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# Physiological responses of cattle to controlled cold exposure.

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PHYSIOLOGICAL RESPONSES OF CATTLE TO CONTROLLED COLD EXPOSURES

by

SF 768  
OL8p  
C.2

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A Thesis Submitted to the  
Graduate Faculty in Partial Fulfillment of  
The Requirements for the Degree of  
MASTER OF SCIENCE

Major Subject: Veterinary Physiology

Approved: \_\_\_\_\_

Signatures have been redacted for privacy

Iowa State University  
Of Science and Technology  
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## I. INTRODUCTION

The influence of environmental factors on domestic animals has been studied by others to measure the effect of these factors on production characteristics of cattle. An improvement in production capabilities is the fundamental goal of most agricultural researchers. However, measuring the level of production as it is affected by environmental factors is not enough. A more specialized study of the fundamental physiological functions, as they are influenced by various environmental conditions, is needed. The knowledge obtained can then be used to establish why productive capabilities of domestic animals are influenced by certain environmental factors so that specific methods of husbandry can be best utilized to ameliorate the effect of environmental factors to increase animal productivity.

In recent years considerable research has been conducted on the problem of heat in relation to performance and production of livestock. Lee (1959), while reviewing the status of animal climatology, has written, "For the purpose of obtaining a balanced picture it is unfortunate that most of the discussion has centered around the reactions of animals to hot conditions, while insufficient attention has been given to the problems of cold climates, to which a large proportion of the domestic animal population is frequently exposed."

This study was designed to observe the responses of cattle, acclimated to warm and cold temperatures, when exposed to a range of cold environmental temperatures.

The primary objective has been to study physiological responses to abrupt cold exposure, thereby establishing a pattern of reaction which may be used for comparison in studies utilizing pathogenic agents.

## II. REVIEW OF LITERATURE

### A. Response of Cattle to Cold Exposure

European cattle (Bos taurus) are considered to be quite tolerant to cold. This tolerance has been attributed to the conservation of body heat, by reduction in respiratory frequency, growth of denser hair coats, increase of coat insulation by erection of hairs, higher metabolic rate, removal of water from circulating blood, increased muscle tone and shivering, reduction of blood flow to superficial tissues, and possibly by special arrangement of venae comites and arterio-venous anastomoses (Hess and Bailey, 1961; Lee and Phillips, 1948; Goodall and Yang, 1954; Goodall, 1955).

#### 1. Blood pressure

Little has been reported concerning the measurement of blood pressure of cattle during controlled cold exposure. Changes of arterial blood pressure associated with changes of body temperature in cattle have been studied during short exposures of 3-7 hours in environments of 23, 50, 59, 68, and 104°F (Ingram and Whittow, 1963). No mention was made of environmental conditioning prior to the exposures. In the 23°F environment the mean arterial blood pressure increased immediately before the rise in ear temperatures noted in the study. However, no values were reported for the level of blood pressure throughout the entire exposure period. It was noted that blood pressure increased in response to heating the hypothalamus during exposure to environmental temperatures of 23, 59, and 104°F. The effect of heating the hypothalamus on the skin temperatures of the ears

during the 23°F exposure depended upon whether the skin temperature of the ear was increasing or decreasing spontaneously. When the skin temperature was increasing, heating the hypothalamus caused the temperature of the ear to increase. When the skin temperature of the ear was decreasing, heating the hypothalamus had no effect. Infrared radiation of the rump and flanks during 50 and 59°F exposure was accompanied by an increase of mean blood pressure, at times independent of changes in hypothalamus temperature.

The increase in arterial blood pressure which followed localized heating of the hypothalamus or the skin was at times (6 in 20) associated with an increase in the skin temperature of the extremities. This was accepted as evidence that the increased blood pressure was not due to an increase in cutaneous vascular resistance in the extremities. The possibility was presented that the effect of the increased pressure was to cause an increase in the blood flow through the ear by the opening of arterio-venous anastomoses, which have been shown to be present in the ear of the ox (Goodall, 1955). An increase in cardiac output was suggested as a possible factor causing the increased blood pressure reported in that study.

## 2. Heart rate

A cardiovascular response to stress in general, and to cold exposure in particular, is an alteration in heart rate (Horvath and Howell, 1964). A close correlation between pulse rate and heat production has been generally known for a long time (Read, 1924). "This similarity is not surprising considering that the blood pumped by the heart supplies oxygen and nutrients for heat production, and also carries heat from the interior

to the surface of the body for dissipation" (Kibler and Brody, 1949). Some factors affecting heart rate also affect heat production.

An apparent "comfort zone" for European cattle under controlled laboratory conditions has been reported to lie between 30 and 60°F. The "normal" heart rate within this temperature range was 58 to 66 beats per minute for lactating dairy cows (Worstell and Brody, 1953). This compares favorably with the normal heart rate of 60-70 given by Dukes (1955) for dairy cattle.

When cattle in a controlled environment were subjected to a "gradual" change (2 weeks at a given temperature level) of environmental temperature from 50 to 5°F, the pulse rate ratio (ratio of experimental to control data) increased approximately 8% (Kibler and Brody, 1949). A similar experiment exposing cattle to a gradual change of environmental temperature from 50 to 9°F revealed only a slight increase in pulse rate. However, considerable fluctuation of environmental temperature occurred below 20°F (Kibler and Brody, 1950).

During another study designed to observe the response to different levels of humidity at temperatures of 50, 40, and 12°F, the heart rate was slightly slower at 12°F than at higher temperatures (Kibler and Brody, 1953).

In a later experiment (Kibler and Brody, 1954) designed to observe the effect of wind at environmental temperatures of 50 and 17°F, the average heart rate of all animals at low wind velocity (0.5 mph) was 56 and 64 beats per minute, respectively. At medium wind velocity (3-6 mph) the average heart rate was 56 and 64, and for high wind velocity (7-10 mph) the average rate was 56 and 67. If one ignores the breed, temperature, and wind velocity interaction, which no doubt introduces considerable error,

the average increase in heart rate is about 10 beats per minute at 17°F as compared with 50°F environmental temperature. At 17°F, increasing air velocity from 0.5 to 10 mph caused a statistically significant increase of 4 to 9 beats per minute in the dairy cattle.

Kibler and Brody (1956) also studied the effect of daily variation in environmental temperature. During periods of 3 to 5 weeks the mean daily temperature was controlled at about 25, 55, and 85°F with a daily range of 30°F. Maximal temperatures occurred between 3 and 4 p.m. and minimal temperatures occurred between 3 and 7 a.m. If one compares the 55 and 25°F groups during the minimal temperature period of each day, viz. 3 a.m. observations, at about 40 and 15°F, respectively, the heart rate at 15°F is increased approximately 8%.

Several field studies have been made to observe the effect of cold exposure on heart rate.

Holstein-Friesian cows in a loose-housing shed (MacDonald and Bell, 1958), where the inside minimum air temperature ranged from -5 to 38°F, had an increase in heart rate as the ambient temperature decreased, with a regression significance level of 9%. An average heart rate of 67 beats per minute was observed above 40°F environmental temperature and an average rate of 70 beats per minute was observed below 10°F environment. It was concluded, also, that low temperature fluctuations per se do not affect heart rate changes in lactating, mature Holstein-Friesian cows that have been acclimatized to low temperatures and high relative humidity.

A similar study (Williams and Bell, 1964) observed that a significant relationship of heart rate and relative humidity existed at low fluctuating

environmental temperatures and confirmed the previous observation that heart rate is not affected by fluctuation of low environmental temperature.

Graf and Petersen (1953) reported that when animals were placed outside in a low environmental temperature at  $-16$  to  $+16^{\circ}\text{F}$ , the heart rates increased 12.4% of the rate of 97 observed inside the barn at  $45^{\circ}\text{F}$ ; however, these results were not statistically significant. The heart rates of the control animals at  $45^{\circ}\text{F}$  were subject to considerable variation.

### 3. Respiratory frequency

Alterations in respiratory activity, such as change in depth and frequency of respiration, occur in thermoregulation. Respiratory activity may be envisioned as respiratory frequency and respiratory volume. The respiratory volume is determined, in part, by the depth of respiration. Tidal volume is the amount of air breathed in or out during one respiratory cycle. In the normal animal at rest, respiration is regulated to maintain a constant alveolar ventilation (*i.e.*, an adequate amount of "fresh" air entering the alveoli). Alveolar ventilation rate is affected by the frequency and depth of respiration, as well as anatomic dead space, in response to the chemical and nervous control mechanisms of the body. Hence, control of pulmonary ventilation is actually an additive or multiplicative effect of many factors acting simultaneously on the respiratory centers, with the amount of interplay determined by environmental conditions (Guyton, 1966).

It is recognized that the frequency of the respiratory cycle is subject to numerous variations. Dukes (1955) lists the respiratory frequency of cattle as ranging from 10 to 30. The "normal" respiratory frequency of mature cattle in a controlled environment of  $40$  to  $60^{\circ}\text{F}$  (within the "comfort zone" of cattle) has been reported as 14 to 25 (Worstell and Brody, 1953).

When cattle in a controlled environment were subjected to a "gradual" change of environmental temperature from 50 to 5°F, their respiratory frequency was approximately 40% of that at 50°F. At low temperatures there was no evidence of breed difference in respiratory response. Minimal frequencies of 10 to 15 respirations were observed at 4°F. The thermoneutrality range (as judged by heat production) was 40 to 60°F for lactating Jersey and Holstein cows of that study (Kibler and Brody, 1949). During a similar experiment exposing cattle to a gradual change of environmental temperature from 50 to 9°F, the respiratory frequency per minute decreased from about 25 to 15 in Holsteins, from 20 to 14 in Jerseys, and from 14 to 12 in Brahmans (Kibler and Brody, 1950).

Another study was designed to observe the response to different levels of humidity at temperatures of 50, 40, and 12°F, the respiratory frequency (average of all animals at all humidity levels for a given temperature) at 50, 40, and 12°F was 29, 24, and 17 per minute, respectively.

In a later experiment designed to observe the effect of wind at environmental temperatures of 17, 50 and 65°F, the average respiratory frequency of all animals at low wind velocities (0.5 mph) was 12 (range 10 to 18), 20 (range 12 to 27), and 33 (range 10 to 51) per minute, respectively. At 17°F the respiratory frequency showed very slight changes with increased air velocity of 0.5 to 10 mph (Kibler and Brody, 1954).

The effect of daily variation in environmental temperature has been studied, the conclusion being that respiratory frequency was insensitive to temperature fluctuations between 10 to 40°F, become moderately sensitive between 40 and 70°F. The respiratory frequency ranged from about 12 to 27

per minute between 10 to 40°F, and from about 22 to 47 per minute between 40 to 70°F (Kibler and Brody, 1956).

Dairy cattle stanchioned in a loose-housing, uninsulated pole barn showed a decrease in respiratory frequency from 25 to 15.7 as the daily minimum air temperature decreased from 40 to 0°F (MacDonald and Bell, 1958). A similar study by Williams and Bell (1964) showed very close agreement of the relationship of respiratory frequency and air temperature at low fluctuating temperatures (approximately -3 to 34°F).

#### 4. Blood glucose

The concentration of blood glucose in adult ruminants under ordinary circumstances is relatively lower than monogastric animals (Dukes, 1955). The level of blood glucose in adult ruminants is relatively constant but is known to vary under certain circumstances (Reid, 1950). Temperature "stress" reported by Selye (1950) increases blood sugar level in experimental animals, especially during the resistance phase of the General Adaptation Syndrome.

In cattle, few studies have been made of the effect of controlled cold exposure on blood glucose. Blincoe and Brody (1951) noted a slight increase in blood glucose of dairy cows at low temperatures (0 to 105°F). Kamal et al. (1962) reported the effect of 35°F cold exposure for two weeks on dairy heifers that had been raised from 1 to 13 months of age at 50 and 80°F. When sampled on the ninth day of exposure, higher blood glucose concentration was observed in the warm acclimated heifers than in cold acclimated animals. These results indicated the occurrence of "stress" in cattle at cold exposure if they were previously raised in a warm climate.

Fish (1928) studied the seasonal change of blood sugar concentration of five cows noting that little fluctuation occurred during October, November and December. However, there was a uniform decrease in blood sugar during January and February with an increase in March, followed by fluctuation down in April, up in May and June, and a decline in July. Hodgson et al. (1932) reported that blood sugar levels throughout the winter months were lower than that previously observed during July and August. Horrocks and Paterson (1957) also noted a seasonal variation in blood levels of glucose in cattle, with the highest concentration occurring in July, progressively decreasing until December and then increasing from January to March.

#### 5. Total blood plasma proteins

Blood plasma proteins may be classified (Dukes, 1955) as total protein, fibrinogen, albumin and globulin, and various fractions thereof. The albumin-globulin ratio is approximately 1:1 for cattle. The total plasma proteins of cattle have been observed to be 8.32 gm/100 ml of plasma and the albumin-globulin fraction being 7.60 gm/100 ml of plasma.

It is generally accepted that the liver is the primary source of plasma proteins, with some of the globulin fractions originating extra-hepatically. Plasma proteins contribute to the viscosity of the blood, and aid in maintaining normal hemodynamics. In addition they aid in maintaining the acid-base equilibrium, transport of biologically active compounds, facilitate the blood clotting process, and aid in the animal's ability to resist disease.

Kamal et al. (1962) have reviewed the effect of environmental temperature on total plasma proteins. They cite reported concentrations of plasma

proteins for cattle ranging from 5.8 to 9.12 gm/100 ml plasma. They also conclude that surprisingly little study has been made of the influence of environmental temperature on the concentration of blood proteins in cattle.

Selye (1950) reported that in the General Adaptation Syndrome most stressors have a common effect on plasma protein; viz., to produce catabolic phases wherein plasma protein tends to fall below normal levels, with a change in relative concentrations.

Plasma protein concentrations may be changed either by alteration of synthesis or catabolism; or due to hemoconcentration or hemodilution.

Concurrent consideration of the plasma protein level and packed cell volume of blood may give some general indication of the plasma volume, since several studies have shown an inverse relationship between plasma protein concentration and plasma volume (Bazett et al., 1940; Conley and Nickerson, 1945; Spealman et al., 1947; Macfarlane, 1961; English, 1966).

#### 6. Blood packed cell volume

The packed cell volume (P.C.V.) is the percentage of blood that is erythrocytes. This percentage is commonly determined by measuring the relative height of packed cells in a centrifuged sample of blood. The size and number of the red blood cells circulating in the blood influence the packed cell volume. A change in the plasma volume of blood may also alter the P.C.V.

Various values of the venous P.C.V. of cattle have been reported. Hansard et al. (1953) reported a P.C.V. (corrected for trapped plasma) of 41% for four beef cattle. Reynolds (1953) reported a corrected P.C.V. of

32.4% (range 30.3-34.9) and plasma volume of 38.8 ml/kg body weight (range 36.3-40.6) for 10 dairy cows. Dale et al. (1957) reported a P.C.V. of 40.9% and plasma volume of 36.6 ml/kg for three dairy cows. Jones et al. (1956) reported an average P.C.V. of  $35.08 \pm 3.30$  std. dev. for 92 mature dairy cows. No significant breed differences were noted. Albritton (1952) cited a P.C.V. of 40 for the cow. Gartner et al. (1965) studied the influence of excitement on P.C.V. values of 16 beef cattle between the ages of 6 and 43 months. They reported a P.C.V. of 39.09 by venipuncture, 29.43 at rest (blood drawn through a jugular catheter), 33.06 after visual stimulation, and 39.16 after forced running for 100 meters.

It has been known for some time that an abrupt change of environmental temperature may cause rapid change in P.C.V. and plasma volume (Adolph and Molnar, 1946; Bass et al., 1951; Eliot et al., 1949). Blincoe and Brody (1951) reported little change in P.C.V. for dairy cows as the environmental temperature changed from 0 to 65°F.

## 7. Blood plasma electrolytes

a. Potassium Hammarsten et al. (1963) have discussed the normal metabolism of potassium. Potassium is one of the most abundant cations in the body and plays a part in many physiological processes. The high concentration of intracellular potassium aids in maintaining a suitable osmolarity and electroneutrality as well as playing a part in enzyme action associated with production of energy, storage, and cellular growth. The effect of potassium on enzyme systems is commonly antagonized by sodium. Changes in intracellular or extracellular pH or osmotic pressure disturb the normal potassium equilibrium and potassium tends to move in or out of the cells

in exchange for sodium and hydrogen ions. The concentration of potassium in extracellular water is also a function of extracellular water volume. The gradient between extracellular and intracellular potassium concentrations is influenced by, among other things, adrenal steroids, oxygen saturation of the blood, hyponatremia, and disturbances in hydrogen ion regulation or carbohydrate metabolism. The kidney does not conserve potassium as well as it does sodium. Ionic concentration of potassium in the urine is the result of tubular secretion. Adrenal steroids are believed to increase tubular secretion of potassium in exchange for sodium. A decrease in serum potassium may result from movement of fluid into the extracellular or intracellular space or by loss through the kidney or digestive tract.

Ward (1966) recently reviewed potassium metabolism of domestic ruminants. He stated that available literature did not indicate that the metabolism of potassium after absorption or at any cellular level was any different in ruminants than in other species, with the exception of the potassium content of sheep red blood cells, which seems to be under genetic control.

McSherry and Grinyer (1954) found no significant differences in the blood serum potassium of 126 dairy animals of various ages.

From the data of Ward (1956) it was calculated that urinary potassium as a percentage of total output for nonlactating cows was 86% and for lactating cows urine represented 75, feces 13, and milk 12% of the total. The ready absorption of potassium from the fore-gut would indicate that fecal potassium arises principally from endogenous sources.

There appears to be an obligatory excretion of potassium by cattle, both in the feces and urine (Campbell and Roberts, 1965) perhaps in part as a

consequence of the equilibrium exchange in the kidney between potassium and hydrogen ions (Pickering, 1965).

Kamal et al. (1962) reviewed the climatic influence on plasma and urine potassium levels. Reported studies pertaining to cattle are scarce and unsatisfactory. Blincoe and Brody (1951) found no marked difference in plasma potassium between 5 and 50°F. Kamal et al. (1962) did not observe a significant change in plasma potassium when heifers previously acclimated to 50 and 80°F were exposed to 35°F for 14 days (sampled on 9th day of exposure).

b. Sodium Sodium is the most abundant cation of extracellular fluid. The homeostatic "mechanism" (Cort, 1963) maintaining a constant osmotic pressure in the extracellular fluid acts primarily by regulating sodium balance. Regulation of sodium balance is accomplished primarily by the regulation of the amount of sodium filtered at the glomerulus and the amount of sodium reabsorbed along the tubular route to the pelvis of the kidney. Extracellular osmotic pressure is the most potent determinant of neurohumoral control of water excretion even though extracellular and blood volume changes may influence the rate of antidiuretic hormone secretion. Antidiuretic hormone may or may not have an effect on sodium transport in the renal tubules and mineralocorticoids definitely do influence sodium transport, but these two factors cannot explain the entirety of regulation of sodium reabsorption in the kidney tubule (Cort, 1963). In acute diseases associated with sodium depletion up to 20% of the total body sodium may be lost, almost all of which comes from plasma and interstitial fluid sodium (Cooke, 1952).

Blincoe and Brody (1951) found no marked change in plasma sodium of cattle due to change of environmental temperature (0 to 105°F). Kamal et al. (1962) reported a statistically significant difference in plasma sodium between heifers raised at 50 and 80°F when they were exposed to 35°F for 14 days (sampled on the 9th day of exposure). They concluded this difference was probably due to hemoconcentration in the 80°F group in the 35°F environment.

## III. MATERIALS AND METHODS

## A. Animals Used: Their Care and Management

The experimental animals used are listed in Table 1. The cows were neither lactating nor pregnant during the experiments.

The animals were stanchioned in a room (approximate dimensions 11' wide, 12' long, and 10' high) throughout the experiments. The animals stood on elevated, slatted wooden platforms covered with heavy rubber mats.<sup>1</sup> No bedding was used, with the exception of occasional use of wood shavings to absorb moisture. The air temperature, humidity, and hours of light (incandescent) were controlled throughout the experiments. The fresh air exchange rate of the room was about fifteen complete changes per hour throughout most of the experiment, with an estimated 102 feet/minute movement<sup>2</sup> around the animal's body.

A controlled maintenance diet was fed twice daily through the experiments. The daily diet consisted of 2.25 pounds dairy pellets, 8 pounds alfalfa pellets, 2 pounds alfalfa hay, vitamin-mineral supplement, and water free choice. This ration was intentionally used to provide a low level of nutrition. It was adequate to maintain a fair condition of all but the larger cows which lost some body condition after being fed this diet.

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<sup>1</sup>NASCO cow mats, NASCO, Fort Atkinson, Wisconsin 53538.

<sup>2</sup>Measured at various positions around the animal's body using a Hastings-Raydist air-meter with an N-7B probe. Hastings-Raydist, Inc., Hampton, Virginia 23361.

Table 1. Experimental animals

| Animal No. | Breed                            | Initial <sup>a</sup><br>body<br>weight<br>(lbs) | Age when<br>placed in<br>chamber<br>(years) |
|------------|----------------------------------|---|---|
| 1          | Hereford X Holstein <sup>b</sup> | 989   | 2   |
| 2          | Holstein X Guernsey <sup>c</sup> | 992   | 2   |
| 3          | Hereford X Holstein              | 985   | 2   |
| 4          | Holstein X Guernsey              | 949   | 2   |
| 5          | Holstein-Friesian                | 1,100 <sup>d</sup>                              | 6   |
| 6          | Holstein-Friesian <sup>e</sup>   | 1,000 <sup>d</sup>                              | 7   |
| 7          | Holstein-Friesian                | 1,000 <sup>d</sup>                              | 7   |
| 8          | Holstein-Friesian                | 1,100 <sup>d</sup>                              | 5   |
| 9          | Holstein-Friesian                | 1,150   | 5   |
| 10         | Holstein-Friesian                | 1,075   | 5   |

<sup>a</sup>Initial measurements were taken when the animal entered the environmental chamber, or prior to the first observations.

<sup>b</sup>Animals 1 and 3 were monozygous twins.

<sup>c</sup>Animals 2 and 4 were monozygous twins.

<sup>d</sup>Estimated.

<sup>e</sup>Animals 6 and 7 were monozygous twins.

## B. Program of Environmental Temperature

The program of environmental temperature is listed in Table 2. In general, the procedure was to acclimate the cattle to a base temperature. Then periodically the air temperature of the room was changed at a rate of 35°F per hour from the base temperature to the exposure temperature. The air temperature ranged  $\pm 4^\circ\text{F}$  during the exposure. After a given duration of exposure the air temperature was returned to the base condition, with the exception that exposure temperature of Experiment 1 served as base temperature for Experiment 8. Animals 9 and 10 of Experiment 8 were housed in a laboratory barn during the base period. This barn was maintained at 50 to 55°F during the winter and the cows were exercised outside daily for 6 hours irrespective of weather conditions. See Table 2 for the range of outside temperatures. After animals 9 and 10 had been surgically prepared for blood pressure measurements, they were maintained at 33°F for two days to obtain pre-exposure measurements.

## C. Surgical Technique for Catheterization of Aorta

A technique was developed for implanting vinyl tubing<sup>1</sup> into the abdominal aorta of cattle to allow continuous direct measurement of blood pressure under various environmental conditions. The route of catheterization was chosen to allow placement of the pressure transducer<sup>2</sup> upon the

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<sup>1</sup>Vinyl (medical grade), Becton Dickinson & Co., Rutherford, New Jersey.

