potassium Excretion During Stimulation of Endogenous Aldosterone Production. *J. Clin. Endocrinol. and Met.,* 18:1178, 1958.

Mills, J. N., and Thomas, S., The Acute Effect of Adrenal Hormones and Carbohydrate Metabolism Upon Plasma Phosphate and Potassium Concentrations in Man. *J. Endocrinol.,* 16:164, 1957.

Mills, J. N., and Thomas, S., The Acute Effects of Cortisone and Cortisol Upon Renal Function in Man. *J. Endocrinol.,* 17:41, 1958.

Mitchesll, H. H., Hamilton, T. S., and Haines, W. T., The Dermal Excretion Under Controlled Environmental Conditions of Nitrogen and Minerals in Human Subjects, with Particular Reference to Calcium and Iron. *J. Biol. Chem.,* 178:345, 1949.

Montastruc, P., Contribution Experimentale a l'Etude de la Regulation Hormonale du Metabolisme de L'Eau. Etude des Effects de al Suppression Simultanee des Hormones Antidiuretiques et Cortico-Surrenales. *Imp. du Sud,* Toulouse, 1954.

Morrison, S. D., The Nitrogen Balance of Pregnant Rats. *J. Physiol.,* 133:167, 1956.
Newburgh, L. H., *Physiology of Heat Regulation and the Science of Clothing.* Philadelphia: W. B. Saunders Co., 1949.
Nicholls, D., and Rossiter, R. J., Phosphorus Metabolism of the Adrenal Gland of the Rat. Acclimatization to Cold. *Am. J. Physiol.,* 187:11, 1956.
Nicholls, D., and Rossiter, R. J., The Role of Pituitary and Thyroid Glands in the Phosphorus Metabolism of the Adrenal Gland During Cold Stress. *Endocrinology,* 66:547, 1955.
Noall, M. W., Riggs, T. R., Walker, L. M., and Christensen, H. N., Endocrine Control of Amino Acid Transfer. *Science,* 126:1002, 1957.
Nungesser, W. C., Acute Cold and Renal Function in Dogs. *Fed. Proc.,* 14:108, 1955.
OBara, J., and Watase, H., Effects of Administration of Some Carbohydrates and Fatty Acids Upon the Blood Glucose Level of Goats. *Bul. Natl. Inst. Animal Health,* 32:373, 1957.
Onchi, T., Influence of Cold on the Blood-Sugar Level in a Cat Under Insulin. *Tokoku, J. Exper. Med.,* 41:97, 1941.
Oppel, T. W., and Hardy, J. D., Studies in Temperature Sensation. II. The Temperature Changes Responsible For The Stimulation of Heat End Organs. *J. Clin. Investigation,* 16:525, 1937.
Pappinheimer, J. R., and Kinter, W. B., Hematocrit Ratio of Blood Within Mammalian Kidney and its Significance for Renal Hemodynamics. *Am. J. Physiol.,* 185:377, 1956.
Pechet, M. M., The Metabolic Effects of Metacortandracin and Metacortandralone in Man. A New Series of diene Steroids, 47th Meet. *Am. Soc. Clin. Invest.,* p. 8, 1955.
Peirce, A. W., Studies on Salt Tolerance of Sheep. I. The Tolerance of Sheep for Sodium Chloride in the Drinking Water. *Australian J. Agr. Res.,* 8:711, 1957.
Potter, V. R., Possible Biochemical Mechanisms Underlying Adaptation to Cold. *Fed. Proc.,* 17:1060, 1958.
Premachandra, B. N., The Thyroxine Secretion Rate of Domestic Animals Utilizing Radioactive Iodine as a Tracer. Ph.D. Thesis, University of Missouri, 1958.
Premachandra, B. N., Pipes, G. W., and Turner, C. W., A Study of Thyroxine Secretion in Cattle. *J. Animal Sci.,* 16:1063, 1957.
Premachandra, B. N., Pipes, G. W., and Turner, C. W., Variation in the Thyroxine-Secretion Rate of Cattle. *J. Dairy Sci.,* 41:1609, 1958.
Querido, A., Kassenaar, A. A. H., and Cats, A., Variability of Adrenocortical Response to ACTH in Different Persons, and the Influence of Variations in Administration of ACTH Preparations on the Level of 17-Hydroxycorticosteroids in the Blood. *Ciba Foundation Colloquia on Endocrinology,* 8:309, 1955.
Ragsdale, A. C., Thompson, H. J. Worstell, D. M., and Brody, S., Influence of Increasing of Temperature, 40° to 105°F. on Milk Production in Brown Swiss Cows, and on Feed and Water Consumption and Body Weight in Brown Swiss and Brahman Cows and Heifers. *Mo. Agr. Exp. Sta. Res. Bul.,* 471, 1951.
Ragsdale, A. C., Thompson, H. J. Worstell, D. M., and Brody, S., Milk Production and Feed and Water Consumption Responses of Brahman, Jersey, and Holstein Cows to Changes in Temperature, 50° to 105°F. and 50° to 8°F., *Mo. Agr. Exp. Sta. Res. Bul.,* 460, 1950.
Raisz, L. S., McNeely, W. F., Saxon, L., and Rosenbaum, J. D., The Effects of Hydrocortisone on Water Diuresis and Renal Function in Man. *J. Clin. Inv.,* 36:767, 1957.
Rasmussen, H., Thyroxine Metabolism in the Nephrotic Syndrome. *J. Clin. Inv.,* 35: 792, 1956.
Reichlin, S., The Effect of Starvation and Dehydration on Radioiodine Excretion Following Injection of I^{131} Labelled Thyroxine. *Endocrinology,* 62:868, 1958.