<sup>2</sup>Statham pressure transducer, Model P23AA, Statham Instruments, Inc., Los Angeles, California.

Table 2. Program of environmental temperatures

| Experiment | a | Base temp. (°F) | Length of time at base temp. (days) | Exposure temp. (°F) | Duration of exposure (days) | Animals exposed (See Table 1) |
|------------|---|-----------------|-------------------------------------|---------------------|-----------------------------|-------------------------------|
| 1          | 6 | 74°             | 180                                 | 33°                 | 21                          | 5, 6, 7, 8                    |
| 2          | 1 | 74°             | 159                                 | 0°                  | 1 <sup>b</sup>              | 1, 2, 3, & 4                  |
| 3          | 2 | 74°             | 110                                 | -5°                 | 3                           | 1 & 2                         |
| 4          | 3 | 74°             | 130                                 | -25°                | 4                           | 3 & 4                         |
| 5          | 4 | 33°             | 61                                  | -5°                 | 7                           | 1, 2, 3, & 4                  |
| 6          | 7 | 33°             | 21                                  | -12°                | 14                          | 5, 6, 7, & 8                  |
| 7          | 5 | 33°             | 178                                 | -20°                | 14                          | 1 & 3                         |
| 8          | 8 | c               | c                                   | -20°                | 11                          | 9 & 10                        |

<sup>a</sup>Chronological order of experiments.

<sup>b</sup>Three one-day exposures were made with 7 days between each exposure.

<sup>c</sup>Outside winter exposure 6 hours per day. Outside air temperature averaged 14°F (range -13 to 25°F) for one month before exposure and averaged 35°F (range 14 to 59°F) for the second month before exposure. Inside barn air temperature was controlled at 50 to 55°F. See text concerning base temperature recording periods.

animal with a minimum length of tubing and to allow protection of the exposed parts from adverse environmental temperatures. Because of these considerations, previous techniques for arterial catheterization (Buck et al., 1965; Dougherty et al., 1965; Ingram and Whittow, 1963) were not satisfactory for the desired use in cattle.

Silicone-coated<sup>1</sup> vinyl tubing, varying in size from 0.044 inch i.d. (0.05 o.d.) to 0.067 inch i.d. (0.087 o.d.), was used. Luer-lock adapters<sup>2</sup> and valves<sup>3</sup> were fitted to the end of the tubing as described by Buck (1965). Vetafil<sup>4</sup> suture material was used throughout the surgery.

Initially, a standing operation was successfully performed on two animals using local infiltration of procaine for anesthesia. Because of excessive movement, the remaining 13 operations were carried out with general anesthesia using Halothane<sup>5</sup> as the anesthetic agent.

A surgical field centered about 8 inches below the tuber coxa was prepared. Beginning 7 inches ventral to the tuber coxa, an 8-inch incision was made ventrally along the cranial border of the tensor fascia latae muscle (Figure 45). Caudal and lateral reflection of muscle exposed the caudal branch of the circumflex iliac artery, vein, and lymph duct as they course ventrally to become superficial in the region of the prefemoral (subiliac) lymph node.

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<sup>1</sup>Siliclad<sup>®</sup>, Clay Adams, Inc., New York, New York.

<sup>2</sup>Clay-Adams, Inc., New York, New York.

<sup>3</sup>M.S. 09, Becton Dickinson & Co., Rutherford, New Jersey.

<sup>4</sup>Dr. S. Jackson, Importer, Washington, D.C.

<sup>5</sup>Fluothane<sup>®</sup>, Ayerst Laboratories, Inc., New York, New York.

After the circumflex iliac artery was isolated, a temporary occlusive ligature was placed proximal and a tight ligature was placed distal to the site of entry of the tubing into the artery (Figure 46). The temporary ligature was made by passing suture material around the artery, inserting the free ends of the suture through an 8-inch glass tube. The end of the glass tube was reduced in size and fire polished to form a rounded, smooth surface. By putting tension on the suture material hemorrhage was controlled while the artery was cut and the tubing introduced. A transverse incision was made through one-half of the artery and the proximal edge of the arterial incision was grasped with a splinter forceps to facilitate introduction of the tubing.

The saline-filled catheter was advanced until the tip was estimated to lie near the end of the aorta as judged by a pulse pressure monitoring system.<sup>1</sup> (Care should be exercised when introducing the tubing to insure that the catheter does not pass down the contralateral external iliac artery.) The catheter was fixed in place by tying and wrapping the occlusive ligature about the tubing. The ligature was then sutured to fascia on the medial side of the muscle for fixation.

The heat-sealed exposed end of the catheter was put through the eye of a large, solid, stainless steel needle which was inserted dorsally beneath the skin (Figure 47). The catheter emerged dorsomedial to the tuber coxa, with approximately 4 inches protruding. Both subcutaneous tissue and the skin were sutured.

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<sup>1</sup>Sanborn Model 769 Viso-scope, Sanborn Company, Waltham, Massachusetts.

The valve adaptor was attached to the end of the catheter and fixed midway between the tuber coxa and sacrum by a short length of vinyl tubing looped beneath approximately 2 inches of skin (Figure 48). Vetafil was threaded through the tubing and tied about the valve. Careful fixation of the valve was made to minimize movement of the catheter in and out of the skin. Without movement, the skin healed around the catheter with minimal or no suppuration.

The catheter was flushed periodically with physiologic saline and then filled with heparin solution (2,000 IU/ml). If no leakage from the valve occurred, the catheter did not require flushing for as long as 6 hours of recording.

A similar approach was used to catheterize the vena cava of animals 5, 8, 9, and 10.

Repetitive blood samples were readily obtained with no apparent discomfort or excitement of the animal.

#### D. Methods of Recording Physiologic Events

Measurements were usually begun 1.5 hours after the morning feeding, but on occasion continuous measurements were made beginning 2 hours before feeding and continued throughout the day. A continuous recording for a period of at least 1.5 hours was taken each recording day. The animals were trained to stand with their heads tied during the recording period (Figure 49). Readings were taken from sections of the record where no person was in the room and the animal was standing quietly. Several readings taken from the daily record were averaged to compute a daily value for each individual animal.

1. Blood pressure

Continuous recordings<sup>1</sup> of blood pressure (Figure 51) were obtained by utilizing a pressure transducer<sup>2</sup> connected to the heparin-filled catheter implanted into the abdominal aorta. The blood pressure transducer was placed horizontally upon the lumbo-sacral area and adjacent to the valve of the catheter. The temperature of the pressure transducer and external part of the catheter was controlled by covering the area with a conventional heating pad (Figure 50). The temperature of the transducer was monitored by the instrument<sup>3</sup> used to record room temperatures (Figure 52). Control of the pad temperature was accomplished using a Variac<sup>®</sup><sup>4</sup> to alter the electrical current through the heating pad. The blood pressure recording system was calibrated using a mercury manometer. The manometer was attached to the 7-inch interconnecting tube between the heparin-filled transducer and valve of the catheter. After the temperature of the heparin-filled transducer had stabilized, calibration was done with the tip of the connecting tube at the level of the catheter valve. The pressure of the fluid column within the aortic catheter was not added to the values reported here. Therefore, the reported values for aortic blood pressure are slightly lower than what actually existed.

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<sup>1</sup>Sanborn Model 350 oscillographic recording system, Sanborn Company, Waltham, Massachusetts.

<sup>2</sup>Statham pressure transducer, Model P23AA, Statham Instruments, Inc., Los Angeles, California.

<sup>3</sup>Honeywell Electronik 15 potentiometric recorder. Minneapolis-Honeywell Regulator Company, Philadelphia, Pennsylvania.

<sup>4</sup>Type W5MT3, General Radio Company, Concord, Massachusetts.

## 2. Heart rate

Continuous recordings of heart rate were obtained from the EKG record for Experiment 2. For other experiments the heart rate was taken from the pulse pressure trace.

## 3. Respiration

Continuous recordings of respiratory rate were obtained with a pneumograph around the thorax at the level of the eleventh rib.

### E. Analysis of Blood

Repetitive blood samples for analysis were readily obtained by way of the aortic catheter with no apparent discomfort or excitement of the animal.

#### 1. Glucose

Fluoridated blood samples collected during Experiment 2 were analyzed for glucose levels. Whole blood glucose levels were determined by the glucose oxidase method first proposed by Keston (1956) and developed by Teller (1956) using commercial reagents.<sup>1</sup> The filtrate was made immediately after blood collection by mixing 0.4 ml of blood, 3.6 ml distilled water, 2.0 ml of a 5 percent  $\text{Ba}(\text{OH})_2$  solution and 2.0 ml of a 4.5 percent  $\text{ZnSO}_4$  solution. This mixture was filtered through Whatman No. 1 paper. The filtrate was then frozen at  $-40^\circ\text{F}$  until all of the samples were collected. The entire collection of samples from one animal was thawed and analyzed at one time. Duplicate determinations were made for each sample of filtrate. One ml of

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<sup>1</sup>Glucostat Special <sup>®</sup>, Worthington Biochemical Corp., Freehold, New Jersey.

filtrate was incubated at 99°F with 4 ml of the reagent for 30 minutes. The reagent was prepared by diluting the powdered material in the chromogen and glucostat vials to 80 ml with distilled water. After incubation, a drop of 0.4 N HCl was added and immediately mixed. After 5 minutes the optical density was measured at 400 mμ wave length in a spectrophotometer (Buck, 1963).<sup>1</sup>

2. Total plasma proteins

Total plasma protein levels were determined for experiments 2, 3, and 4. The biuret method of Gornall et al. (1949) was used to estimate the total plasma proteins concentration. Standard beef albumin<sup>2</sup> was used to make the calibration curve. Physiological saline was used in place of sodium sulfate solution for blank and serum diluent.

3. Packed cell volume (P.C.V.)

Packed cell determinations were made of freshly drawn blood samples during all experiments except 7. Duplicate samples were obtained for each determination using the microhematocrit centrifuge.<sup>3</sup> The values were not corrected for trapped plasma.

4. Plasma electrolytes

Plasma sodium and potassium determinations were made for all experiments except 5 and 7. Plasma samples for electrolytes were obtained by

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<sup>1</sup>Spectronic 20

<sup>2</sup>Armour

<sup>3</sup>International centrifuge model MB and model MB and model CR reader, International Equipment Co., Boston, Massachusetts.

immediate refrigerated centrifugation of the blood sample at 13,000 rpm (20,000 G) for 10 to 20 minutes. The plasma was stored at -40°F until chemical determinations were carried out.

Plasma sodium and potassium were determined by the flame photometric method<sup>1</sup> by Teloh (1959) modified for use with samples prepared in a 5% trichloroacetic acid and 10% isopropyl alcohol solution as described by Beckman (ca. 1957).

#### F. Statistical Analysis

The least squares analysis of data (Harvey, 1960) was utilized throughout all experiments. Least squares means for equally balanced data (i.e. equal numbers of observations for each classification) are the same as the arithmetic means; however, when the data are unbalanced (i.e. different numbers of observations in some classifications), the least squares means are adjusted means based on the number of observations and variability of the observations. The standard errors of the least squares means were utilized to make approximate tests of significant differences between specific sets of means. The least squares means of data from each animal have been presented graphically for each experiment.

The results of all experiments in general were grouped into periods, viz. a period before cold exposure, one or more periods during cold exposure, and in some cases, a recovery period following exposure. Comparisons between periods were made within each experiment for all animals

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<sup>1</sup>Model B spectrophotometer with flame attachment, Beckman Instruments, Inc., Fullerton, California.

observed. Animal differences across all periods and animal by period interactions within an experiment were also tested for statistical significance. Specific period means of periods 2, 3, and 4 were compared to period 1 using the standard errors of the least squares means to make an approximate test.

## IV. RESULTS

The results of all experiments were arbitrarily grouped into periods. The number of days within any particular period varied within most experiments and also varied between experiments. In general, there was a period before exposure, one or more periods during cold exposure, and in some cases, a recovery period following the cold exposure.

Average values for each day of the experiment were calculated. The daily averages for blood pressure, heart rate, and respiratory frequency were calculated from 2 or more readings taken from a chart of continuous recordings (see Methods) for each animal. The daily average for each animal was the experimental observation value reported for each day. The appropriate daily observations within an arbitrary period were analyzed for differences that were statistically significant. Four fundamental questions were asked:

1. Is there a significant difference in response of individual animals that is consistent throughout the entire experiment (tested statistically by significant animal differences)?
2. Is there a significant difference in the average values of the observations made before, during, and after cold exposure (tested statistically by significant period differences)?
3. Do all the animals of an experiment respond in the same way throughout all of the periods (tested statistically by significant animal by period interaction)?

4. Is the average response for a particular cold exposure or recovery period significantly different from the base temperature period (an approximation test was made utilizing the standard error of the particular means)?

#### A. Experiment 1

Animals 5, 6, 7, and 8 were acclimated to a base temperature of 74°F for 180 days. The environmental temperature was then abruptly changed to 33°F and remained at this temperature for 21 days. Period 1 environmental temperature was 74°F and measurements were made 1 and 3 days before cold exposure. Period 2 environmental temperature was 33°F and measurements were made during days 1 through 7 of cold exposure. Period 3 environmental temperature was 33°F and measurements were made during days 13, 17, and 21 of cold exposure. Physiological measurements were made of blood pressure, heart rate, respiratory frequency, blood packed cell volume, and blood plasma potassium and sodium.

##### 1. Blood pressure

The average blood pressure responses of animals 5, 6, and 7 have been illustrated by Figure 1. The means for each period have been listed in Table 3. Animal 5's blood pressure was significantly ( $P < .05$ ) higher than animals 6 and 7 throughout the experiment. The average blood pressure was significantly higher ( $P < .005$ ) during periods 2 and 3. Animal by period

interaction was not significant even though animal 1 showed little response except for a slight rise during period 3.

## 2. Heart rate

The heart rates of animals 5, 6, and 7 have been illustrated by Figure

8. The means for each period have been listed in Table 3.

No significant animal differences were noted. The means for periods 2 and 3 were significantly higher ( $P < .05$ ) than period 1. There was no significant animal by period interaction.

## 3. Respiratory frequency

The frequencies of the respiratory cycle of cows 5, 6, and 7 have been illustrated by Figure 15. The means for each period have been listed in Table 3.

Animal differences were highly significant ( $P < .005$ ). Respiratory frequency of animal 5 was consistently higher than animals 6 and 7. The means for periods 2 and 3 were significantly lower ( $P < .01$ ) than period 1. Animal 7 did not respond to the same extent as animals 5 and 6 (animal by period interaction highly significant  $P < .005$ ), probably because of the lower respiratory rate during period 1 before cold exposure.

## 4. Packed blood cell volume

The packed cell volumes of cows 5, 6, 7, and 8 have been illustrated by Figure 26. The mean values for each period have been listed in Table 3.

Animal differences were not significant. The differences in mean values for periods were statistically significant ( $P < .05$ ) with a slight rise in packed cell volume occurring during cold exposure.

Table 3. Mean values for responses of cattle (Nos. 5, 6, 7, and 8) exposed to environmental temperatures of Experiment 1

|  | Period 1<br>(74°F)       | Period 2<br>(33°F)        | Period 3<br>(33°F)        |
|--|--------------------------|---------------------------|---------------------------|
| Day when observations were made <sup>a</sup> | 1, 2                     | 1-7                       | 13, 17, 20                |
| Average blood pressure (mm Hg)               | 83.7 ± 1.86 <sup>b</sup> | 92.6 ± 1.16 <sup>**</sup> | 91.6 ± 1.64 <sup>**</sup> |
| Heart rate (beats per minute)                | 43.5 ± 1.33              | 56.8 ± 0.83 <sup>**</sup> | 54.9 ± 1.17 <sup>**</sup> |
| Respiratory frequency (per minute)           | 14.2 ± 0.51              | 6.6 ± 0.32 <sup>**</sup>  | 6.8 ± 0.45 <sup>**</sup>  |
| Day when observations were made <sup>a</sup> | 1, 2, 3                  | 8, 10, 11, 13             | 18-21                     |
| Packed blood cell volume (%)                 | 32.4 ± 0.34              | 33.6 ± 0.37 <sup>*</sup>  | 33.8 ± 0.34 <sup>*</sup>  |
| Blood plasma potassium (mEq/L)               | 3.82 ± 0.06              | 3.60 ± 0.06               | 3.75 ± 0.06               |
| Blood plasma sodium (mEq/L)                  | 130.0 ± 1.50             | 125.0 ± 1.64              | 131.4 ± 1.50              |

<sup>a</sup>See text for number of animals sampled for each physiological function.

<sup>b</sup>Standard error of the mean.

\*Statistically significant (P<.05).

\*\*Statistically significant (P<.01).

#### 5. Blood plasma potassium

Blood plasma potassium levels of cows 5, 6, 7, and 8 have been illustrated in Figure 33. The mean values for each period have been listed in Table 3.

Animal differences in blood potassium level were highly significant ( $P < .05$ ). The plasma potassium concentration of animal 8 was consistently higher than all other animals and that of animal 6 was consistently the lowest. The mean value of the potassium concentration was slightly decreased (significant at  $P < .10$ ) during 33°F exposure. Animal by period interaction was not significant.

#### 6. Blood plasma sodium

Blood plasma sodium levels of cows 5, 6, 7, and 8 have been shown in Figure 39 and the mean values for each period have been listed in Table 3.

Consistent animal differences were not significant. Period differences in mean plasma sodium concentration were significant ( $P < .05$ ); however, the response to 33°F cold was not the same for all animals. Animal by period interaction was highly significant ( $P < .005$ ).

### B. Experiment 2

Animals 1, 2, 3, and 4 were acclimated to 74°F for 159 days. The environmental temperature was then abruptly changed to 0°F for 24 hours and then rapidly raised to 74°F again. Three such exposures were made at one-week intervals. Period 1 environmental temperature was 74°F and measurements were made the second day of each week. Period 2 environmental temperature was 0°F and measurements were made on day 3 and 4 of each week.

Period 3 environmental temperature was 74°F and measurements were made on day 5 of each week. Physiological measurements were made 3 times during each recording day of blood pressure, heart rate, respiratory frequency, blood glucose, total blood plasma protein concentration, packed blood cell volume, and blood plasma potassium and sodium. The weekly results of Experiment 2 were individually analyzed for differences in response to each consecutive exposure and collectively analyzed for period differences.

1. Blood pressure

The average blood pressures of animals 1, 2, 3, and 4 have been illustrated by Figure 2. The means for each period have been listed in Table 4.

All animals did not have the same blood pressure as indicated by highly significant ( $P < .005$ ) animal differences. The blood pressure of animal 3 was significantly ( $P < .05$ ) lower throughout the entire experiment. The mean for period 2 was significantly ( $P < .005$ ) increased over periods 1 and 3. The means for periods 1 and 3 were not significantly different. The means for weeks 1, 2, and 3 were significantly ( $P < .01$ ) different. The mean for the first week was  $81.2 \pm 1.58$  mm Hg; the second week was  $84.2 \pm 1.58$ ; and the third week was  $89.4 \pm 1.58$ . However, all animals did not show a consistent increase of weekly mean as indicated by a significant ( $P < .025$ ) animal by week interaction.

2. Heart rate

The mean values for the heart rate of animals 1, 2, 3, and 4 have been illustrated by Figure 9. The means for each period have been listed in Table 4.

Table 4. Mean values for responses of cattle (Nos. 1, 2, 3, and 4) exposed to environmental temperatures of Experiment 2

|  | Period 1<br>(74°F)       | Period 2<br>(0°F)              | Period 3<br>(74°F)        |
|--|--------------------------|--------------------------------|---------------------------|
| Day when observations were made <sup>a</sup> | 2 <sup>b</sup>           | 3, <sup>c</sup> 4 <sup>d</sup> | 5                         |
| Average blood pressure (mm Hg)               | 74.2 ± 1.46 <sup>e</sup> | 102.7 ± 1.79 <sup>**</sup>     | 77.9 ± 1.46               |
| Heart rate (beats per minute)                | 50.7 ± 0.78              | 73.0 ± 0.96 <sup>**</sup>      | 52.4 ± 0.78               |
| Respiratory frequency (per minute)           | 14.7 ± 0.47              | 8.9 ± 0.57 <sup>**</sup>       | 11.6 ± 0.47 <sup>**</sup> |
| Packed blood cell volume (%)                 | 30.4 ± 0.32              | 33.5 ± 0.40 <sup>**</sup>      | 31.4 ± 0.32               |

<sup>a</sup>See text for number of animals sampled for each physiological function.

<sup>b</sup>Day of week - cold exposure once weekly for 3 weeks (see Table 2).

<sup>c</sup>One sample when environmental temperature near 0°F.

<sup>d</sup>One sample after environmental temperature 0°F for 15 hours.

<sup>e</sup>Standard error of the mean.

<sup>\*\*</sup>Statistically significant (P<.01).

Table 4 (Continued)

|  | Period 1<br>(74°F) | Period 2<br>(0°F) | Period 3<br>(74°F) |
|--|--------------------|-------------------|--------------------|
| Day when observations were made <sup>a</sup> | 2                  | 4 <sup>f</sup>    | 5                  |
| Blood glucose (mg %)                         | 38.6 ± 12.0        | 44.0 ± 14.7       | 36.9 ± 12.0        |
| Total blood plasma proteins (gm/100 ml)      | 6.82 ± 0.10        | 6.65 ± 0.10       | 6.40 ± 0.10*       |
| Blood plasma potassium (mEq/L)               | 3.96 ± 0.04        | 3.71 ± 0.04*      | 3.70 ± 0.04*       |
| Blood plasma sodium (mEq/L)                  | 130.4 ± 1.14       | 130.0 ± 1.14      | 124.0 ± 1.14*      |

<sup>f</sup>First sample when 0°F; second sample while temperature rising; third sample after reaching 74°F.

\*Statistically significant (P<.05).

Animal differences were highly significant ( $P < .005$ ). The mean for animal 1 was consistently significantly ( $P < .05$ ) lower throughout the experiment. The difference between period means was highly significant ( $P < .005$ ). Although all animals showed highly significant ( $P < .005$ ) increases in heart rate during cold exposure, there were no consistent significant differences in heart rate during the recovery period after each exposure of the 3 weeks.

### 3. Respiratory frequency

The mean values for the respiratory frequency of animals 1, 2, 3, and 4 have been illustrated by Figure 16. The period means have been listed in Table 4.

The animals had significantly different ( $P < .005$ ) respiratory rates throughout the experiment with animal 3 having the highest respiratory rate and animal 4 having the lowest.

The respiratory rate was decreased during cold exposure with the differences between period means being highly significant ( $P < .005$ ). The mean for period 3 was also significantly lower ( $P < .005$ ) than before exposure, with this effect being the greatest during the week 3 for all animals except No. 3.

### 4. Blood glucose

The mean values for the blood glucose of animals 1, 2, 3, and 4 have been illustrated by Figure 22. The period means have been listed in Table 4.

Animal differences were not significant. The mean for period 2 during cold exposure was higher than before or after cold exposure. However,

highly significant ( $P < .01$ ) interactions (animal by period by week) and excessive variability made interpretation difficult.

5. Total blood plasma proteins

The mean values for the total blood plasma protein concentration of animals 1, 2, 3, and 4 have been illustrated by Figure 23. The period means have been listed in Table 4.

Animal differences were not statistically significant. Period differences in total plasma proteins were significant ( $P < .025$ ); however, the interaction (period by weeks) was highly significant ( $P < .005$ ) making interpretation difficult. All animals except No. 1 had a slight decrease in plasma proteins during cold exposure. The plasma proteins of animals 1 and 3 decreased during the recovery period, while that of animal 2 increased.