Reid, R. L., Studies on the Carbohydrate Metabolism of Sheep. I. The Range of Blood-Sugar Values Under Several Conditions. *Aust. J. Agr. Res.,* 1:182, 1950a.

Reid, R. L., Studies on the Carbohydrate Metabolism of Sheep. II. The Uptake by the Tissues of Glucose and Acetic Acid from the Peripheral Circulation. *Aust. J. Agr. Res.,* 1:338, 1950b.

Reid, R. L., Studies on the Carbohydrate Metabolism of Sheep III. The Blood Glucose During Insulin Hypoglycaenia. *Aust. J. of Agr. Res.,* 2:132, 1951a.

Reid, R. L., Studies on the Carbohydrate Metabolism of Sheep. VI. Interrelationship Between Changes in the Distribution and Levels of Glucose and Levels of Volatile Fatty Acids in the Blood of Lambs. *Aust. J. Agr. Res.* 4:213, 1953.

Reid, R. L., Studies on the Carbohydrate Metabolism of Sheep. VII. Intravenous Glucose and Acetate Tolerance Test. *Aust. J. Agr. Res.,* 9:788, 1958.

Reid, R. L., and Hogan, J. P., Studies on the Carbohydrate Metabolism of Sheep. VIII. Hypoglycaemia and Hyperketonaemia in Under-nourished and Fasted Pregnant Ewes. *Aust. J. Agr. Res.,* 10:81, 1959.

Reinberg, A., and Stolkowski, J., Research on the Mode of Action of Some Corticosteroids on Cellular Potassium. II. Relationship Between Glucose Metabolism and Acid-Base Equilibrium. *Ann. Endocrinol.,* 18:681, 1957.

Reinhold, J. G., *Standard Methods of Clinical Chemistry.* Vol. I. New York: Acad. Press Inc., 1953.

Renold, A. E., Ashmore, J., and Hastings, B., Regulation of Carbohydrate Metabolism in Isolated Tissues. *Vitamins and Hormones,* 14:139, 1956.

Resnick, O., and Hechter, O., Studies on the Permeability of Galactose in Muscle Cells of the Isolated Rat Diaphragm. *J. Biol. Chem.,* 224:941, 1957.

Riek, R. F., and Lee, D. H. K., Reactions to Hot Atmosphere of Jersey Cows in Milk. *J. Dairy Res.,* 15:219, 1948.