6. Packed blood cell volume

The mean values for animals 1, 2, 3, and 4 have been illustrated by Figure 27. The means for each period have been listed in Table 4.

The individual animal P.C.V. was significantly different ( $P < .005$ ). The P.C.V. of animal 2 was consistently higher than animals 3 and 4. Animal 1 had a higher P.C.V. than all the other animals during periods 1 and 3 and did not show an increase on cold exposure. The P.C.V. was significantly ( $P < .005$ ) increased during cold exposure. The mean P.C.V. for all animals was slightly lower (significant at  $P < 0.1$ ) during the third week.

7. Blood plasma potassium

The means for animals 1, 2, 3, and 4 have been illustrated by Figure 34. The period means have been listed in Table 4.

The animal differences in plasma potassium were not statistically significant. The period differences were highly significant ( $P < .005$ ). All animals had a decrease in plasma potassium during cold exposure. The recovery period mean was also significantly lower than the mean before exposure. The response for each period was not the same for each of the 3 weeks as indicated by significant ( $P < .025$ ) period by week interaction. The mean of period 2 was lower than period 1, except during the third week when a rise occurred. Period 3 means were lower than period 1.

#### 8. Blood plasma sodium

The means for animals 1, 2, 3, and 4 have been illustrated by Figure 40. The period means for plasma sodium concentration have been listed in Table 4.

The individual animal differences in plasma sodium were not statistically significant. Differences in period means were highly significant ( $P < .005$ ). Period 3 mean was significantly lower than period 1 and 2. The differences in plasma sodium were highly significant ( $P < .005$ ) for each of the 3 weeks. The mean for week 1 was 132.4, week 2 was 124.6, and week 3 was 127.3 mEq/L. The differences between each period were not the same for each of the 3 weeks as indicated by highly significant ( $P < .005$ ) period by week interaction. The mean for period 2 cold exposure was lower than period 1 during weeks 1 and 2 but was higher than period 1 during week 3.

#### C. Experiment 3

Animals 1 and 2 were acclimated to 74°F for 110 days. The environmental temperature was abruptly changed to -5°F for 3 days and then

rapidly raised again to 74°F. Period 1 measurements were made 1, 2, and 3 days before cold exposure. Period 2 measurements were made during days 1, 2, and 3 of cold exposure and period 3 measurements were made 1 and 2 days after exposure. Physiological measurements were made of blood pressure, heart rate, respiratory frequency, total blood plasma proteins, packed blood cell volume, and blood plasma potassium and sodium concentration.

1. Blood pressure

The mean values for animals 1 and 3 have been illustrated by Figure

3. The means for each period have been listed in Table 5.

The differences between animals were highly significant ( $P < .01$ ), primarily due to the higher blood pressure of animal 2 during and following cold exposure. The rise in blood pressure during cold exposure was highly significant ( $P < .005$ ). The response was the same for all animals (animal by period interaction was not significant).

2. Heart rate

The mean values for animals 1 and 2 have been illustrated by Figure

10. The means for each period have been listed in Table 5.

The animal differences were statistically significant ( $P < .025$ ), primarily due to the higher increase in heart rate of animal 2 during and following cold exposure. The increase in heart rate during cold exposure was highly significant ( $P < .005$ ). The heart rate was also significantly ( $P < .05$ ) increased during the recovery period.

Table 5. Mean values for responses of cattle (Nos. 1 and 2) exposed to environmental temperatures of Experiment 3

|  | Period 1<br>(74°F)       | Period 2<br>(-5°F)         | Period 3<br>(74°F)        |
|--|--------------------------|----------------------------|---------------------------|
| Day when observations were made <sup>a</sup> | 1, 2, 3                  | 1, 2, 3                    | 1, 2                      |
| Average blood pressure (mm Hg)               | 76.3 ± 2.45 <sup>b</sup> | 119.3 ± 2.45 <sup>**</sup> | 86.25 ± 3.00              |
| Heart rate (beats per minute)                | 51.7 ± 2.34              | 81.2 ± 2.34 <sup>**</sup>  | 65.2 ± 2.86 <sup>*</sup>  |
| Respiratory frequency (per minute)           | 15.3 ± 0.43              | 8.5 ± 0.43 <sup>**</sup>   | 11.2 ± 0.53 <sup>**</sup> |
| Total blood plasma proteins (gm/100 ml)      | 7.75 ± 0.23              | 8.02 ± 0.21                | 7.82 ± 0.26               |
| Packed blood cell volume (%)                 | 33.0 ± 0.63              | 35.1 ± 0.58                | 29.8 ± 0.71 <sup>*</sup>  |
| Blood plasma potassium (mEq/L)               | 4.26 ± 0.07              | 4.27 ± 0.07                | 3.96 ± 0.08 <sup>*</sup>  |
| Blood plasma sodium (mEq/L)                  | 129.4 ± 2.16             | 140.1 ± 1.97 <sup>**</sup> | 138.9 ± 2.41 <sup>*</sup> |

<sup>a</sup>See text for number of animals sampled for each physiological function.

<sup>b</sup>Standard error of the mean.

<sup>\*</sup>Statistically significant (P<.05).

<sup>\*\*</sup>Statistically significant (P<.01).

### 3. Respiratory frequency

The mean values for animals 1 and 2 have been illustrated by Figure 17. The means for each period have been listed in Table 5.

The animal differences were statistically significant ( $P < .025$ ). The decrease in respiratory frequency during cold exposure was highly significant ( $P < .005$ ). The frequency during the recovery period was also significantly ( $P < .01$ ) lower. Animal 1 had a greater decrease in frequency during cold exposure and returned to a higher frequency than animal 2 during the recovery period (animal by period interaction was significant at  $P < .025$ ).

### 4. Total blood plasma proteins

The mean values for animals 1 and 2 have been illustrated by Figure 24. The means for each period have been listed in Table 5.

Animal and period differences were not statistically significant. The response of each animal was opposite to the other (animal by period interaction was significant at  $P < .025$ ). Animal 1 had a definite increase in total blood plasma proteins both during and following cold exposure while animal 2 had a continued decrease.

### 5. Packed blood cell volume

The mean values for animals 1 and 2 have been illustrated in Figure 28. The means for each period have been listed in Table 5.

Animal differences were highly significant ( $P < .005$ ). The P.C.V. of animal 1 was consistently higher than that of animal 2. Animal 1 did not have an increase in P.C.V. during cold exposure but had a marked decline following cold exposure. Animal 2 had an increase in P.C.V. during cold

cold exposure with a return to before exposure level. The difference between the exposure and recovery period was highly significant ( $P < .01$ ).

6. Blood plasma potassium

The mean values for animals 1 and 2 have been illustrated in Figure 35. The means for each period have been listed in Table 5.

The difference between periods was significant ( $P < .05$ ). However, this significance was primarily due to the decrease in plasma potassium concentration of animal 2 after cold exposure. The animal by period interaction was significant ( $P < .05$ ).

7. Blood plasma sodium

The mean values for animals 1 and 2 have been illustrated by Figure 41. The means for each period have been listed in Table 5.

The difference between periods was highly significant ( $P < .01$ ). Both animals had increased plasma sodium concentrations during cold exposure. Plasma sodium of animal 2 decreased in period 3 and animal 1 continued to increase (animal by period interaction significant at  $P < .05$ ).

D. Experiment 4

Animals 3 and 4 were acclimated to 74°F for 130 days. The environmental temperature was abruptly changed to -25°F and maintained there for 4 days and then rapidly raised to 74°F. Period 1 environmental temperature was 74°F and measurements were made 1, 2, 3, and 4 days before cold exposure. Period 2 environmental temperature was -25°F and measurements were made on days 1, 2, 3, and 4. Period 3 environmental temperature was 74°F and

measurements were made on days 1, 2, and 3. Physiological measurements were made of total blood plasma proteins, packed blood cell volume, and blood plasma potassium and sodium.

1. Total blood plasma proteins

Mean values for animals 3 and 4 have been illustrated by Figure 25. Period means have been listed in Table 6.

The mean for total plasma protein concentration of animal 1 was  $7.76 \pm 0.19$  gm/100 ml and for animal 2 the mean was  $7.84 \pm 0.19$ . No significant change was detected during cold exposure, although there was a slight increase in total plasma protein concentration during and following cold exposure.

2. Packed blood cell volume

Mean values for animals 3 and 4 have been illustrated by Figure 29. Period means have been listed in Table 6.

The difference in period means was highly significant ( $P < .005$ ). Although both cows showed an increase in P.C.V. during cold exposure, animal 4 had a much larger increase than animal 3.

3. Blood plasma potassium

Mean values for animals 3 and 4 have been illustrated by Figure 36. Period means have been listed in Table 6.

The blood plasma potassium concentration was slightly lower after cold exposure than before exposure but the change was not statistically significant. The response was not consistent in that animal 3 had a decrease in plasma potassium on cold exposure while animal 4 had an increase.

Table 6. Mean values for responses of cattle (Nos. 3 and 4) exposed to environmental temperatures of Experiment 4

|  | Period 1<br>(74°F)       | Period 2<br>(-25°F)       | Period 3<br>(74°F) |
|--|--------------------------|---------------------------|--------------------|
| Day when observations were made <sup>a</sup> | 1, 2, 3, 4               | 1, 2, 3, 4                | 1, 2, 3            |
| Total blood plasma proteins (gm/100 ml)      | 7.53 ± 0.22 <sup>b</sup> | 7.86 ± 0.22               | 8.02 ± 0.23        |
| Packed blood cell volume (%)                 | 30.1 ± 1.62              | 38.2 ± 1.62 <sup>**</sup> | 34.5 ± 1.87        |
| Blood plasma potassium (mEq/L)               | 3.89 ± 0.09              | 3.80 ± 0.09               | 3.66 ± 0.10        |
| Blood plasma sodium (mEq/L)                  | 133.6 ± 2.71             | 128.8 ± 2.71              | 130.23 ± 3.12      |

<sup>a</sup>See text for number of animals sampled for each physiological function.

<sup>b</sup>Standard error of the mean.

<sup>\*\*</sup>Statistically significant (P<.01).

#### 4. Blood plasma sodium

Mean values for animals 3 and 4 have been illustrated by Figure 42. The period means have been listed in Table 6.

The difference between period means was not statistically significant. The animals did not react the same to the cold exposure. Animal 3 had a decrease in blood plasma sodium during cold exposure and animal 4 had a decrease after cold exposure. Excessive variation of values made interpretation difficult.

### E. Experiment 5

Animals 1, 2, 3, and 4 were acclimated to 33°F for 61 days. The environmental temperature was abruptly changed to -5°F for 7 days after which it was rapidly raised to 33°F. Period 1 environmental temperature was 33°F and measurements were made 1, 2, 3, and 4 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, 3, 5, 6, and 7. Period 3 environmental temperature was 33°F and measurements were made on days 1 through 6. Physiological measurements were made once daily of blood pressure, heart rate, respiratory rate, and packed blood cell volume.

#### 1. Blood pressure

The mean values for average blood pressure of animals 1, 2, and 3 have been illustrated by Figure 4. The period means have been listed in Table 7.

The animal differences were highly significant ( $P < .005$ ). The blood pressure of animal 2 was consistently higher than that of animals 1 and 3.

Table 7. Mean values for responses of cattle (Nos. 1, 2, 3, and 4) exposed to environmental temperatures of Experiment 5

|  | Period 1<br>(33°F)        | Period 2<br>(-5°F)         | Period 3<br>(33°F)       |
|--|---------------------------|----------------------------|--------------------------|
| Day when observations were made <sup>a</sup> | 1, 2, 3, 4 <sup>b</sup>   | 1,2,3,5,6,7                | 1,2,3,4,5,6 <sup>c</sup> |
| Average blood pressure (mm Hg)               | 83.42 ± 3.04 <sup>d</sup> | 114.5 ± 2.32 <sup>**</sup> | 88.6 ± 2.36              |
| Heart rate (beats per minute)                | 56.7 ± 1.90               | 79.0 ± 1.45 <sup>**</sup>  | 67.2 ± 1.47 <sup>*</sup> |
| Respiratory frequency (per minute)           | 6.7 ± 0.32                | 6.5 ± 0.24                 | 7.1 ± 0.25               |
| Packed blood cell volume (%)                 | 35.0 ± 0.88               | 34.3 ± 0.50                | 32.2 ± 0.55 <sup>*</sup> |

<sup>a</sup>See text for number of animals sampled for each physiological function.

<sup>b</sup>Packed cell volume on days 1 and 2 only.

<sup>c</sup>Packed cell volume on days 1-5 only.

<sup>d</sup>Standard error of the mean.

<sup>\*</sup>Statistically significant (P<.05).

<sup>\*\*</sup>Statistically significant (P<.01).

The difference between periods was highly significant ( $P < .005$ ) with all animals having an increased blood pressure during cold exposure. The blood pressure returned to pre-exposure levels during the six-day recovery period.

## 2. Heart rate

The mean values for heart rates of animals 1, 2, 3, and 4 have been illustrated by Figure 11. The period means have been listed in Table 7.

The animal differences were highly significant ( $P < .005$ ). The heart rate of animal 2 was consistently higher than that of animals 1 and 3. The difference in period means was highly significant ( $P < .005$ ) with all animals showing an increased heart rate during cold exposure. The heart rate during the six-day recovery period was also significantly ( $P < .05$ ) higher than before exposure.

## 3. Respiratory frequency

The mean values for respiratory frequency of animals 1, 2, and 3 have been illustrated by Figure 18. The period means have been listed in Table 7.

The animal differences were highly significant ( $P < .005$ ). The respiratory frequency of animal 2 was consistently lower than that of animals 1 and 3 and the frequency did not change during or following cold exposure. The differences in period means were not statistically significant. Animals 1 and 3 showed a very slight decrease in respiratory frequency during cold exposure with a return to pre-exposure level during the recovery period.

#### 4. Packed blood cell volume

The mean values for P.C.V. of cows 2 and 3 have been illustrated by Figure 30. The period means have been listed in Table 7.

The difference between period means was significant ( $P < .05$ ) with the lowest P.C.V. for both animals occurring during the recovery period.

#### F. Experiment 6

Animals 5, 6, 7, and 8 were acclimated to 33°F for 21 days (see Experiment 1). The environmental temperature was then abruptly changed to -12°F for 14 days after which it was raised to 33°F. Period 1 environmental temperature was 33°F. Measurements were made 1 and 2 days before cold exposure. Period 2 environmental temperature was -12°F and measurements were made on days 1, 2, 3, and 4 of cold exposure. Period 3 environmental temperature was -12°F and measurements were made during days 9 through 14. Period 4 environmental temperature was 33°F and measurements were made on days 1, 2, 4, 5, and 6 after cold exposure. Physiological measurements were made of blood pressure, heart rate, respiratory frequency, packed blood cell volume, and blood plasma potassium and sodium concentrations.

##### 1. Blood pressure

The means for animals 5, 6, and 7 have been illustrated by Figure 5. The period means have been listed in Table 8.

The difference in individual animal blood pressure was highly significant ( $P < .005$ ), with animal 5 being consistently higher than animals 6 and 7.

Table 8. Mean values for responses of cattle (Nos. 5, 6, 7, and 8) exposed to environmental temperatures of Experiment 6

|  | Period 1<br>(33°F)       | Period 2<br>(-12°F)        | Period 3<br>(-12°F)        | Period 4<br>(33°F)       |
|--|--------------------------|----------------------------|----------------------------|--------------------------|
| Day when observations were made <sup>a</sup> | 1, 2                     | 1, 2, 4                    | 9,11,12,13                 | 1,2,5,6                  |
| Average blood pressure (mm Hg)               | 87.5 ± 2.95 <sup>b</sup> | 108.3 ± 2.09 <sup>**</sup> | 115.2 ± 1.81 <sup>**</sup> | 99.1 ± 2.00 <sup>*</sup> |
| Heart rate (beats per minute)                | 56.2 ± 2.49              | 70.9 ± 1.76 <sup>**</sup>  | 76.3 ± 1.53 <sup>**</sup>  | 63.0 ± 1.69              |
| Respiratory frequency (per minute)           | 6.7 ± 0.18               | 7.2 ± 0.13                 | 6.8 ± 0.11                 | 6.6 ± 0.12               |
| Day when observations were made <sup>a</sup> | 1, 2                     | 1, 2, 3                    | 9,11,12,13,14              | 1,2,4,5,6                |
| Packed blood cell volume (%)                 | 34.4 ± 0.39              | 34.0 ± 0.32                | 35.1 ± 0.26                | 35.4 ± 0.32 <sup>*</sup> |
| Blood plasma potassium (mEq/L)               | 3.82 ± 0.08              | 3.92 ± 0.06                | 3.92 ± 0.05                | 3.89 ± 0.06              |
| Blood plasma sodium (mEq/L)                  | 132.4 ± 1.9              | 133.0 ± 1.5                | 134.4 ± 1.3                | 126.2 ± 1.5 <sup>*</sup> |

<sup>a</sup>See text for number of animals sampled for each physiological function.

<sup>b</sup>Standard error of the mean.

\*Statistically significant (P<.05).

\*\*Statistically significant (P<.01).

The difference in period means was highly significant ( $P < .005$ ). The largest increase was noted during period 3. The mean for the recovery period was higher than the pre-exposure value.

## 2. Heart rate

The means for animals 5, 6, and 7 have been illustrated by Figure 12. The period means have been listed in Table 8.

The animal differences were highly significant ( $P < .005$ ). The heart rate of animal 5 was consistently lower than that of animals 6 and 7. The differences in period means were highly significant ( $P < .005$ ). The largest increase in rate occurred during period 3 for all animals. The heart rates of animals 6 and 7 during the recovery period were higher than the pre-exposure values.

## 3. Respiratory frequency

The mean values for animals 5, 6, and 7 have been illustrated by Figure 19. The period means have been listed in Table 8.

The animal differences and period differences were highly significant ( $P < .005$ ). The animal by period interaction was also significant ( $P < .05$ ). The respiratory frequency of animal 5 was consistently higher than that of animals 6 and 7. The respiratory frequency of animal 6 remained unchanged during the exposure. Animal 7 had a very slight increase in respiratory frequency during period 2 followed by a decrease in periods 3 and 4.

## 4. Packed blood cell volume

The mean values for animals 5, 6, 7, and 8 have been illustrated by Figure 31. The period means have been listed in Table 8.

Animal differences were highly significant ( $P < .005$ ). The P.C.V. of animal 8 was consistently lower than that of all other animals and the P.C.V. of animal 7 was consistently higher than that of all other animals. Period differences were significant ( $P < .05$ ). Animal 8 had a decrease in P.C.V. during period 2. Animal 5 also had a decrease in P.C.V. in period 2 but had increased to the pre-exposure level during period 3. Animals 6 and 7 had an increased P.C.V. during cold exposure and the recovery period. Cow 8 died on day 13.

#### 5. Blood plasma potassium

The mean values of animals 5, 6, 7, and 8 have been illustrated by Figure 37. The period means have been listed in Table 8.

The animal differences were highly significant ( $P < .005$ ) due to animals 5 and 8 having considerably higher plasma potassium concentration than that of animals 6 and 7. The period differences were not statistically significant but animals 5, 6, and 8 had a slight increase in plasma potassium concentration while animal 7 had a decrease.

#### 6. Blood plasma sodium

The means for animals 5, 6, 7, and 8 have been illustrated by Figure 43. The period means have been listed in Table 8.

Animal differences were highly significant ( $P < .005$ ). The plasma sodium concentration of animal 5 was consistently higher than that of all other animals. The plasma sodium concentration of animal 7 was also higher except during the recovery period. Period differences were significant ( $P < .025$ ) primarily due to the decrease in plasma sodium concentration of animals 5 and 7 during the recovery period.

## G. Experiment 7

Animals 1 and 3 were acclimated to 33°F for 178 days. The environmental temperature was then changed to -20°F and maintained for 14 days. Period 1 environmental temperature was 33°F and measurements were made 1 and 4 days before cold exposure. Period 2 environmental temperature was -20°F and measurements were made during days 1 through 4. Period 3 environmental temperature was -20°F and measurements were made on days 10 and 14. Physiological measurements were made of the blood pressure, heart rate, and respiratory frequency.

1. Blood pressure

The mean values for the average blood pressure of animals 1 and 3 have been illustrated by Figure 6. The period means have been listed in Table 9.

Animal differences were highly significant ( $P < .005$ ). The blood pressure of animal 1 was consistently higher than that of animal 3. Period differences were highly significant ( $P < .005$ ). There was a slight decrease in blood pressure of both animals during period 3.

2. Heart rate

The mean values for animals 1 and 3 have been illustrated by Figure 13. The period means have been listed in Table 9.

The period differences were highly significant ( $P < .005$ ). The heart rate of all animals was increased during period 2 and a further small increase occurred during period 3. Animal differences were not significant.

Table 9. Mean values for responses of cattle (Nos. 1 and 3) exposed to environmental temperatures of Experiment 7

|  | Period 1<br>(33°F)       | Period 2<br>(-20°F)        | Period 3<br>(-20°F)        |
|--|--------------------------|----------------------------|----------------------------|
| Day when observations were made <sup>a</sup> | 1, 4                     | 1, 2, 3, 4                 | 10, 14                     |
| Average blood pressure (mm Hg)               | 82.5 ± 2.22 <sup>b</sup> | 106.2 ± 1.57 <sup>**</sup> | 100.5 ± 2.22 <sup>**</sup> |
| Heart rate (beats per minute)                | 52.5 ± 1.63              | 80.8 ± 1.15 <sup>**</sup>  | 85.8 ± 1.63 <sup>**</sup>  |
| Respiratory frequency (per minute)           | 11.0 ± 0.60              | 8.5 ± 0.43 <sup>*</sup>    | 8.0 ± 0.60 <sup>**</sup>   |

<sup>a</sup>See text for number of animals sampled for each physiological function.

<sup>b</sup>Standard error of the mean.

\*Statistically significant (P<.05).

\*\*Statistically significant (P<.01).

### 3. Respiratory frequency

The mean values for animals 1 and 3 have been illustrated by Figure 20. The period means have been listed in Table 9.

Animal differences were not statistically significant although animal 1 had a lower respiratory frequency throughout the cold exposure. Period differences were highly significant ( $P < .01$ ) with a decrease in respiratory rate of both animals during period 2 and a slight further decrease occurred in period 3. Animal 1 had the greatest decrease in respiratory frequency.

## H. Experiment 8

Animals 9 and 10 had been housed in a heated barn at approximately 50°F during the fall and early winter. These animals were accustomed to daily outdoor exercise regardless of the weather (see Table 2). Therefore, they represented animals naturally acclimatized to winter weather conditions such as might be expected in certain areas of animal production. Before-exposure measurements were obtained at an environmental temperature of 33°F. The temperature was then abruptly changed to -20°F and maintained for 10 days. Physiological measurements were made of blood pressure, heart rate, respiratory frequency, packed blood cell volume, and blood plasma potassium and sodium.

### 1. Blood pressure

The mean values for average blood pressures of animals 9 and 10 have been illustrated by Figure 7. The period means have been listed in Table 10.

The period differences were highly significant ( $P < .005$ ). The blood pressure was significantly ( $P < .05$ ) increased during period 2 and continued

Table 10. Mean values for responses of cattle (Nos. 9 and 10) exposed to environmental temperatures of Experiment 8

|  | Period 1<br>(33°F) <sup>a</sup> | Period 2<br>(-20°F) | Period 3<br>(-20°F)  | Period 4<br>(-20°F) |
|--|---------------------------------|---------------------|----------------------|---------------------|
| Day when observations were made <sup>b</sup> | 1, 2                            | 1, 2                | 4, 5, 6 <sup>c</sup> | 8, 9                |
| Average blood pressure (mm Hg)               | 98.2 ± 2.35 <sup>d</sup>        | 111.0 ± 2.35*       | 120.2 ± 1.92***      | 124.0 ± 2.35**      |
| Heart rate (beats per minute)                | 58.0 ± 2.34                     | 73.2 ± 2.34**       | 84.2 ± 1.91**        | 79.5 ± 2.34**       |
| Respiratory frequency (per minute)           | 8.8 ± 0.64                      | 9.8 ± 0.64          | 7.7 ± 0.52           | 7.8 ± 0.64          |
| Packed blood cell volume (%)                 | 36.8 ± 1.13                     | 35.2 ± 1.13         | 35.0 ± 1.13          | 37.0 ± 1.13         |
| Blood plasma potassium (mEq/L)               | 3.76 ± 0.18                     | 3.88 ± 0.18         | 3.86 ± 0.18          | 3.77 ± 0.18         |
| Blood plasma sodium (mEq/L)                  | 130.5 ± 2.89                    | 130.4 ± 2.89        | 136.1 ± 2.89         | 132.7 ± 2.89        |

<sup>a</sup>See Table 2, also see text concerning base temperature recording periods.

<sup>b</sup>See text for number of animals sampled for each physiological function.

<sup>c</sup>Only blood pressure, heart rate, and respiration rate on day 6.

<sup>d</sup>Standard error of the mean.

\*Statistically significant (P<.05).

\*\*Statistically significant (P<.01).

to increase as time of exposure increased, except for animal 10 whose blood pressure decreased in period 4. Animal 10 died on day 10 of cold exposure.

## 2. Heart rate

The mean values of animals 9 and 10 have been illustrated by Figure 14. The period means have been listed in Table 10.

The animal differences were highly significant ( $P < .005$ ) with animal 10 having a higher heart rate than animal 9 throughout the exposure periods. Period differences were highly significant ( $P < .005$ ). Both animals showed a continued rise in heart rate as exposure time increased with the highest mean value during period 3. Both animals showed a slight decline in heart rate during period 4.

## 3. Respiratory frequency

The mean values for animals 9 and 10 have been illustrated by Figure 21. The period means have been listed in Table 10.

The animal and period differences were not statistically significant. Animal 9 remained about the same with a very slight decrease in respiratory frequency during periods 3 and 4. Animal 10 showed an increase in frequency during period 2 followed by a decrease in period 3 and a slight rise in period 4.

## 4. Packed blood cell volume

The mean values for animals 9 and 10 have been illustrated by Figure 32. The period means have been listed in Table 10.

Neither animal differences nor period differences were statistically significant. However, animal 10 showed a definite decrease in P.C.V. during period 2 and 3, followed by an increase in period 4.

5. Blood plasma potassium

The mean values for animals 9 and 10 have been illustrated by Figure 38. The period means have been listed in Table 10.

Animal differences were statistically significant ( $P < .025$ ) with animal 10 having a higher plasma potassium concentration than that of animal 9. Animal 10 showed a rise in plasma potassium concentration during period 2 followed by a decline to pre-exposure level during period 4. The plasma potassium concentration of animal 9 remained essentially unchanged.

6. Blood plasma sodium

The mean values for animals 9 and 10 have been illustrated by Figure 44. The period means have been listed in Table 10.

Neither animal nor period differences were significant. However, animal 10 had a definite increase in plasma sodium concentration during periods 3 and 4. Animal 9 had a very slight decline.

## V. DISCUSSION

Prosser (1964) has stated that adaptional physiology as a study depends on certain assumptions, one of which is, that organisms are in dynamic equilibrium with their environment. The living organism functions as an "open thermodynamic system" with both continuing input and output. The maintenance of a "dynamic equilibrium" or steady state constitutes "homeostasis", the ability to survive in a varying environment which is the central physiological characteristic of living things.

Observations of blood pressure, heart rate, and respiratory frequency were made to evaluate the "general homeostasis" of bodily function. The observation of blood glucose, packed blood cell volume, total blood plasma proteins, and blood plasma potassium and sodium concentrations was an attempt to evaluate the "stress" of cold exposure since these factors are generally influenced by the pituitary-adrenal complex.

The cardiovascular system is one of the primary physiological mechanisms first used to control heat loss in a cold environment (Folk, 1966). The rate of heat loss is reduced by cutaneous and peripheral vasoconstriction and a fall in temperature of the skin surface and extremities. It is logical to assume that the vasomotor adjustments will be correlated to changes in systemic blood pressure. Burton (1965) has stated that the homeostasis of arterial blood pressure is the dominant circulatory reflex under the control of the carotid sinus and aortic reflexes. Therefore, the degree of change in blood pressure, established during cold exposure, and

the time required to return to some "normal" pressure (perhaps different than that before exposure) should be one measure of an animal's ability to compensate for cold stress.

The "comfort zone" for cattle has been suggested to be 30 to 60°F (Brody, 1956). The results of this study imply that the average aortic blood pressure of cattle within the "comfort zone" may range from near 74 mm Hg to near 98 mm Hg, as measured by the reported technique.

The computed period means of the average blood pressure during cold exposure were all significantly increased above that recorded at a base temperature. This increase in blood pressure occurred even though significant differences in blood pressure of individual animals were recorded. This would indicate that the increase in blood pressure was a true effect of the cold exposure. The relative increase in blood pressure during cold exposure varied from 10% of the base value in Experiment 1 to 56% in Experiment 3. One might conclude that for a rapid decrease of 38°F of environmental temperature (from a temperature within the "comfort zone" of cattle) one can expect at least a 10% increase in blood pressure of cattle.

It would seem that most of the cattle in this study, with the possible exception of those in Experiments 1 and 7, had not reached a "steady state" or homeostasis of blood pressure during the cold exposures. There was no statistically significant decrease in blood pressure during cold although a slight decrease in blood pressure occurred near the end of the cold exposure in Experiments 1 and 7. It is possible that the heat production (or "cold acclimation") of the animals had not sufficiently increased

during the time period studied to reduce the need for peripheral vasomotor control of heat loss, at least to the extent that a more "normal" blood pressure would result.

It is understood that a knowledge of changes in the heart rate per se does not allow one to state the definite changes occurring in cardiovascular function. However, the heart rate is governed by the integrated activity of brain medullary centers which function in cardiac and peripheral vascular control (Rushmer, 1961). Therefore, many factors causing changes in cardiovascular function might be expected to alter the "steady state" of the heart rate. Observations of the changes in heart rate might then be used as an indicator of when a stress, such as cold exposure, has upset the "steady state" of the cardiovascular system.

The mean heart rates during period 1 base temperatures ranged from 43 beats per minute at 74°F in Experiment 1 to 58 beats per minute at 33°F in Experiment 8. These values were somewhat below those reported by Worstell and Brody (1953) for cattle within their "comfort zone" of environmental temperature. They were also below the values reported by others who have studied the relationship of the heart rate and environmental temperature within the "comfort zone" of cattle (Findlay, 1950; Graff and Peterson, 1953; Ingram and Whittow, 1963; MacDonald and Bell, 1958).

The computed period means of the heart rate during cold exposure were all significantly increased above that recorded at the base temperature. This increase in heart rate occurred even though significant differences in heart rate of individual animals were recorded. This would indicate that the increase in heart rate was a true effect of the cold exposure. The

relative increase in heart rate during cold exposure varied from 26% in Experiments 6 and 8 to 63% in Experiment 7. The highest mean value for the heart rate during cold exposure was  $85.8 \pm 1.63$  beats per minute recorded during period 3 of Experiment 7. With the exception of Experiment 1, the recorded mean heart rates during cold exposure lie within the range of 71 to 86 beats per minute. Most of the reported values for the heart rate of cattle in an environmental temperature below 30°F also lie within or slightly below this range (Kibler and Brody, 1949, 1950, 1953, 1954; MacDonald and Bell, 1958). These investigators report only a small change in heart rate of cattle during cold exposure. It may be that the method of recording used in the present study allows one to obtain lower pre-exposure values; however, animal differences due to nutrition, exercise, and lactation may also account for the higher heart rates reported by other workers at warmer temperatures.

The control of respiratory function is influenced by a myriad of various factors, both endogenous and exogenous to the body, and any attempt to relegate the control of respiration to a few isolated factors is fraught with exceptions. For the purpose of this discussion, respiratory function has been considered in terms of frequency and depth of the respiratory cycle although only the former was actually measured in this study.

In general, the control of frequency and depth of respiration is appropriately altered to satisfy the need for adequate alveolar ventilation (i.e. for the maintenance of the proper blood concentration of CO<sub>2</sub> and O<sub>2</sub>). However, the rate and depth of respiration may also be modified in response to environmental temperature. The respiratory frequency of cattle is known to decrease during cold exposure (Worstell and Brody, 1953).

Calculated means of the respiratory frequency for the base periods varied from  $6.7 \pm 0.18$  during Experiment 6 to  $15.3 \pm 0.43$  during Experiment 3. There was a definite difference between the frequencies observed at 74 and 33°F.

The change in respiratory frequency during cold exposure was dependent upon the base temperature to which the animal had been acclimated. All animals at a base temperature of 74°F had a definite, statistically significant decrease in respiratory frequency when exposed to cold environmental temperatures. Whereas, those animals acclimated to 33°F did not have a significant decrease in respiratory frequency (with the exception of Experiment 7) and at times had an increase in respiratory frequency during cold exposure. These observations, when compared with the summary of respiratory rates of cattle presented by Williams and Bell (1964), imply that the general influence of environmental temperature on cattle resulting in a decreased respiratory frequency does not operate to a significant degree below about 35°F. It is tempting to speculate that the frequency of respiration is decreased to some minimal value to conserve body heat and that any increased need for oxygen is met by a change in tidal volume. However, when the cold exposure is too severe and the oxygen need cannot be met by increased tidal volume alone, the frequency of respiration will also increase, as it did in some experiments.

Some alterations in carbohydrate metabolism are thought to be influenced by changes of environmental temperature. Differences in glycogen content of several tissues of acclimatized rats were related to different environment temperatures; however, skeletal muscle, glycogen content, and

blood glucose levels were not significantly different (Baker and Sellers, 1953). Cold exposure of rats acclimated to warm temperatures resulted in decreased plasma glucose and liver glycogen with increased turnover and oxidation of body glucose (Depocas and Masironi, 1960).

In cattle, few studies have been made where a change in blood glucose has been directly associated with controlled cold exposure. Massey et al. (1958) suggested that quick changes in weather from warm to cold and intense heat over a period of days may have interfered with the response of cattle to injected insulin; however, no specific data on blood glucose were given. Small increases in blood glucose during controlled cold exposure have been reported for cattle previously acclimated to warm temperatures (Blincoe and Brody, 1951; Kamal et al., 1962).

Animals 1, 2, 3, and 4 (Experiment 2) showed a slight increase in blood glucose during cold exposure; however, this change was not statistically significant.

Changes in total blood plasma proteins and packed blood cell volume (P.C.V.) may accompany changes in plasma fluid volume. Plasma protein concentrations may be changed either by alteration of synthesis or catabolism; or due to hemoconcentration or hemodilution. The P.C.V. is influenced by the size and number of erythrocytes as well as the plasma volume of the blood. Although more direct measurements of body fluid volumes are desirable, in general, an inverse relationship exists between plasma protein and plasma volume (Bazett et al., 1940; Conley and Nickerson, 1945; Spealman et al., 1947; Mcfarlane et al., 1961; English, 1966). Concurrent determinations of total plasma protein and P.C.V. were made during

Experiments 2, 3, and 4. Only small changes in plasma proteins occurred during cold exposure and all animals did not respond in the same manner. The P.C.V. was increased during cold exposure throughout Experiments 2, 3, and 4, with increases being statistically significant during Experiments 2 and 4. It was concluded that if the plasma volume changed during cold exposure, as possibly implied by the change in P.C.V., this volume change did not significantly affect the plasma protein concentration. Kamal et al. (1962) reported that heifers reared at 80°F had about 50% higher plasma protein content than that of 50°F animals. Blincoe and Brody (1951) reported no significant change in plasma protein of cattle with declining temperature from 65°F down to about 5°F.

The P.C.V. was significantly increased during cold exposure in all experiments having a 74°F base temperature, except Experiment 3. The high P.C.V. of animal 3 during the pre-exposure measurements of Experiment 3 accounts for the failure of the cold exposure values to be significantly increased. This conclusion seems justified since the period mean for P.C.V. after exposure was significantly lower than the period 1 mean. The mean P.C.V. of the animals at 33°F base temperature was higher than that of the animals at 74°F base temperature. The P.C.V. of the cattle throughout Experiments 5, 6, and 8 did not have a significant increase during cold exposure; perhaps this is related to the higher P.C.V. recorded at the base temperature of 64°F.

The changes in blood plasma electrolytes during cold exposure varied throughout all experiments studied. Statistically significant decreases in plasma potassium concentration occurred during the cold exposure period

of Experiment 2 as well as the recovery periods of Experiments 2 and 3. Blood plasma sodium concentration significantly increased during the cold exposure and recovery periods of Experiment 3. The decrease in plasma sodium during the recovery periods of Experiments 2 and 6 was also statistically significant.

Conclusions concerning electrolyte balance which are made from serum electrolyte concentrations alone are subject to criticism since nothing is known about total fluid volumes; and a change in fluid volumes can be expected to cause variation in plasma electrolyte concentrations.

The lack of a uniform change of plasma electrolyte concentrations in the present study supports the observations of Blincoe and Brody (1951), who reported no significant change of blood electrolytes in cattle while the temperature was declining from 65 to 5°F. Kamal et al. (1962) reported a significant difference in plasma sodium as well as Na/K ratio between heifers reared at 50 and 80°F when exposed to 35°F for 9 days. That response was thought to be due to a hemoconcentration in the 80°F heifers during the cold exposure.

## VI. SUMMARY

This study was designed to observe and record the responses of cattle, acclimated to warm and cold temperatures, when exposed to a range of cold environmental temperatures.

The primary objective was to study physiological responses of cattle to abrupt cold exposure, thereby establishing a pattern of reaction which may be used for comparison in studies utilizing pathogenic agents.

Recorded measurements were made of the following physiological functions: blood pressure, heart rate, respiratory frequency, blood glucose, total blood plasma protein concentrations, packed blood cell volume, and blood plasma potassium and sodium.

The following responses to cold exposure were noted:

1. The blood pressure during cold exposure was significantly increased above that recorded at the base temperature.
2. The heart rate during cold exposure was significantly increased above that recorded at the base temperature.
3. The change in respiratory frequency during cold exposure was dependent upon the base temperature to which the animal had been acclimated. All animals at a base temperature of 74°F had a significant decrease in respiratory frequency during cold exposure. Whereas, those animals acclimated to 33°F did not have a significant decrease in respiratory frequency, with the exception of Experiment 7, and at times they had an increase in respiratory frequency during cold exposure.

4. Only a slight increase in blood glucose occurred during cold exposure in one experiment where measurements were made; however, this change was not statistically significant.
5. Total blood plasma proteins were not significantly changed during cold exposure.
6. Packed blood cell volume of cattle acclimated to 74°F was increased during cold exposure; however, this increase was statistically significant in 3 of 4 experiments. The packed blood cell volume of cattle acclimated to 33°F was slightly higher than those acclimated to 74°F and the small increases during cold exposure were not statistically significant.
7. The blood plasma concentrations of sodium and potassium varied during cold exposure. A statistically significant decrease of potassium occurred during cold exposure in one experiment and a significant increase of sodium occurred during cold exposure of one other experiment.

The results were discussed and compared to previously reported studies on the response of cattle to controlled cold exposure.

The conclusion was that the "general homeostasis" of cattle was disturbed by the abrupt cold exposures used. The degree of "stress" was variable as judged by the measurement of some blood constituents.

## VII. LITERATURE CITED

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IX. APPENDIX A: FIGURES

Figure 1. Average blood pressure of animals 5, 6, and 7 during Experiment 1. Period 1 environmental temperature was 74°F and measurements were made 1 and 3 days before cold exposure. Period 2 environmental temperature was 33°F and measurements were made during days 1 through 7 of cold exposure. Period 3 environmental temperature was 33°F and measurements were made during days 13, 17, and 21 of cold exposure (see Table 3).

Figure 2. Average blood pressure of animals 1, 2, 3, and 4 during Experiment 2. Three 24-hour cold exposures were made at one-week intervals (see Results). Period 1 environmental temperature was 74°F and reported measurements were made the second day of each week. Period 2 environmental temperature is 0°F and measurements were made on day 3 and 4 of each week. Period 3 environmental temperatures are 74°F and measurements were made on day 5 of each week (see Table 4).

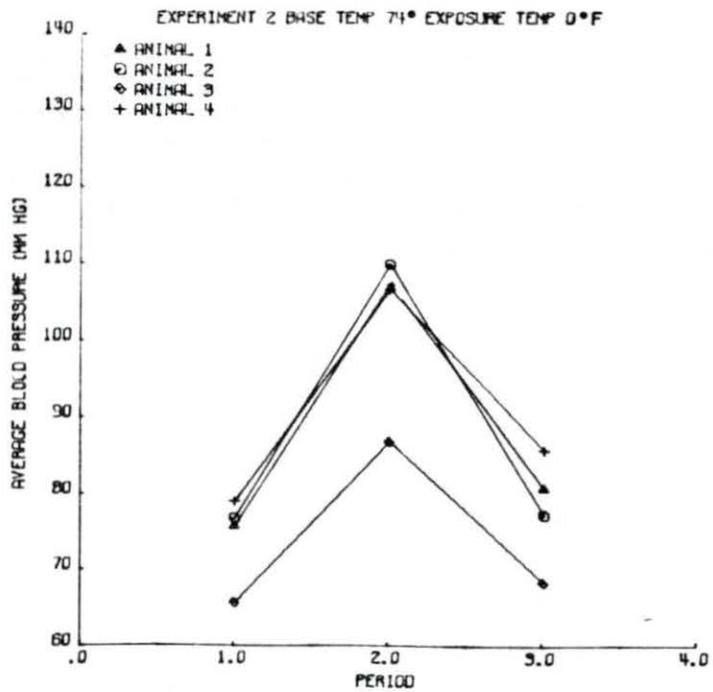
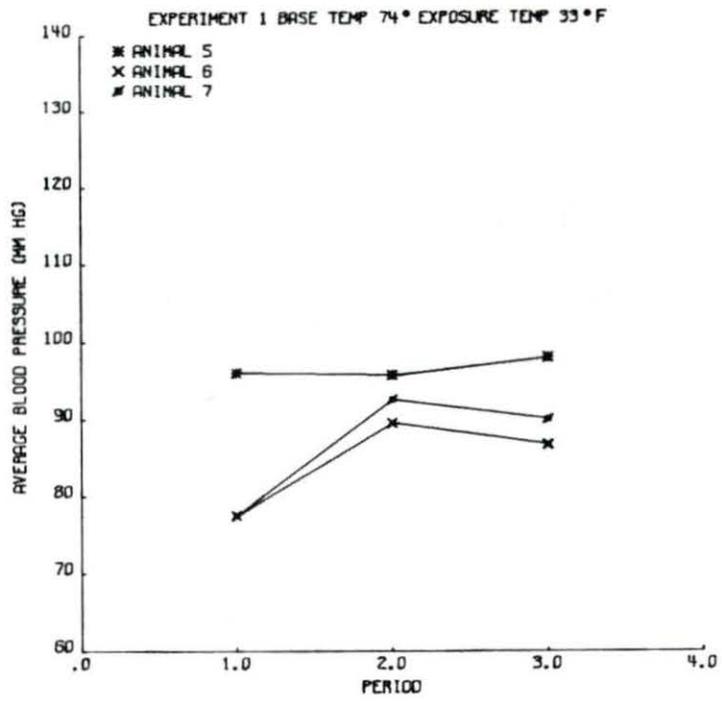


Figure 3. Average blood pressure of animals 1 and 2 during Experiment 3. Period 1 environmental temperature was 74°F and measurements were made 1, 2, and 3 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, and 3. Period 3 environmental temperature was 74°F and measurements were made during days 1 and 2 (see Table 5).

Figure 4. Average blood pressure of animals 1, 2, and 3 during Experiment 5. Period 1 environmental temperature was 33°F and measurements were made 1, 2, 3, and 4 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, 3, 5, 6, and 7. Period 3 environmental temperature was 33°F and measurements were made on days 1 through 6 (see Table 7).

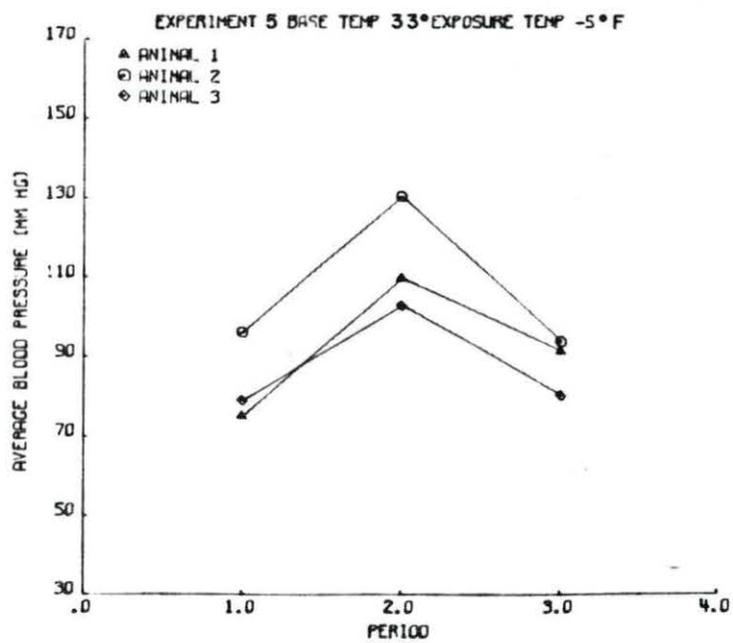
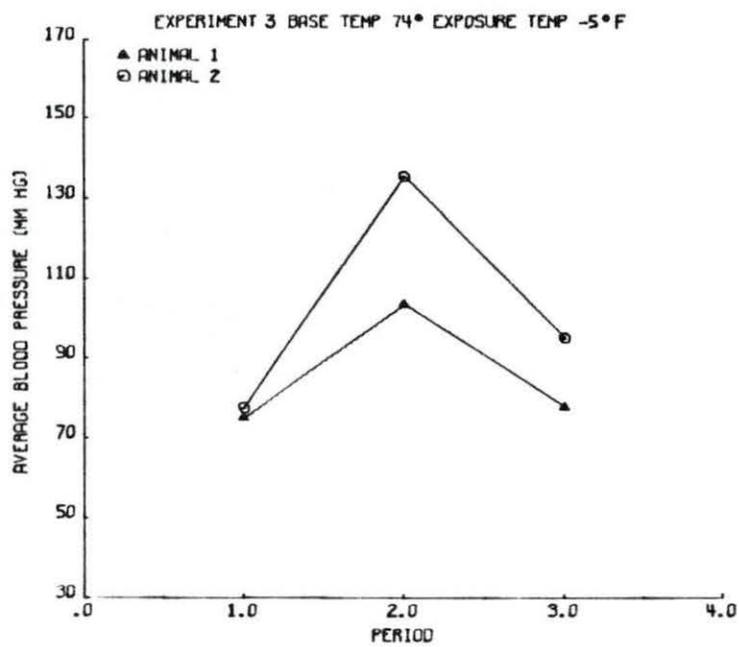


Figure 5. Average blood pressure of animals 5, 6, and 7 during Experiment 6. Period 1 environmental temperature was 33°F and measurements were made 1 and 4 days before cold exposure. Period 2 environmental temperature was -12°F and measurements were made on days 1, 2, and 4. Period 3 environmental temperature was -12°F and measurements were made on days 9, 11, 12, and 13. Period 4 environmental temperature was 33°F and measurements were made on days 1, 2, 5, and 6 (see Table 8).