Robertson, W. G., Mixner, J. P. Bailey, W. W., and Lennon, H. D., Jr., Effect of Certain Acute Stress Conditions on the Plasma Levels of 17-Hydroxycorticosteroids and Protein-Bound Iodine in Dairy Cattle. *J. Dairy Sci.,* 41:302, 1958.

Robinson, S., Physiological Effects of Heat and Cold. *Ann. Rev. Physiol.,* 14:73, 1952.

Robinson, K. W., and Macfarlane, Urinary Excretion of Adrenal Steroids During Exercise in Hot Atmospheres. *J. Appl. Physiol.,* 12:13, 1958.

Robinson, S., and Robinson, A. H., Chemical Composition of Sweat. *Physiol. Revs.,* 34:202, 1954.

Robscheit-Robbins, F. S., Miller, L. L., and Whipple, G. H., Hemoglobin and Plasma Protein. *J. Exp. Med.,* 77:375, 1943.

Rodbard, S., Saili, H., Malin, A., and Young, C., Significance of Changes in Plasma and Extracellular Volumes in Induced Hyperthermia and Hypothermia. *Am. J. Physiol.,* 167:485, 1951.

Rosen, F., Roberts, N. R., Budnick, L. E., and Nichol, C. A., Corticosteroids and Transaminase Activity: The Specificity of the Glutamic-Pyruvic Transaminase Response. *Endocrinology,* 65:256, 1959.

Rosenfeld, C., Rosemberg, E., Ungar, F., and Dorfman, R. I., Regulation of the Secretion of Aldosterone-like Material. *Endocrinlogy,* 58:255, 1956.

Ross, E. J., Reddy, W. J., Rivera, A., and Thorn, W., Effects of Intravenous Infusion of Aldosterone Acetate on Sodium and Potassium Excretion in Man. *J. Clin. Endocrinol. and Med.,* 19:289, 1959.

Russell, J. A., Hormonal Control of Amino Acid Metabolism. *Fed. Proc.,* 14:696, 1955.

Sala, G., and Luetscher, J. A., Jr., The Effect of Sodium-Retaining Corticoid, Electrocortin, Desoxycorticosterone, and Cortisone on Renal Function and Excretion of Sodium, and Water in Adrenalectomized Rats. *Endocrinology,* 55:516, 1954.

Salassa, R. M., Mason, H. L., Mattox, V. R., Orvis, A. L., and Power, M. H., Effects of Aldosterone in Water, Electrolyte, and Nitrogen Metabolism in Addison's Disease. *Proc. Staff Meet Mayo Clinic,* 32:201, 1957.

Salter, J. M., Davidson, J. W. F., and Best, C. H., The Effects of Insulin and Somatotrophin on the Growth of Hypophysectomized Rats. *Canadian J. Biochem. and Physiol.,* 35:913, 1957.

Sampson, J., and Boley, L. E., The Ketone Bodies, Sugar, and Calcium of the Blood of Ewes During Late Pregnancy. *Cornell Vet.,* 30:392, 1940.

Sawyer, W. H., Increased Renal Reabsorption of Osmotically Free Water by the Toad (Bufor Marinus) in Response to Neurohypo-Physial Hormones. *Am. J. Physiol.,* 189:564, 1957.

Sayers, G., Redgate, E. S., Royce, P. C., Hypothalamus, Adenohypophysis and Adrenal Cortex. *Ann. Rev. Physiol.,* 20:243, 1958.

Sayers, G., and Sayers, M. A., The Pituitary-Adrenal System. *Rec. Prog. Hor. Res.,* 2:81, 1948.

Sayers, G., Sayers, M. A., Fry, E. G., White, A., and Long, C. N. H., The Effect of the Adrenotrophic Hormone of the Anterior Pituitary on the Cholesterol Content of the Adrenals. *Yale J. Biol. and Med.,* 16:361, 1944.

Schambye, P., Volatile Acids and Glucose in Portal Blood of Sheep. *Nord. Vet. Med.,* 3:555, 1951.

Schmidt-Nielsen, K., The Physiology of the Camel. *Scientific American,* 201:140, 1959.

Schmidt-Nielsen, K., Schmidt-Nielsen, B., Jarnum, S. A., and Houpt, T. R., Body Temperature of the Camel and its Relation to Water Economy. *Am. J. Physiol.,* 188:103, 1957.