Figure 6. Average blood pressure of animals 1 and 3 during Experiment 7. Period 1 environmental temperature was 33°F and measurements were made 1 and 4 days before cold exposure. Period 2 environmental temperature was -20°F and measurements were made on days 1, 2, 3, and 4. Period 3 environmental temperature was -20°F and measurements were made on days 10 and 14 (see Table 9).

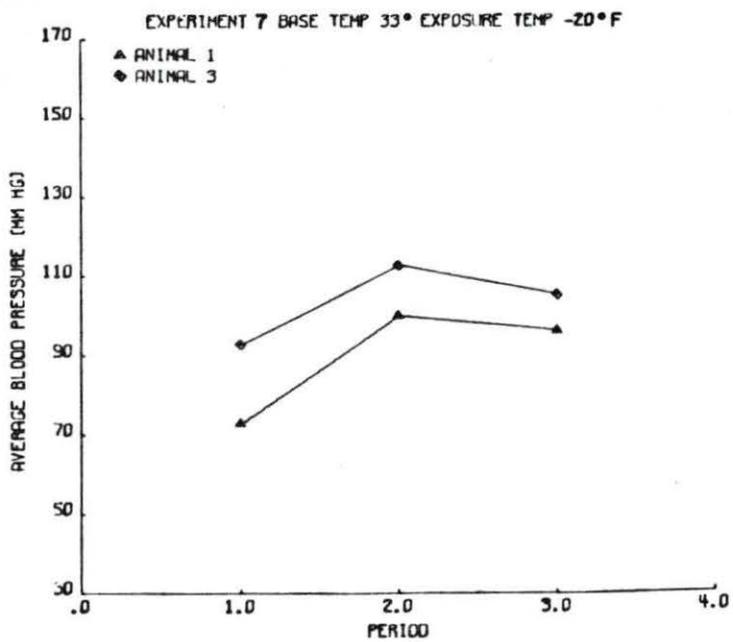
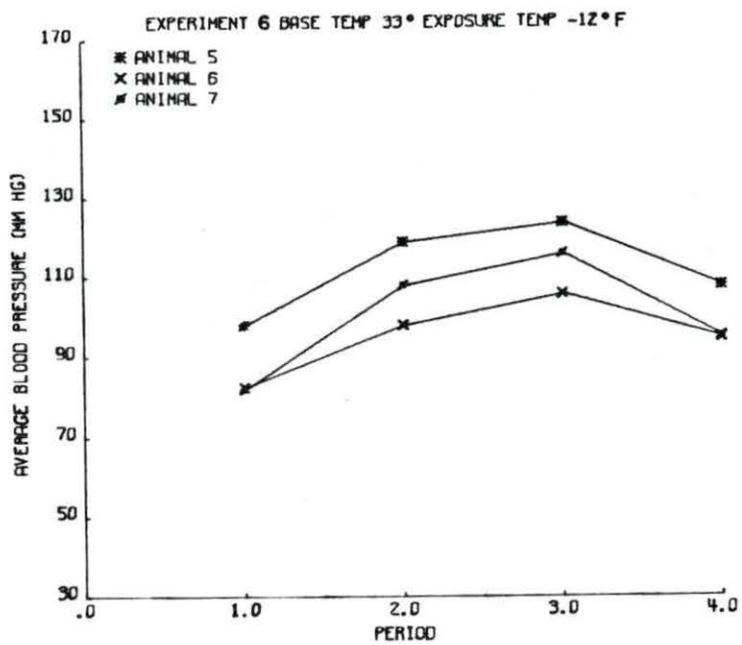


Figure 7. Average blood pressure of animals 9 and 10 during Experiment 8. Period 1 environmental temperature was 33°F and measurements were made 1 and 2 days before cold exposure (see Table 2 for base conditions). Period 2 environmental temperature was -20°F and measurements were made on days 1 and 2. Period 3 environmental temperature was -20°F and measurements were made on days 4, 5, and 6. Period 4 environmental temperature was -20°F and measurements were made on days 8 and 9 (see Table 10). Cow 10 died on day 10 of cold exposure.

Figure 8. Heart rate of animals 5, 6, and 7 during Experiment 1. Period 1 environmental temperature was 74°F and measurements were made 1 and 3 days before cold exposure. Period 2 environmental temperature was 33°F and measurements were made during days 1 through 7 of cold exposure. Period 3 environmental temperature was 33°F and measurements were made during days 13, 17, and 21 of cold exposure (see Table 3).

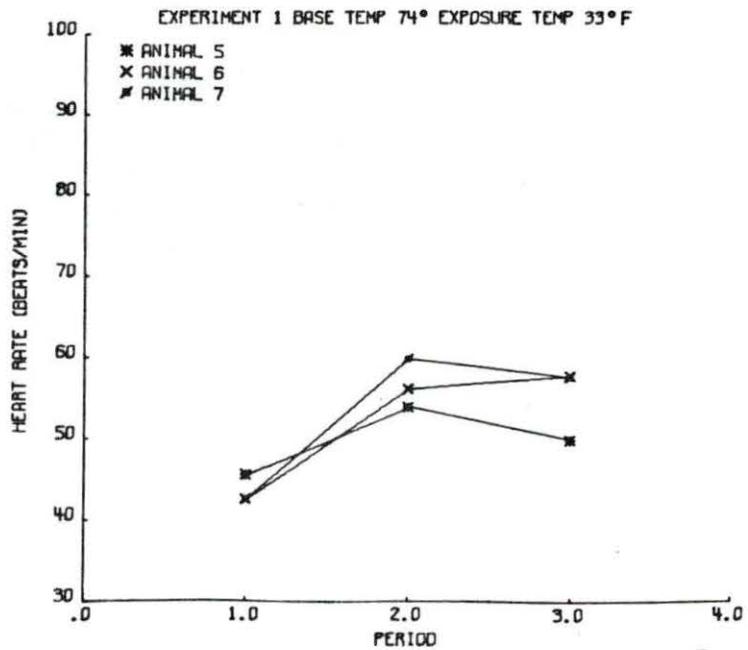
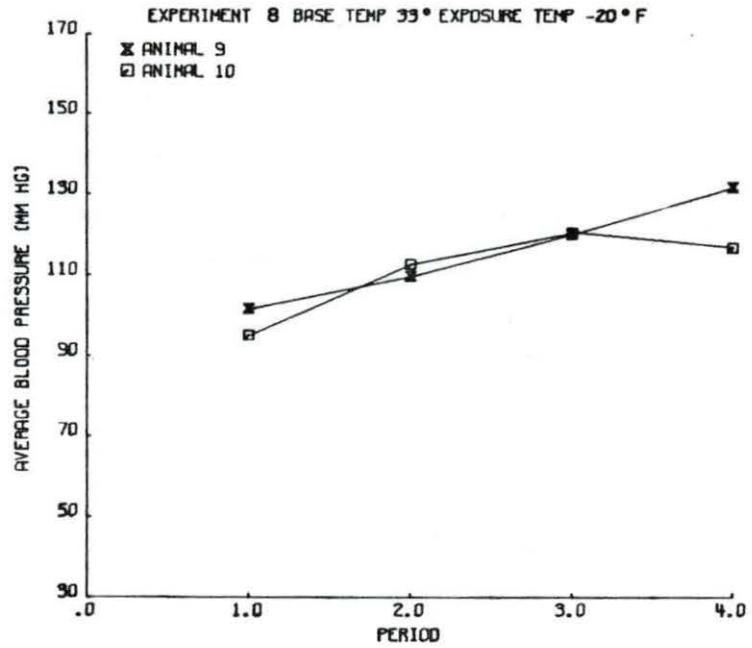


Figure 9. Heart rate of animals 1, 2, 3, and 4 during Experiment 2. Three 24-hour cold exposures were made at one-week intervals (see Results). Period 1 environmental temperature was 74°F and reported measurements were made the second day of each week. Period 2 environmental temperature is 0°F and measurements were made on day 3 and 4 of each week. Period 3 environmental temperatures are 74°F and measurements were made on day 5 of each week (see Table 4).

Figure 10. Heart rate of animals 1 and 2 during Experiment 3. Period 1 environmental temperature was 74°F and measurements were made 1, 2, and 3 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, and 3. Period 3 environmental temperature was 74°F and measurements were made during days 1 and 2 (see Table 5).

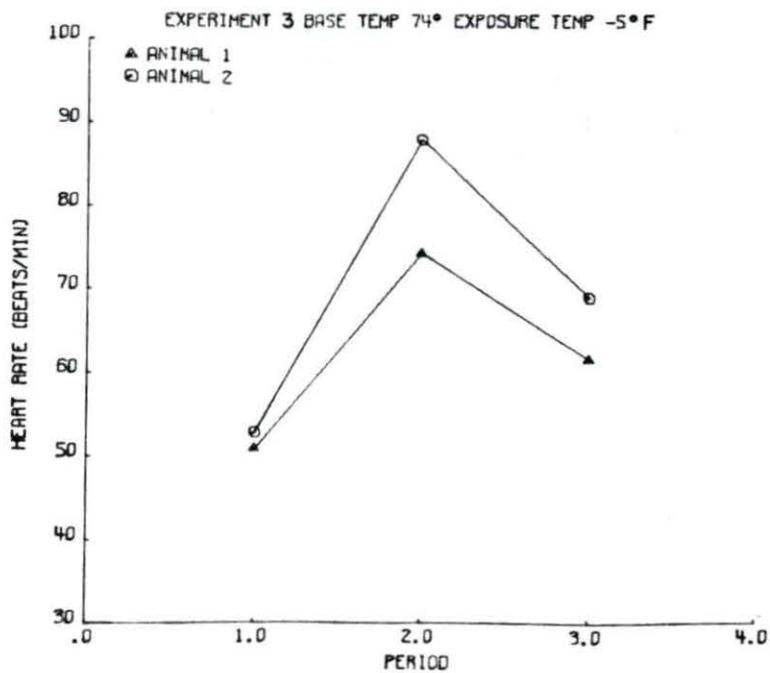
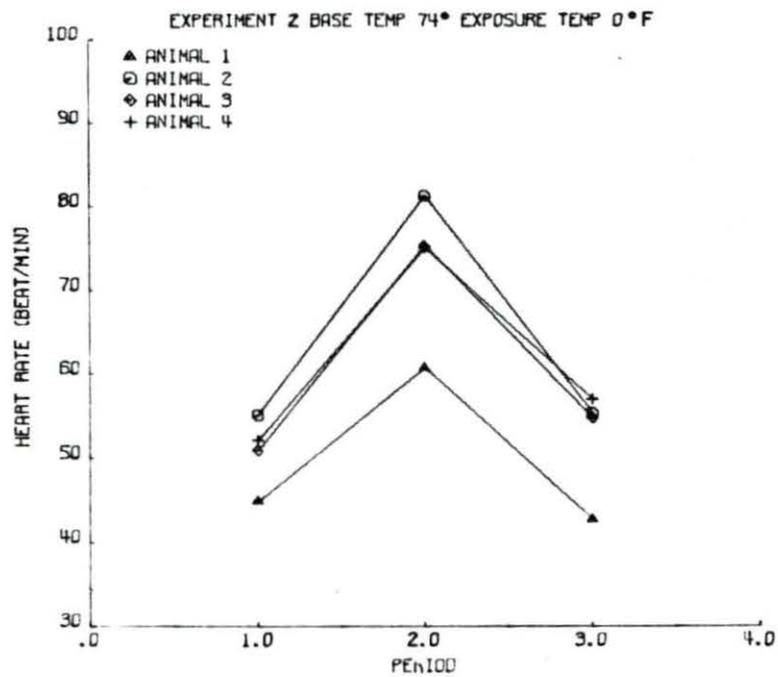


Figure 11. Heart rate of animals 1, 2, and 3 during Experiment 5. Period 1 environmental temperature was 33°F and measurements were made 1, 2, 3, and 4 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, 3, 5, 6, and 7. Period 3 environmental temperature was 33°F and measurements were made on days 1 through 6 (see Table 7).

Figure 12. Heart rate of animals 5, 6, and 7 during Experiment 6. Period 1 environmental temperature was 33°F and measurements were made 1 and 4 days before cold exposure. Period 2 environmental temperature was -12°F and measurements were made on days 1, 2, and 4. Period 3 environmental temperature was -12°F and measurements were made on days 9, 11, 12, and 13. Period 4 environmental temperature was 33°F and measurements were made on days 1, 2, 5, and 6 (see Table 8).

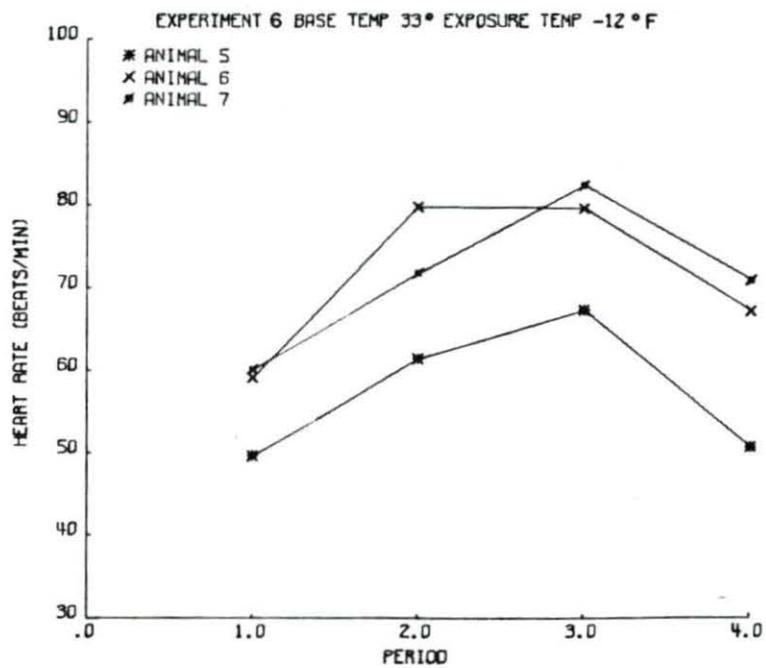
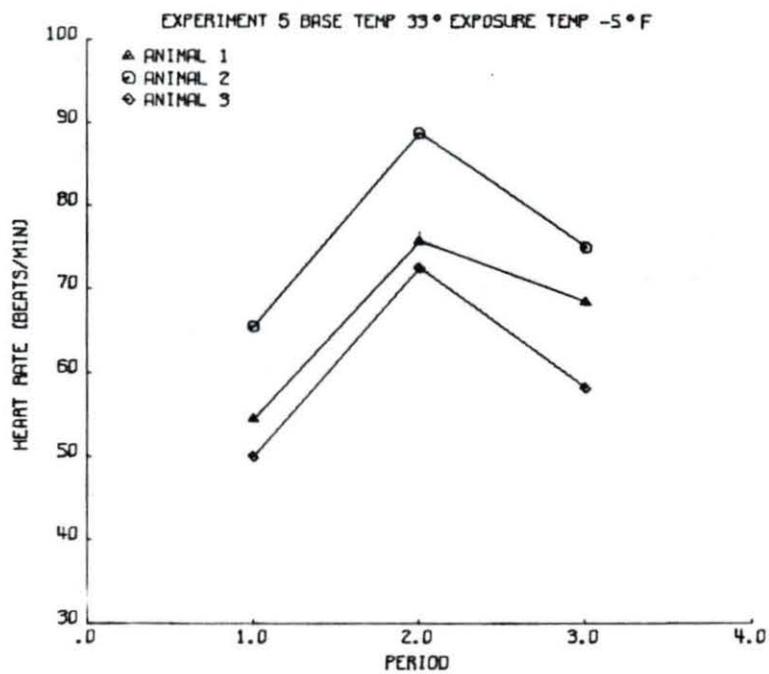


Figure 13. Heart rate of animals 1 and 3 during Experiment 7. Period 1 environmental temperature was 33°F and measurements were made 1 and 4 days before cold exposure. Period 2 environmental temperature was -20°F and measurements were made on days 1, 2, 3, and 4. Period 3 environmental temperature was -20°F and measurements were made on days 10 and 14 (see Table 9).

Figure 14. Heart rate of animals 9 and 10 during Experiment 8. Period 1 environmental temperature was 33°F and measurements were made 1 and 2 days before cold exposure (see Table 2 for base conditions). Period 2 environmental temperature was -20°F and measurements were made on days 1 and 2. Period 3 environmental temperature was -20°F and measurements were made on days 4, 5, and 6. Period 4 environmental temperature was -20°F and measurements were made on days 8 and 9 (see Table 10). Cow 10 died on day 10 of cold exposure.

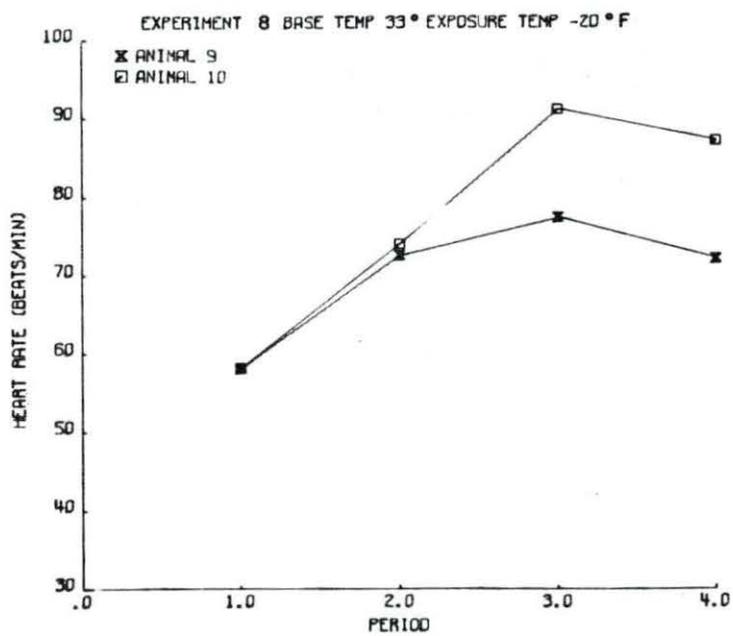
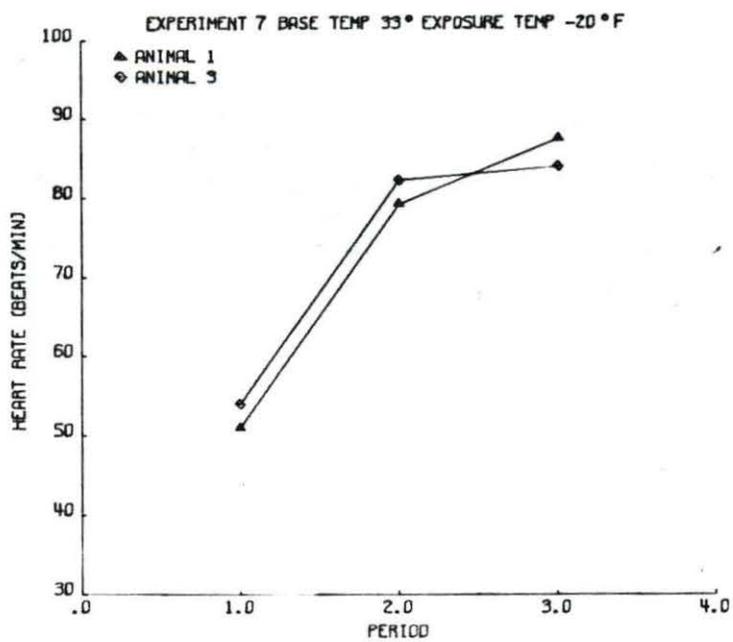


Figure 15. Respiratory frequency of animals 5, 6, and 7 during Experiment 1. Period 1 environmental temperature was 74°F and measurements were made 1 and 3 days before cold exposure. Period 2 environmental temperature was 33°F and measurements were made during days 1 through 7 of cold exposure. Period 3 environmental temperature was 33°F and measurements were made during days 13, 17, and 21 of cold exposure (see Table 3).

Figure 16. Respiratory frequency of animals 1, 2, 3, and 4 during Experiment 2. Three 24-hour cold exposures were made at one-week intervals (see Results). Period 1 environmental temperature was 74°F and reported measurements were made the second day of each week. Period 2 environmental temperature is 0°F and measurements were made on day 3 and 4 of each week. Period 3 environmental temperatures are 74°F and measurements were made on day 5 of each week (see Table 4).

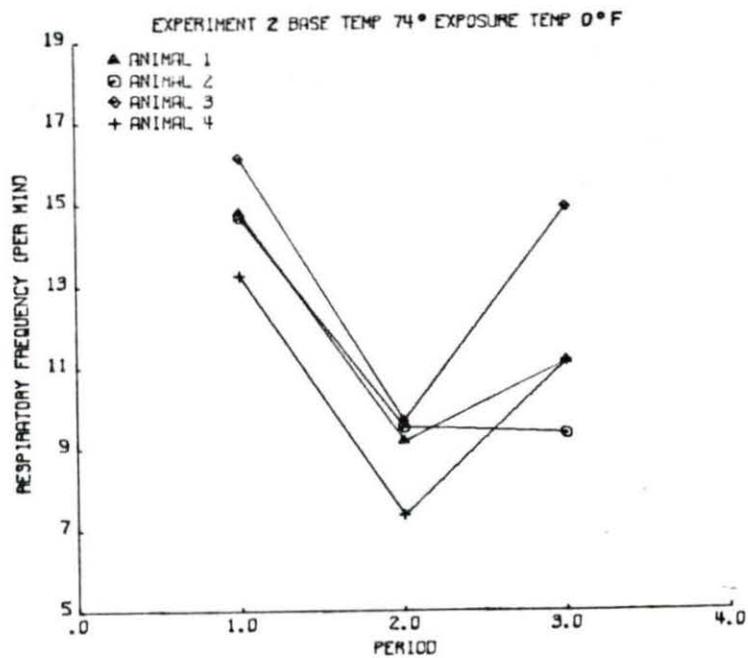
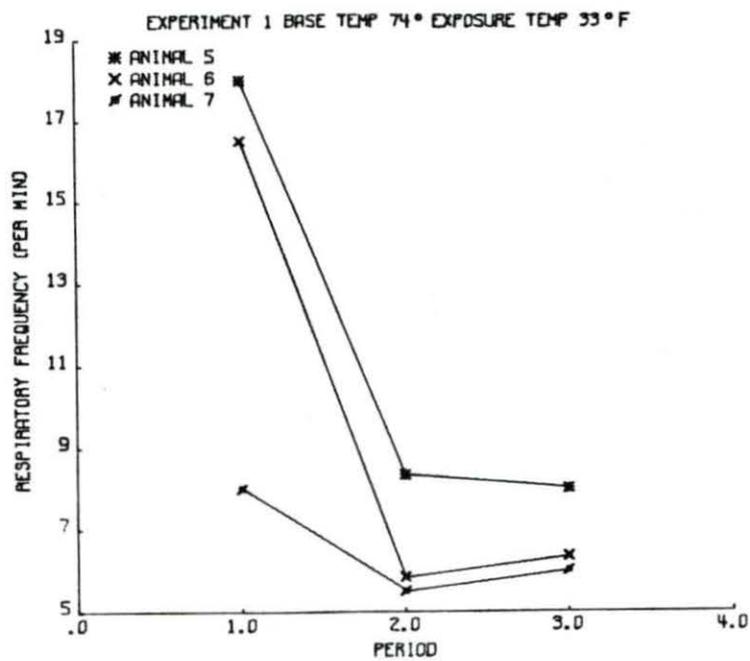


Figure 17. Respiratory frequency of animals 1 and 2 during Experiment 3. Period 1 environmental temperature was 74°F and measurements were made 1, 2, and 3 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, and 3. Period 3 environmental temperature was 74°F and measurements were made during days 1 and 2 (see Table 5).

Figure 18. Respiratory frequency of animals 1, 2, and 3 during Experiment 5. Period 1 environmental temperature was 33°F and measurements were made 1, 2, 3, and 4 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, 3, 5, 6, and 7. Period 3 environmental temperature was 33°F and measurements were made on days 1 through 6 (see Table 7).

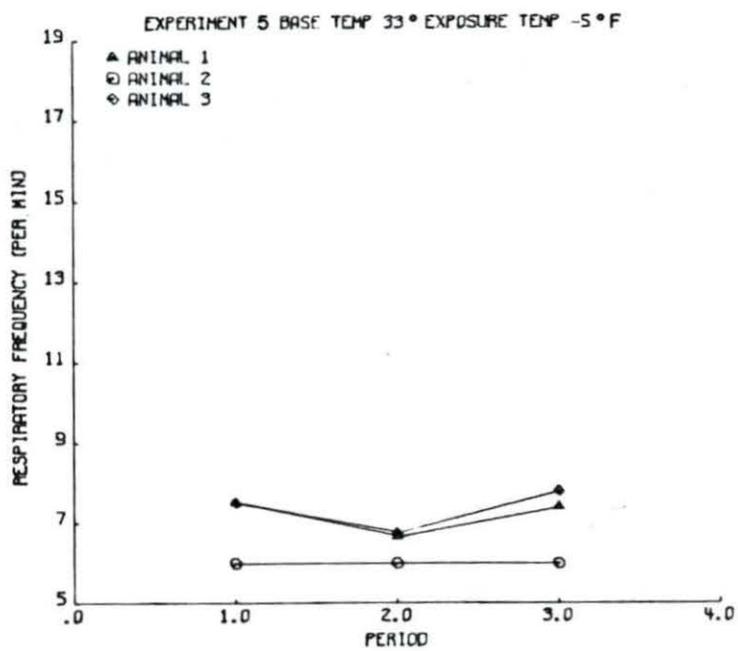
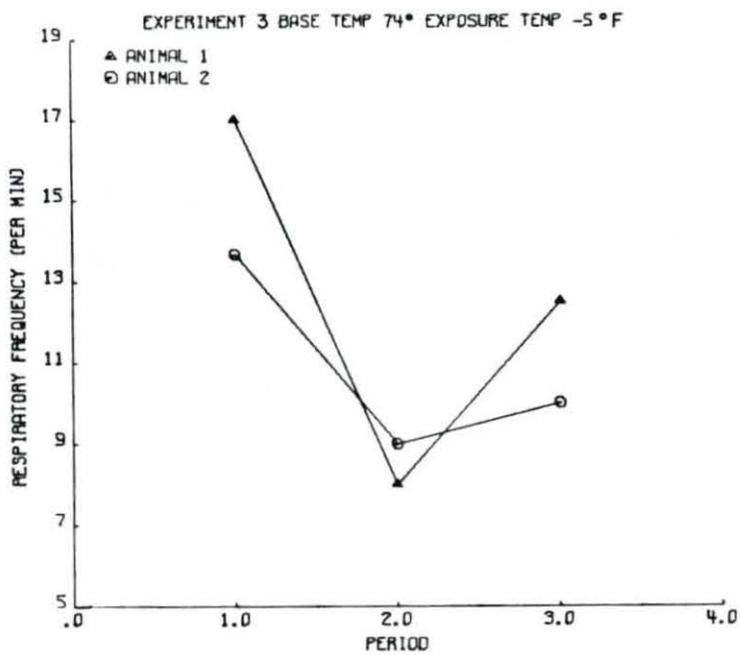


Figure 19. Respiratory frequency of animals 5, 6, and 7 during Experiment 6. Period 1 environmental temperature was 33°F and measurements were made 1 and 4 days before cold exposure. Period 2 environmental temperature was -12°F and measurements were made on days 1, 2, and 4. Period 3 environmental temperature was -12°F and measurements were made on days 9, 11, 12, and 13. Period 4 environmental temperature was 33°F and measurements were made on days 1, 2, 5, and 6 (see Table 8).

Figure 20. Respiratory frequency of animals 1 and 3 during Experiment 7. Period 1 environmental temperature was 33°F and measurements were made 1 and 4 days before cold exposure. Period 2 environmental temperature was -20°F and measurements were made on days 1, 2, 3, and 4. Period 3 environmental temperature was -20°F and measurements were made on days 10 and 14 (see Table 9).

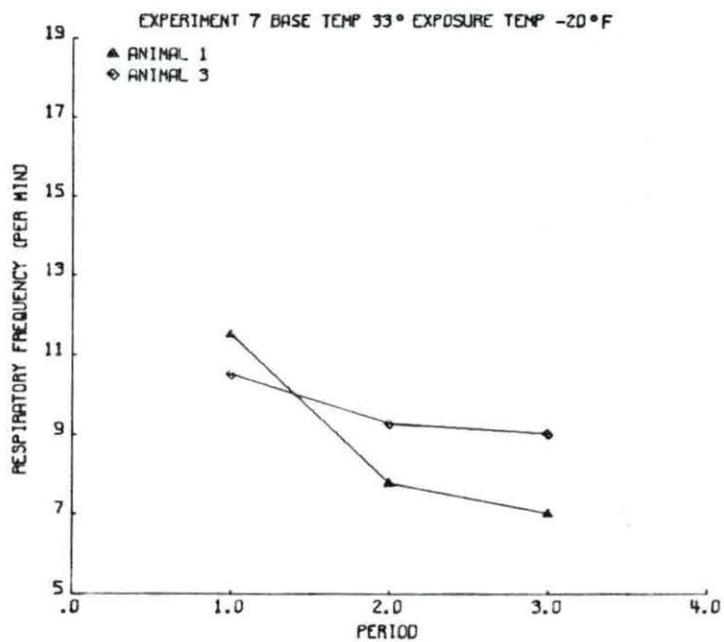
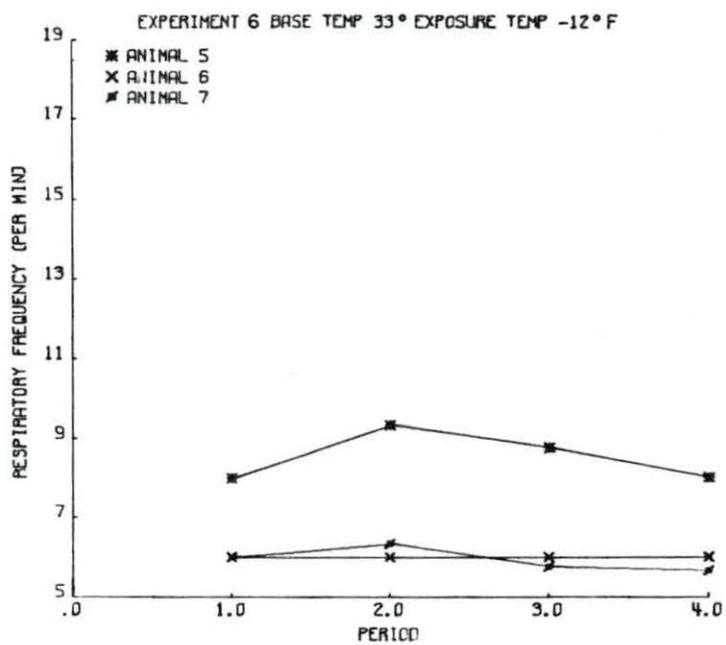


Figure 21. Respiratory frequency of animals 9 and 10 during Experiment 8. Period 1 environmental temperature was 33°F and measurements were made 1 and 2 days before cold exposure (see Table 2 for base conditions). Period 2 environmental temperature was -20°F and measurements were made on days 1 and 2. Period 3 environmental temperature was -20°F and measurements were made on days 4, 5, and 6. Period 4 environmental temperature was -20°F and measurements were made on days 8 and 9 (see Table 10). Cow 10 died on day 10 of cold exposure.

Figure 22. Blood glucose concentration of animals 1, 2, 3, and 4 during Experiment 2. Three 24-hour cold exposures were made at one-week intervals (see Results). Period 1 environmental temperature was 74°F and reported measurements were made the second day of each week. Period 2 environmental temperature is 0°F and measurements were made on day 4 of each week. Period 3 environmental temperatures are 74°F and measurements were made on day 5 of each week (see Table 4).

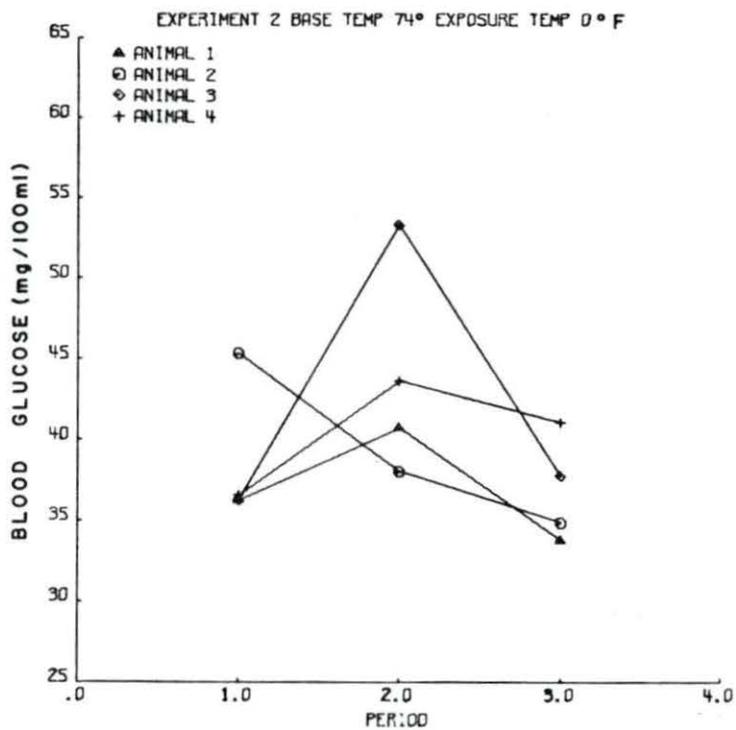
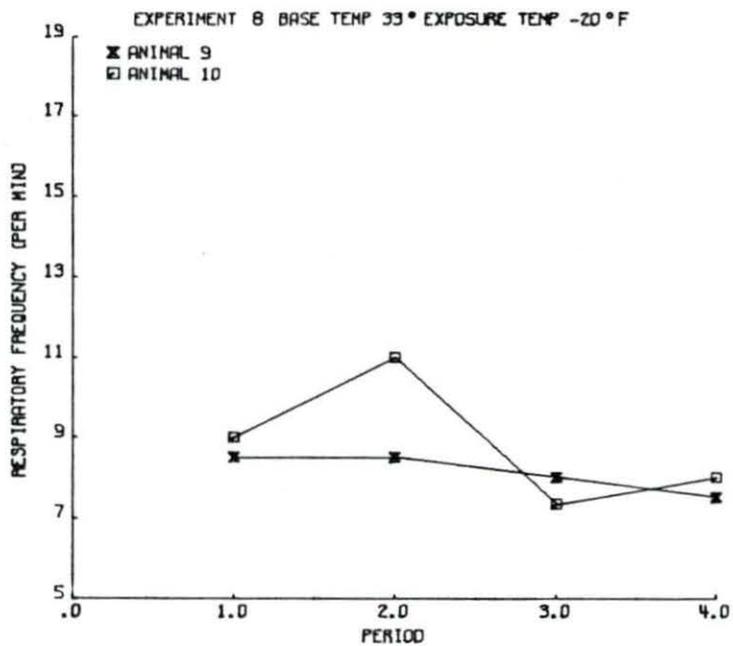


Figure 23. Total blood plasma proteins of animals 1, 2, 3, and 4 during Experiment 2. Three 24-hour cold exposures were made at one-week intervals (see Results). Period 1 environmental temperature was 74°F and reported measurements were made the second day of each week. Period 2 environmental temperature is 0°F and measurements were made on day 4 of each week. Period 3 environmental temperatures are 74°F and measurements were made on day 5 of each week (see Table 4).

Figure 24. Total blood plasma proteins of animals 1 and 2 during Experiment 3. Period 1 environmental temperature was 74°F and measurements were made 1, 2, and 3 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, and 3. Period 3 environmental temperature was 74°F and measurements were made during days 1 and 2 (see Table 5).

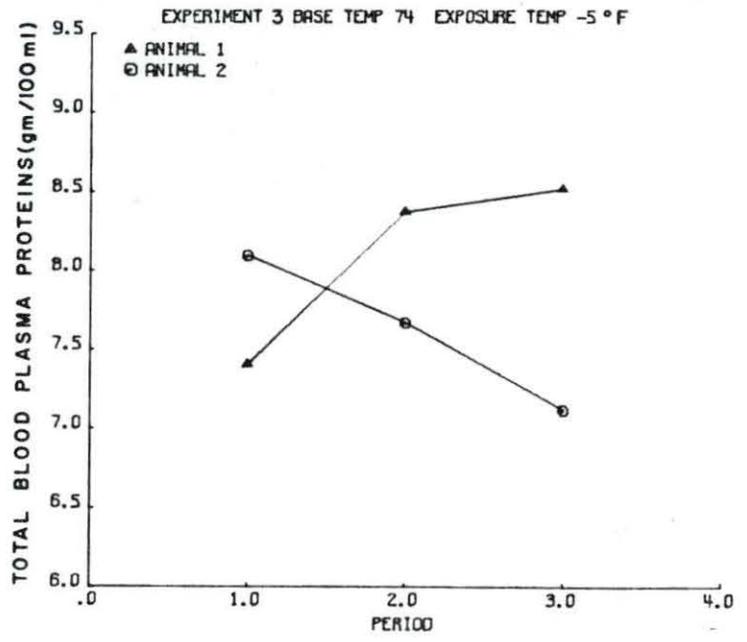
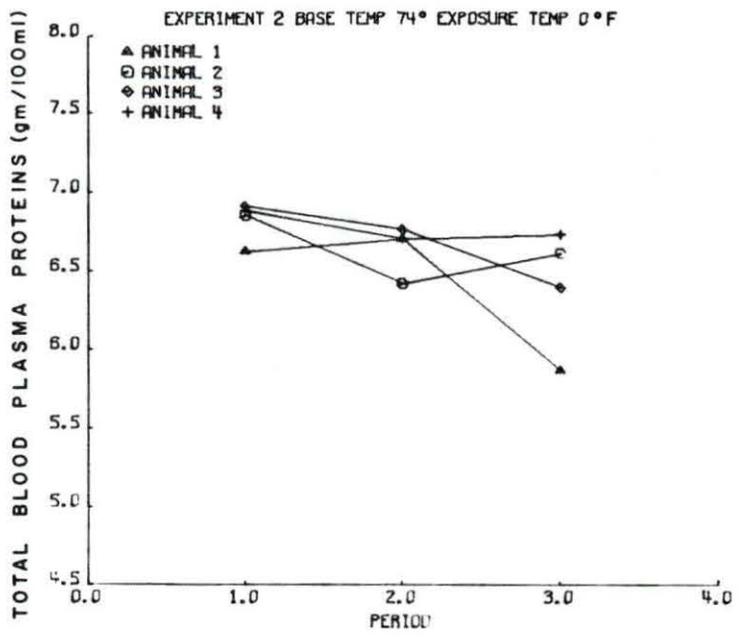


Figure 25. Total blood plasma proteins of animals 3 and 4 during Experiment 4. Period 1 environmental temperature was 74°F and measurements were made on 1, 2, 3, and 4 days before cold exposure. Period 2 environmental temperature was -25°F and measurements were made on days 1, 2, 3, and 4. Period 3 environmental temperature was 74°F and measurements were made on days 1, 2, and 3 (see Table 6).

Figure 26. Packed blood cell volume of animals 5, 6, 7, and 8 during Experiment 1. Period 1 environmental temperature was 74°F and measurements were made 1, 2, and 3 days before cold exposure. Period 2 environmental temperature was 33°F and measurements were made on days 8, 10, 11, and 13. Period 3 environmental temperature was 33°F and measurements were made on days 18, 19, 20, and 21 (see Table 3). Cow 8 died on day 13 of cold exposure.

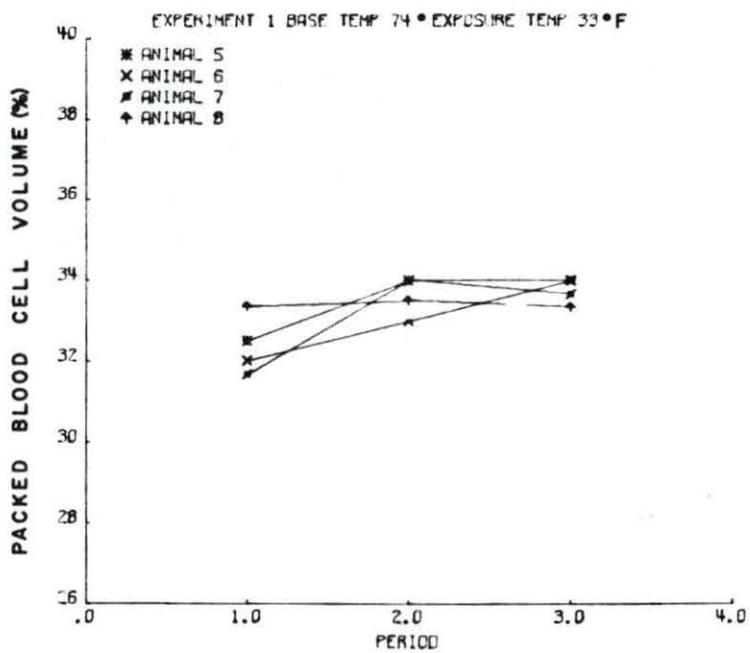
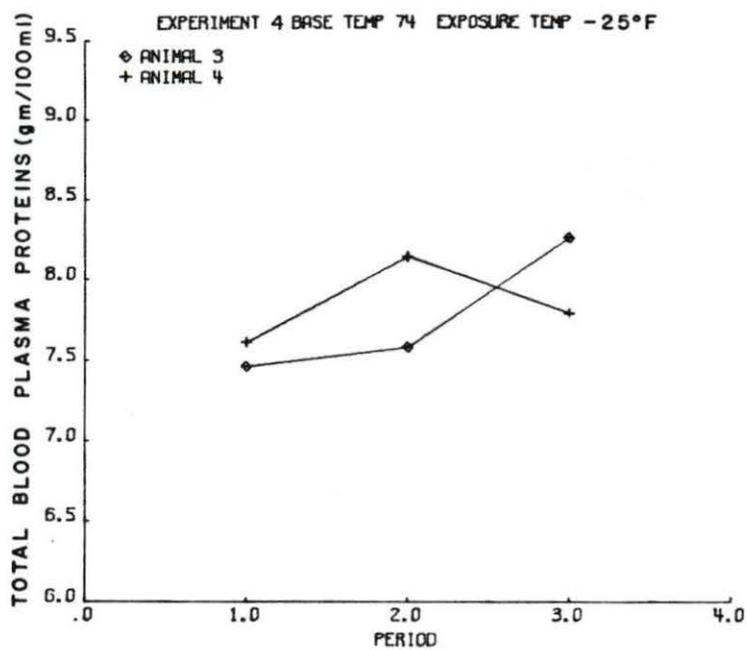


Figure 27. Packed blood cell volume of animals 1, 2, 3, and 4 during Experiment 2. Three 24-hour cold exposures were made at one-week intervals (see Results). Period 1 environmental temperature was 74°F and reported measurements were made the second day of each week. Period 2 environmental temperature is 0°F and measurements were made on day 4 of each week. Period 3 environmental temperatures are 74°F and measurements were made on day 5 of each week (see Table 4).

Figure 28. Packed blood cell volume of animals 1 and 2 during Experiment 3. Period 1 environmental temperature was 74°F and measurements were made 1, 2, and 3 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, and 3. Period 3 environmental temperature was 74°F and measurements were made during days 1 and 2 (see Table 5).

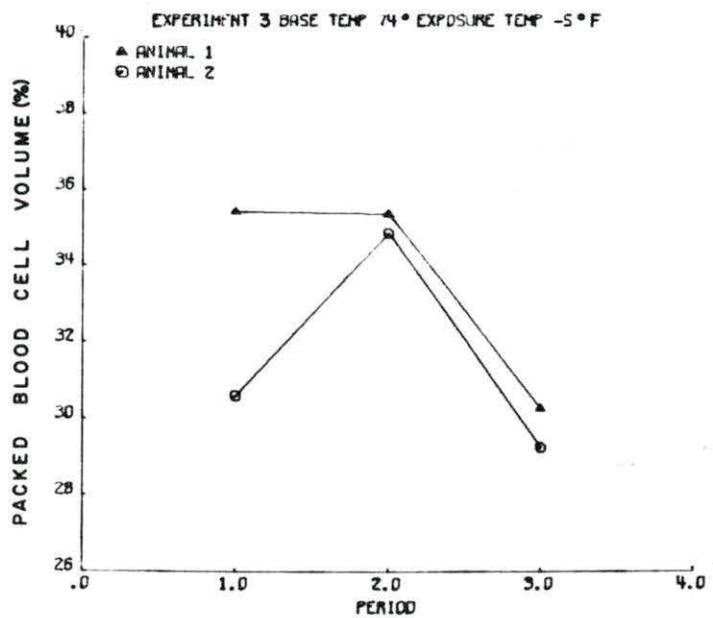
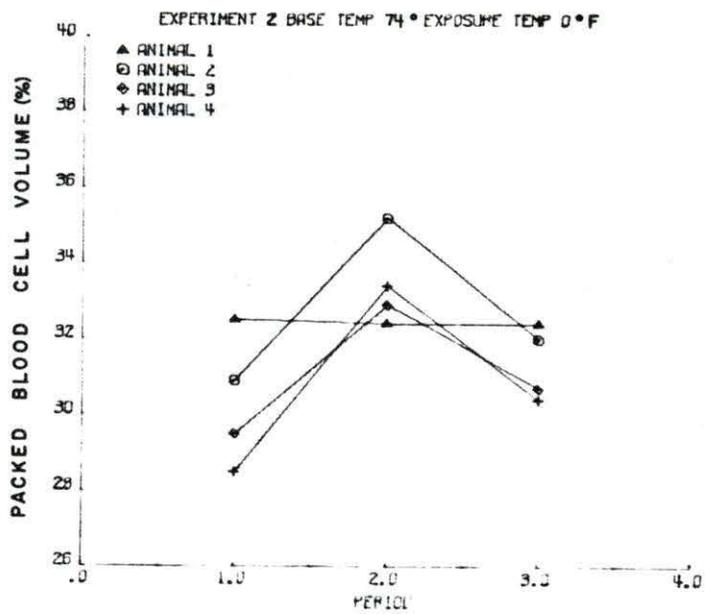


Figure 29. Packed blood cell volume of animals 3 and 4 during Experiment 4. Period 1 environmental temperature was 74°F and measurements were made on 1, 2, 3, and 4 days before cold exposure. Period 2 environmental temperature was -25°F and measurements were made on days 1, 2, 3, and 4. Period 3 environmental temperature was 74°F and measurements were made on days 1, 2, and 3 (see Table 6).