Schmidt-Nielsen, B., Schmidt-Nielsen, K., Houpt, T. R., and Jarnum, S. A., Urea Excretion in the Camel. *Am. J. Physiol.,* 188:477, 1957a.

Schmidt-Nielsen, Murdaugh, H. F., Osaki, H., and O'Dell, R., Urea Excretion in Sheep and Dog. *Fed. Proc.,* 16:113, 1957b.

Schoenheimer, R., Ratner, S., Rittenberg, D., and Heidelberger, M., The Interaction of the Blood Proteins of the Rat with Dietary Nitrogen, *J. Biol. Chem.,* 144:541, 1942.

Scholander, P. F., Studies on Man Exposed to Cold. *Fed. Proc.,* 17:1054, 1958.

Schultz, A. B., Influence of Adrenaline Administration on Some Blood Constituents in Holstein Calves. *J. Dairy Sci.,* 42:166, 1959.

Scow, R. O., Wagner, E. M., and Ronov, E., Effect of Growth Hormone and Insulin on Body Weight and Nitrogen Retention in Pancreatectomized Rats. *Endocrinology,* 62:593, 1958.

Selle, W. A., *Body Temperature.* Springfield, Ill.: Charles C. Thomas Pub., 1952.

Selye, H., Stress: *The Physiology and Pathology of Exposure to Stress.* Acta, Inc., Montreal, Canada, 1950.

Selye, H., The General Adaptation Syndrome and the Diseases of Adaptation. *J. Clinic. Endocrinol.,* 6:117, 1956.

Selye, H., What is Stress. *Metabolism,* 5:525, 1956a.

Selye, H., and Heuser, G., *Fifth Annual Report on Stress.* New York, N. Y.: MD Publications, Inc., 1955-1956.

Sereni, F., Kenney, F. T., and Kretchmer, N., Factors Influencing the Development of Tyrosine-α-Ketoglutarate Transaminase Activity in Rat Liver. *J. Biol. Chem.,* 234:609, 1959.

Shaw, J. C., Hatziolos, B. C., and Chung, A. C., Studies on Ketosis in Dairy Cattle, XV: Response to Treatment with Cortisone and ACTH. *Science,* 114:574, 1951.

Shaw, W. N., and Stadie, W. C., Coexistence of Insulin-Responsive and Insulin-non-Responsive Glycolytic Systems in Rat Diaphragm. *J. Biol. Chem.,* 227:115, 1957.

Shull, K. H., and Mayer, J., Experimental Hyperglycemic States Not Primarily Due to a Lack of Insulin. *Vitamins and Hormones,* 14:187, 1956.

Simpson, S. A., and Tait, J. F., Recent Progress in Methods of Isolation, Chemistry, and Physiology of Aldosterone. *Recent Prog. Hormone Research,* 11:183, 1955b.

Simpson, S. A., and Tait, J. E., The Possible Role of Electrocortin in Normal Human Metabolism. *Ciba Foundation Colliquia on Endocrinology,* 8:204, 1955a.

Singer, B., and Stack-Dunne, M. P., Secretion of Aldosterone and Corticosterone by the Rat Adrenal. *Nature,* 174:790, 1954.

Singer, B., Venning, E. H., Method of Assay of a Sodium-Retaining Factor in Human Urine. *Endocrinology,* 52:623, 1953.

Smith, J. H., Robinson, S., Pearcy, M., Renal Responses to Exercise, Heat, and Dehydration. *J. Appl. Physiol.,* 4:659, 1952.

Spealman, C. R., Newton, M., and Post, R. L., Influence of Environmental Temperature and Posture on Volume and Composition of Blood. *Am. J. Physiol.,* 150:628, 1947.

Stadie, W. C., Current Concept of the Action of Insulin. *Physiol. Rev.,* 34:52, 1954.

Stewart, C. P., Aspects of Recent Work on Electrolyte Metabolism. *Clin. Chem.,* 3:294, 1957.

Strauss, M. B., *Body Water in Man.* 1st Ed., Boston-Toronto: Brown and Company, 1957.