Figure 30. Packed blood cell volume of animals 2 and 3 during Experiment 5. Period 1 environmental temperature was 33°F and measurements were made 1 and 2 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, 3, 5, 6, and 7. Period 3 environmental temperature was 33°F and measurements were made on days 1, 2, 3, 4, and 5 (see Table 7).

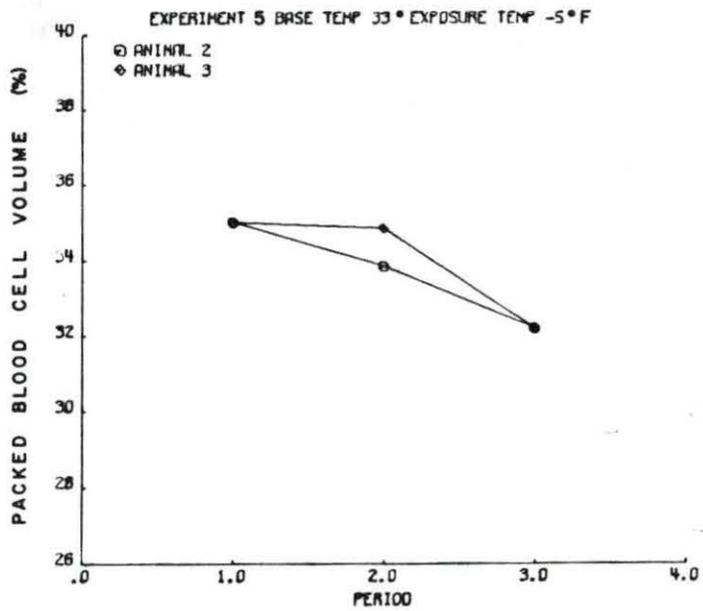
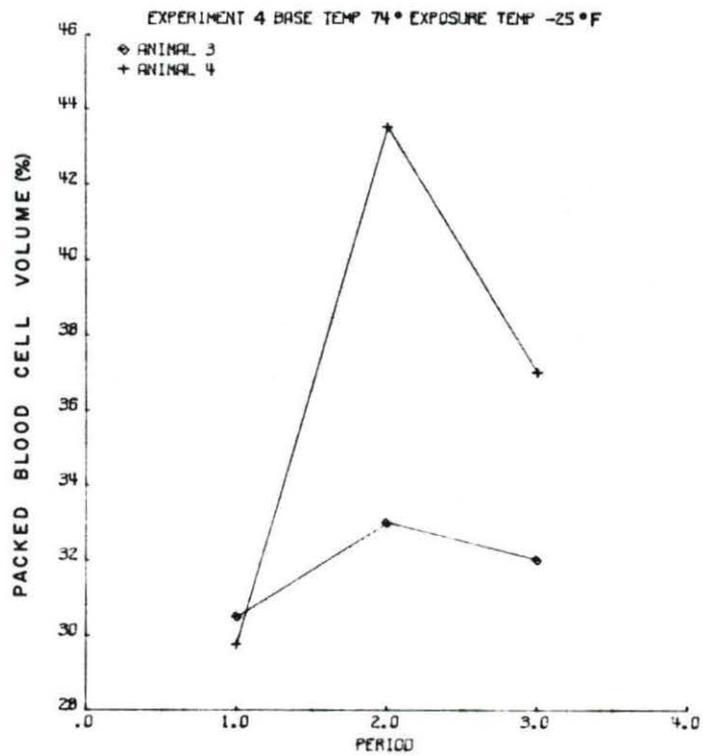


Figure 31. Packed blood cell volume of animals 5, 6, 7, and 8 during Experiment 6. Period 1 environmental temperature was 33°F and measurements were made 1 and 2 days before cold exposure. Period 2 environmental temperature was -12°F and measurements were made on days 1, 2, and 3. Period 3 environmental temperature was -12°F and measurements were made on days 9, 11, 12, 13, and 14. Period 4 environmental temperature was 33°F and measurements were made on days 1, 2, 4, 5, and 6 (see Table 8). Cow 8 died on day 13 of cold exposure.

Figure 32. Packed blood cell volume of animals 9 and 10 during Experiment 8. Period 1 environmental temperature was 33°F and measurements were made 1 and 2 days before cold exposure. Period 2 environmental temperature was -20°F and measurements were made on days 1 and 2. Period 3 environmental temperature was -20°F and measurements were made on days 4 and 5. Period 4 environmental temperature was -20°F and measurements were made on days 8 and 9 (see Table 10). Cow 10 died on day 10 of cold exposure.

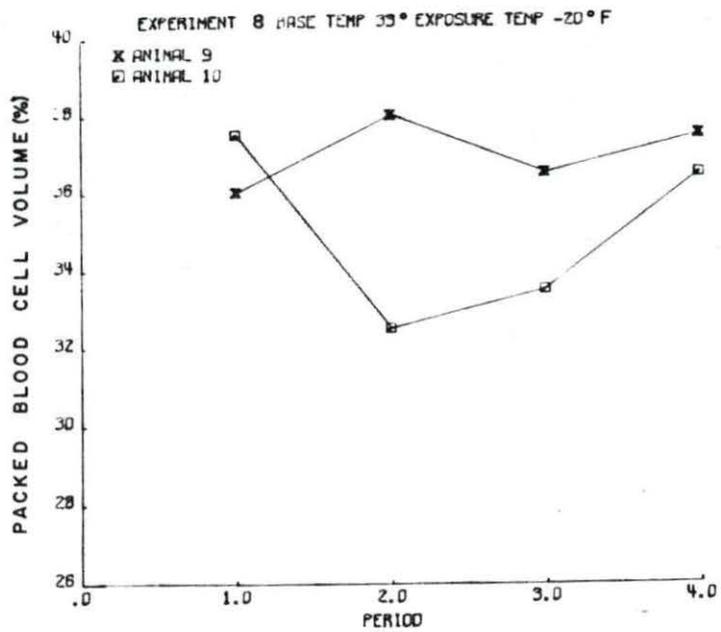
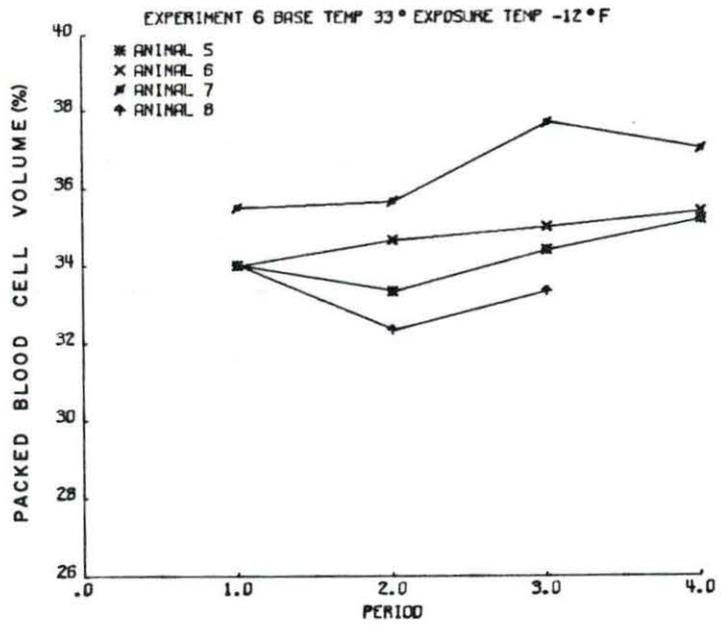


Figure 33. Blood plasma potassium of animals 5, 6, 7, and 8 during Experiment 1. Period 1 environmental temperature was 74°F and measurements were made 1, 2, and 3 days before cold exposure. Period 2 environmental temperature was 33°F and measurements were made on days 8, 10, 11, and 13. Period 3 environmental temperature was 33°F and measurements were made on days 18, 19, 20, and 21 (see Table 3).

Figure 34. Blood plasma potassium of animals 1, 2, 3, and 4 during Experiment 2. Three 24-hour cold exposures were made at one-week intervals (see Results). Period 1 environmental temperature was 74°F and reported measurements were made the second day of each week. Period 2 environmental temperature is 0°F and measurements were made on day 4 of each week. Period 3 environmental temperatures are 74°F and measurements were made on day 5 of each week (see Table 4).

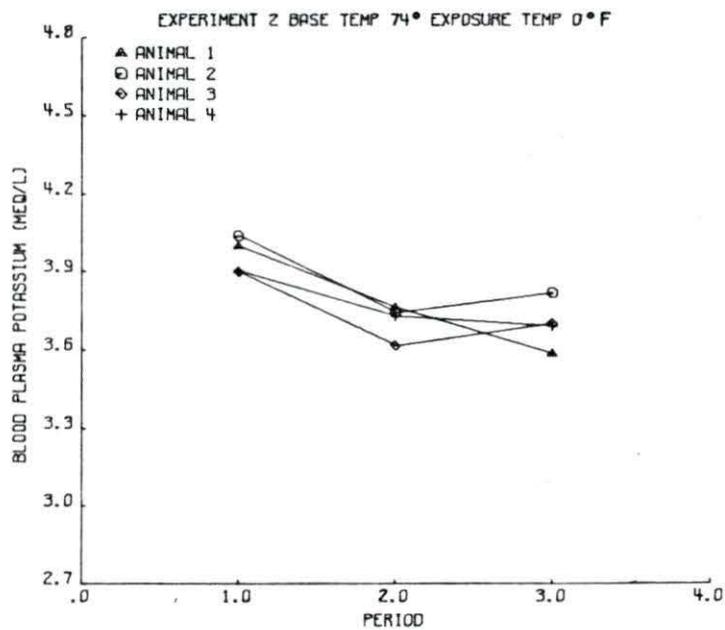
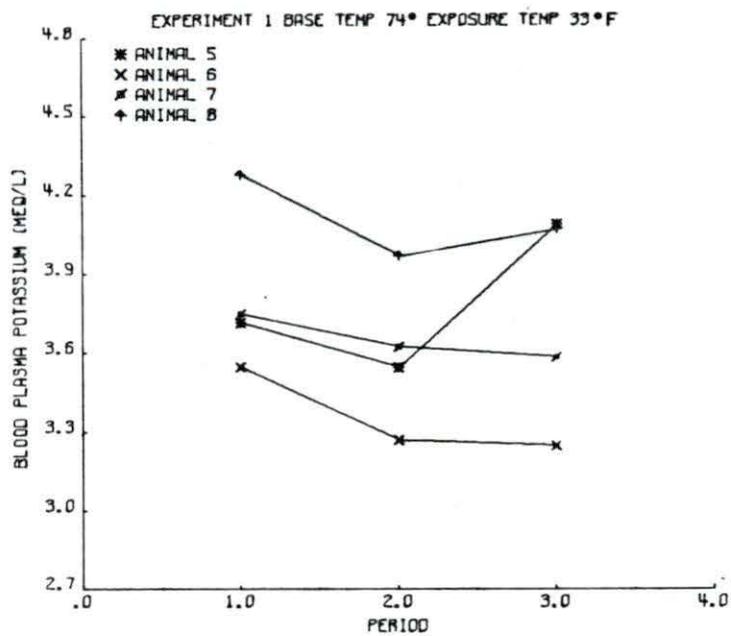


Figure 35. Blood plasma potassium of animals 1 and 2 during Experiment 3. Period 1 environmental temperature was 74°F and measurements were made 1, 2, and 3 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, and 3. Period 3 environmental temperature was 74°F and measurements were made during days 1 and 2 (see Table 5).

Figure 36. Blood plasma potassium of animals 3 and 4 during Experiment 4. Period 1 environmental temperature was 74°F and measurements were made on 1, 2, 3, and 4 days before cold exposure. Period 2 environmental temperature was -25°F and measurements were made on days 1, 2, 3, and 4. Period 3 environmental temperature was 74°F and measurements were made on days 1, 2, and 3 (see Table 6).

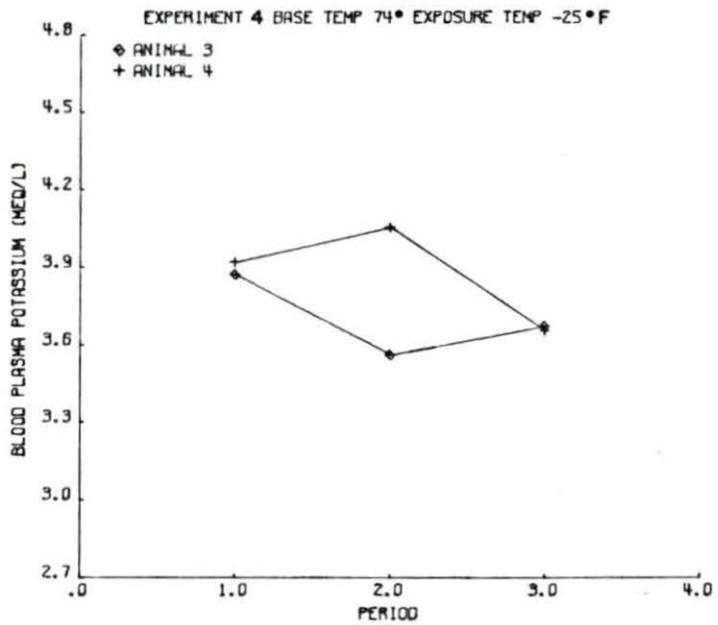
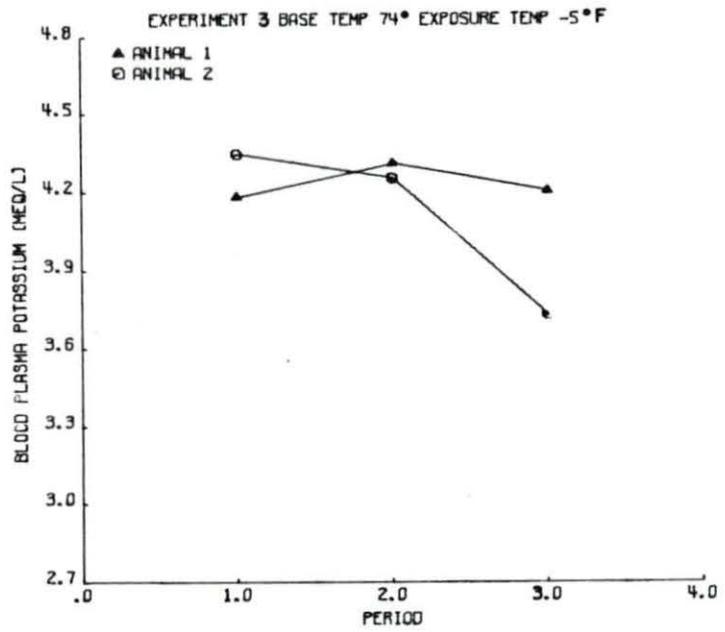


Figure 37. Blood plasma potassium of animals 5, 6, 7, and 8 during Experiment 6. Period 1 environmental temperature was 33°F and measurements were made 1 and 2 days before cold exposure. Period 2 environmental temperature was -12°F and measurements were made on days 1, 2, and 3. Period 3 environmental temperature was -12°F and measurements were made on days 9, 11, 12, 13, and 14. Period 4 environmental temperature was 33°F and measurements were made on days 1, 2, 4, 5, and 6 (see Table 8). Cow 8 died on day 13 of cold exposure.

Figure 38. Blood plasma potassium of animals 9 and 10 during Experiment 8. Period 1 environmental temperature was 33°F and measurements were made 1 and 2 days before cold exposure. Period 2 environmental temperature was -20°F and measurements were made on days 1 and 2. Period 3 environmental temperature was -20°F and measurements were made on days 4 and 5. Period 4 environmental temperature was -20°F and measurements were made on days 8 and 9 (see Table 10). Cow 10 died on day 10 of cold exposure.

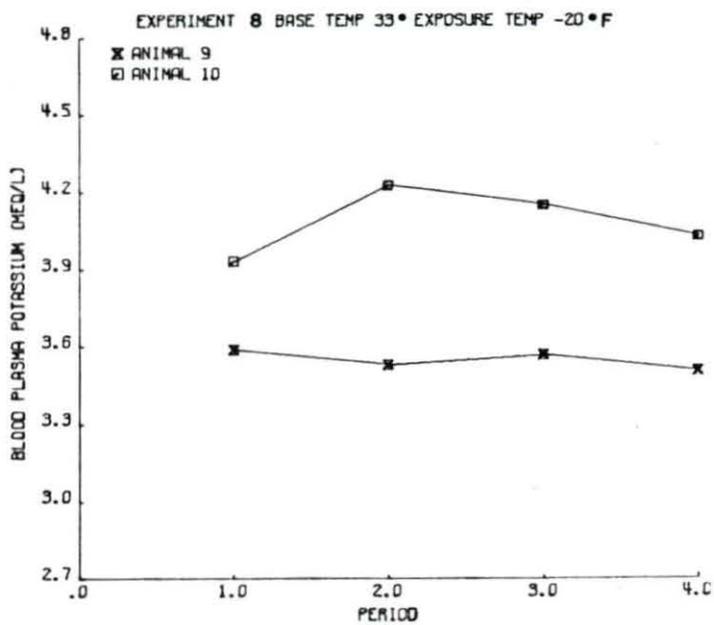
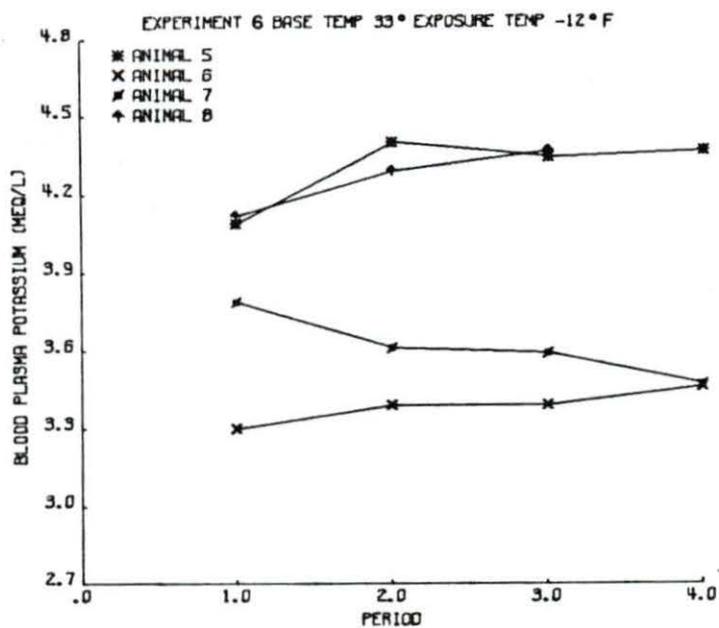


Figure 39. Blood plasma sodium of animals 5, 6, 7, and 8 during Experiment 1. Period 1 environmental temperature was 74°F and measurements were made 1, 2, and 3 days before cold exposure. Period 2 environmental temperature was 33°F and measurements were made on days 8, 10, 11, and 13. Period 3 environmental temperature was 33°F and measurements were made on days 18, 19, 20, and 21 (see Table 3).

Figure 40. Blood plasma sodium of animals 1, 2, 3, and 4 during Experiment 2. Three 24-hour cold exposures were made at one-week intervals (see Results). Period 1 environmental temperature was 74°F and reported measurements were made the second day of each week. Period 2 environmental temperature is 0°F and measurements were made on day 4 of each week. Period 3 environmental temperatures are 74°F and measurements were made on day 5 of each week (see Table 4).

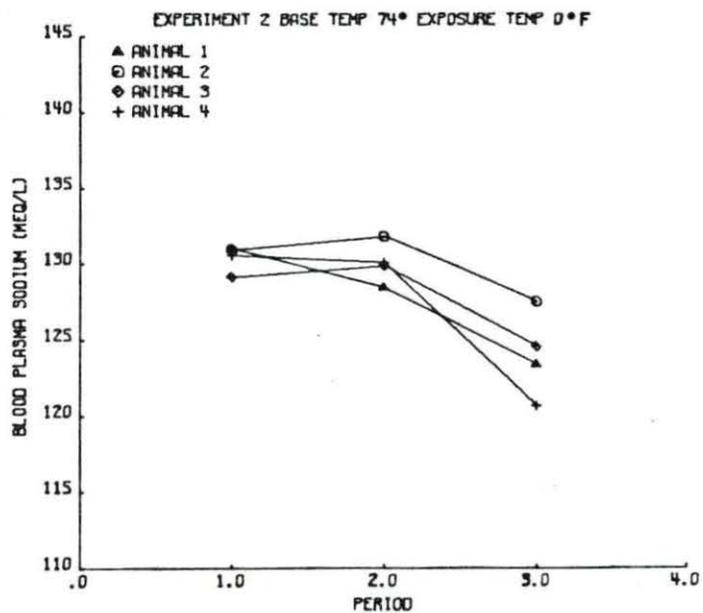
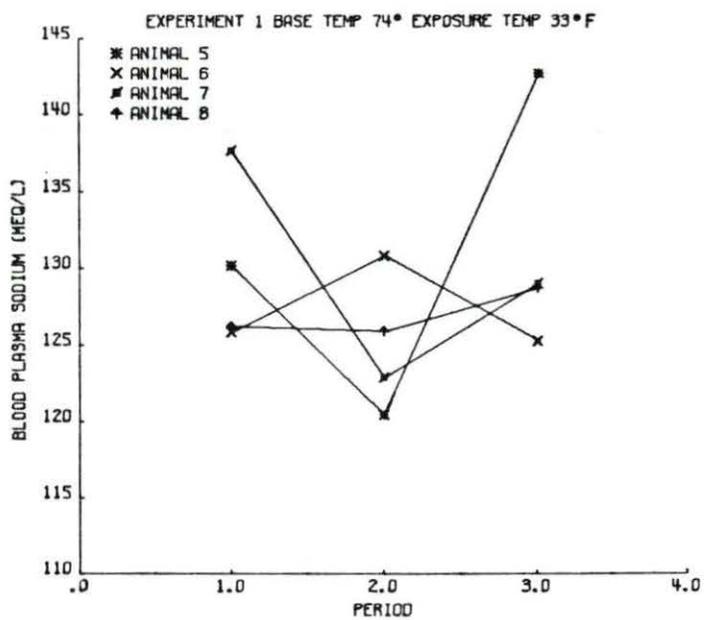


Figure 41. Blood plasma sodium of animals 1 and 2 during Experiment 3. Period 1 environmental temperature was 74°F and measurements were made 1, 2, and 3 days before cold exposure. Period 2 environmental temperature was -5°F and measurements were made during days 1, 2, and 3. Period 3 environmental temperature was 74°F and measurements were made during days 1 and 2 (see Table 5).

Figure 42. Blood plasma sodium of animals 3 and 4 during Experiment 4. Period 1 environmental temperature was 74°F and measurements were made on 1, 2, 3, and 4 days before cold exposure. Period 2 environmental temperature was -25°F and measurements were made on days 1, 2, 3, and 4. Period 3 environmental temperature was 74°F and measurements were made on days 1, 2, and 3 (see Table 6).