Streeten, D. H. P., Phil, D., Pont, M. E., and Conn, J. W., Effects of Hydrocortisone on Urinary Excretion of Sodium in Adrenalectomized Rats. *J. Lab. and Clin. Med.,* 46:729, 1955.

Struempler, A. W., and Burroughs, W., Stilbesterol Feeding and Growth Hormone Stimulation in Inmature Ruminants. *J. Animal Sci.,* 18:427, 1959.

Swanson, E. W., Lengemann, F. W., and Monroe, R. A., Factors Affecting the Thyroid Uptake of I^{131} in Dairy Cows. *J. Animal Sci.,* 16:318, 1957.

Swingle, W. W., Brannick, L. J., Osborn, M., and Glenister, D., Effect of Gluco- and Mineralocorticoid Adrenal Steroids on Fluid and Electrolytes of Fasted Adrenalectomized Dogs. *Proc. Soc. Expt. Biol. and Med.,* 96:446, 1957.

Swingle, W. W., DaVanzo, J. P., Glenister, D., Crossfield, H. C., and Wagle, C., Role of Gluco- and Mineralocorticoids in Salt and Water Metabolism of Adrenalectomized Dogs. *Am. J. Physiol.,* 196:283, 1959.

Taneja, G. C., Sweating in Cattle. *J. Agr. Sci.,* 52:168, 1959.

Tasker, J. B., Roberts, S. J., Fox, F. H., and Hall, C. E., Abomasal Ulcers in Cattle, Recovery of One Cow After Surgery. *Am. Vet. M. Assoc.* J., 133:365, 1958.

Taurog, A., and Chaikoff, I. L., the Nature of the Circulating Thyroidal Hormone. *J. Biol. Chem.,* 176:639, 1948.

Teeri, A. E., Virchow, W., Colovos, N. F., and Greeley, F., Blood Composition of White-Tailed Deer. *J. Mammal,* 39:269, 1958.

Thorn, G. W., Laidlaw, J. C., and Goldfien, A., Studies on the Sodium-Retaining Effect of Adrenal Cortical Steroids. *Ciba Foundation Colliquia on Endocrinology,* 8: 343, 1955.

Thorn, N. A., Mammalian Antidiuretic Hormone. *Physiol. Revs.,* 38:169, 1958.

Treichler, R., and Mitchell, H. H., The Influence of Plane of Nutrition and of Environmental Temperature on the Relationship Between Basal Metabolism and Endogenous Nitrogen Metabolism Subsequently Determined. *J. Nutrition,* 22:338, 1941.

Vander, A. J., Malvin, R. L., Wilde, W. S., Lapides, J., Sullivan, L. P. and McMurray, V. M., Effects of Adrenalectomy and Aldosterone on Proximal and Distal Tubular Sodium Reabsorption. *Proc. Soc. Expt. Biol. & Med.,* 99:323, 1958.

Watanabe, G. I., and Yoshida, S., Climatic Effect on Urinary Output of Neutral 17-Ketosteroids. *J. Appl. Physiol.,* 9:456, 1956.

Welt, I. D., Stetten, Dew, Jr., Ingle, D. J., and Morely, E. H., Effects of Cortisone Upon Rates of Glucose Production and Oxidation in the Rat. *J. Biol. Chem.,* 197: 57, 1952.

Wolf, A. V., Thirst, *Scientific American,* Jan. p. 70, 1956.

Wool, I. C., and Goldstein, M. S., Role of Neurohumors in the Action of the Adrenal Cortical Steroids. Protein Mobilization. *Am. J. Physiol.,* 194:481, 1958.

Yulle, C. L., Lucas, F. V., Olson, J. P., and Shapiro, A. B., Plasma Protein Turnover And Tissue Exchange. Influence of Dietary Protein and Protein Depletion. *J. Expt. Med.,* 109:173, 1959.

You, S. S., You, R. W., and Sellers, E. A., Effect of Thyroidectomy, Adrenalectomy and Burning on the Urinary Nitrogen Excretion of the Rat Maintained in a Cold Environment. *Endocrinology,* 47:156, 1950.