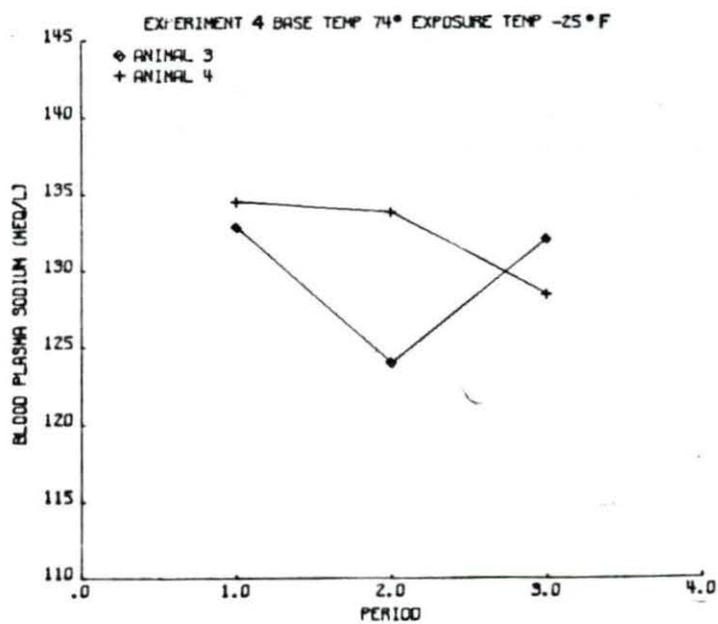
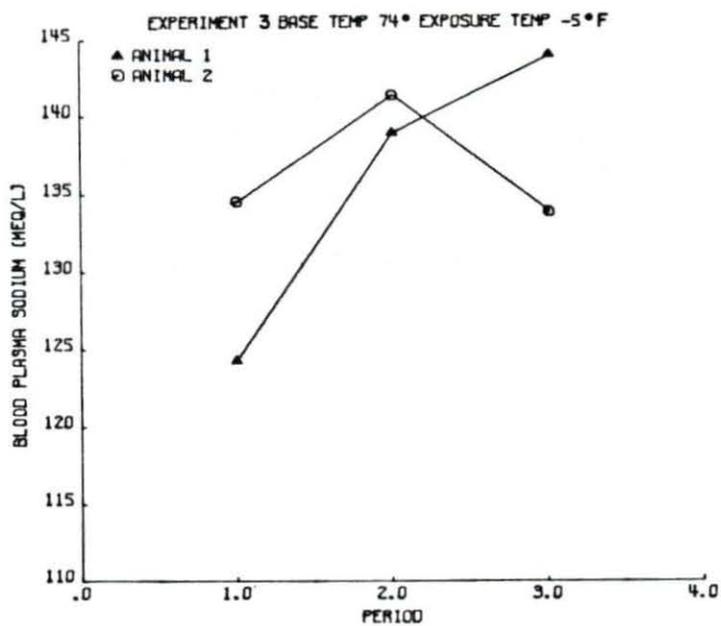


Figure 43. Blood plasma sodium of animals 5, 6, 7, and 8 during Experiment 6. Period 1 environmental temperature was 33°F and measurements were made 1 and 2 days before cold exposure. Period 2 environmental temperature was -12°F and measurements were made on days 1, 2, and 3. Period 3 environmental temperature was -12°F and measurements were made on days 9, 11, 12, 13, and 14. Period 4 environmental temperature was 33°F and measurements were made on days 1, 2, 4, 5, and 6 (see Table 8). Cow 8 died on day 13 of cold exposure.

Figure 44. Blood plasma sodium of animals 9 and 10 during Experiment 8. Period 1 environmental temperature was 33°F and measurements were made 1 and 2 days before cold exposure. Period 2 environmental temperature was -20°F and measurements were made on days 1 and 2. Period 3 environmental temperature was -20°F and measurements were made on days 4 and 5. Period 4 environmental temperature was -20°F and measurements were made on days 8 and 9 (see Table 10). Cow 10 died on day 10 of cold exposure.

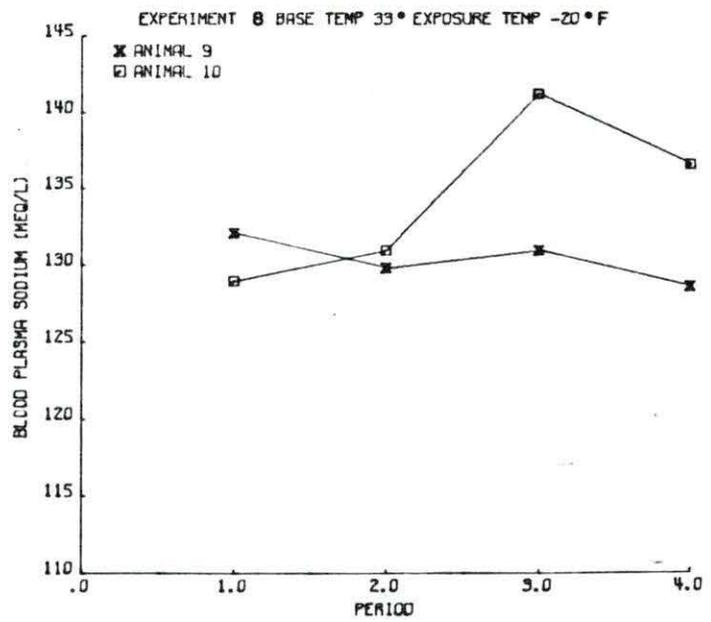
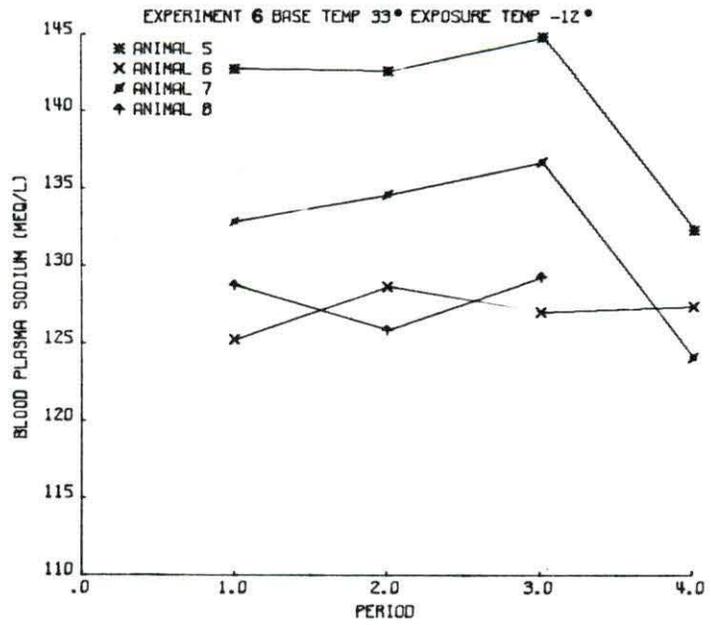


Figure 45. Surgical site after arterial catheterization.

Figure 46. Surgical isolation of the caudal branch of the circumflex iliac artery.

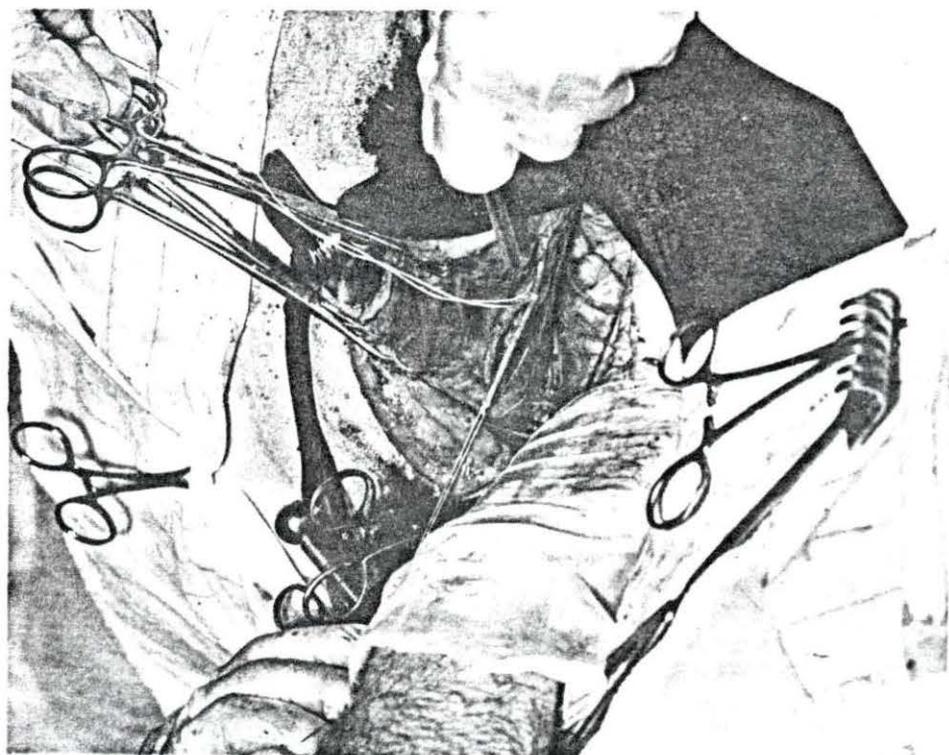
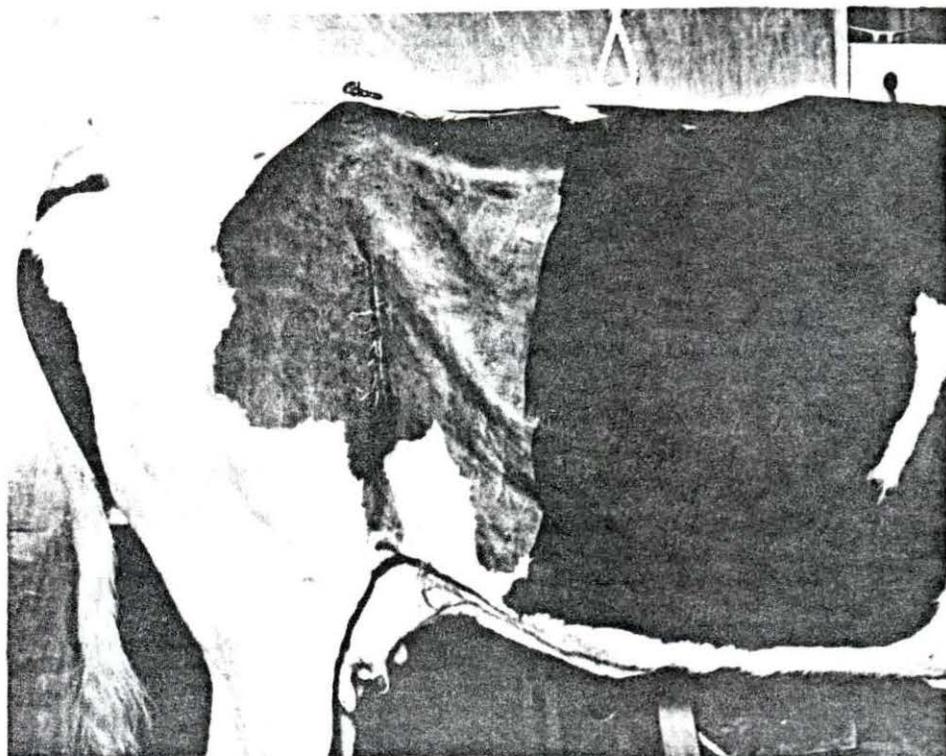
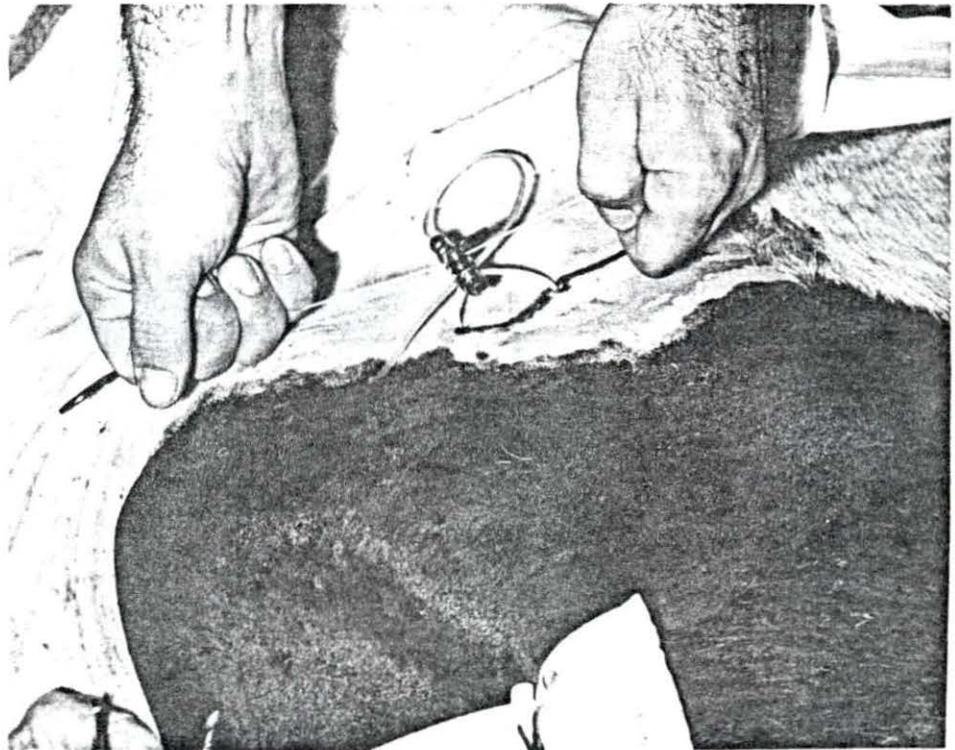
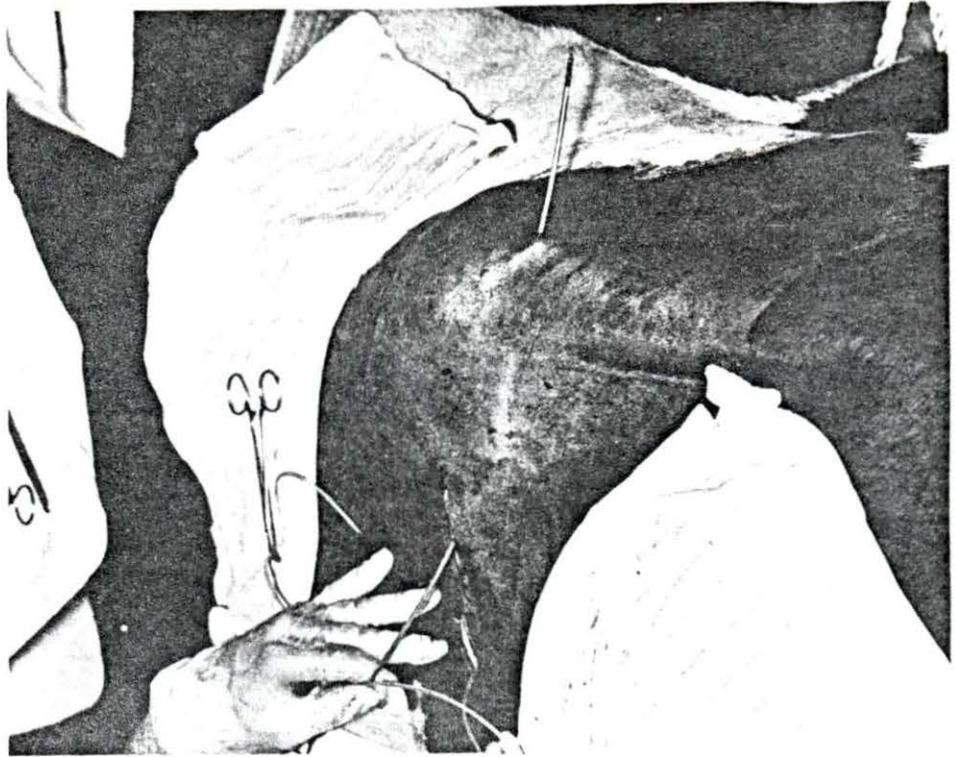


Figure 47. Procedure for placing the catheter beneath the skin.

Figure 48. Procedure for fastening the adaptor and valve to the skin.



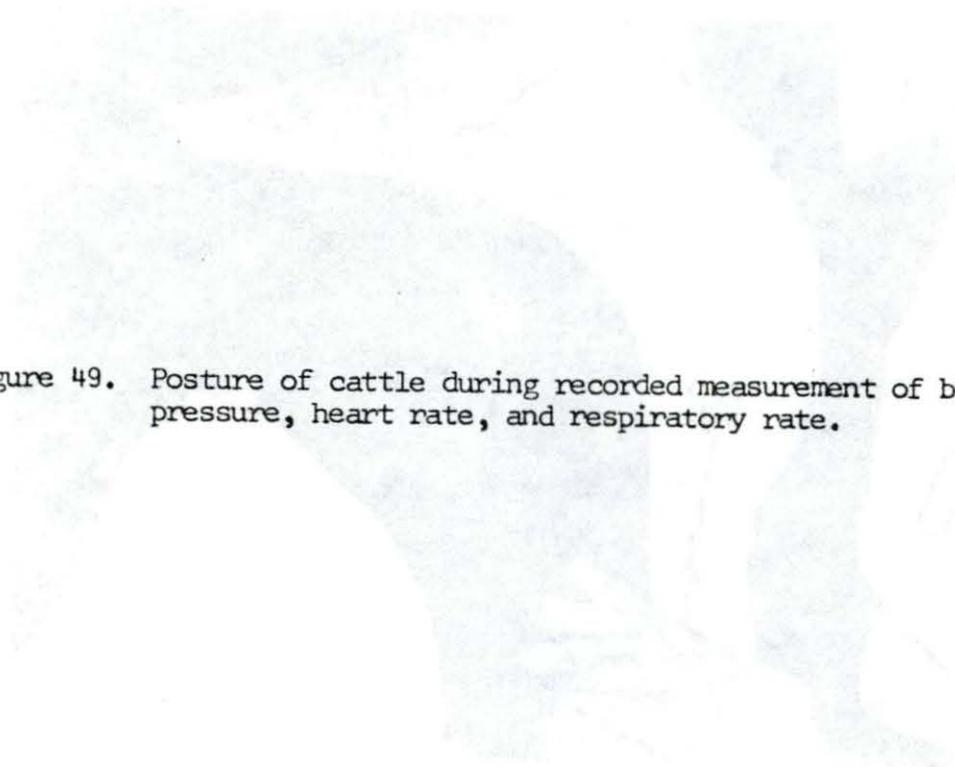


Figure 49. Posture of cattle during recorded measurement of blood pressure, heart rate, and respiratory rate.

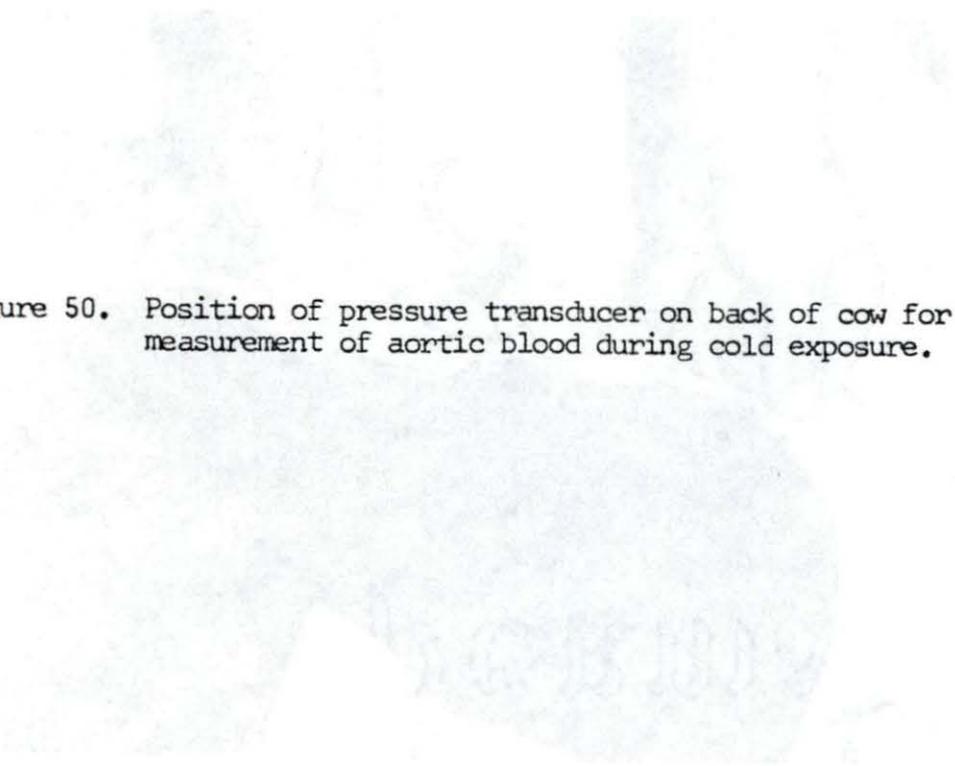


Figure 50. Position of pressure transducer on back of cow for direct measurement of aortic blood during cold exposure.

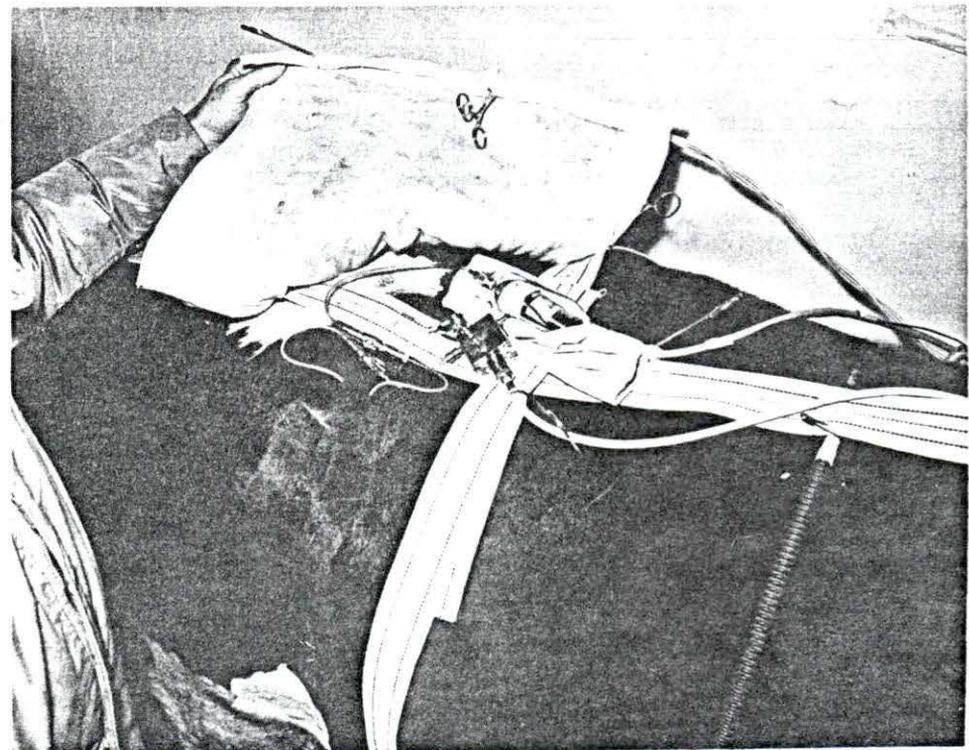


Figure 51. Tracing of the aortic blood pressure of a cow using a permanent catheter.

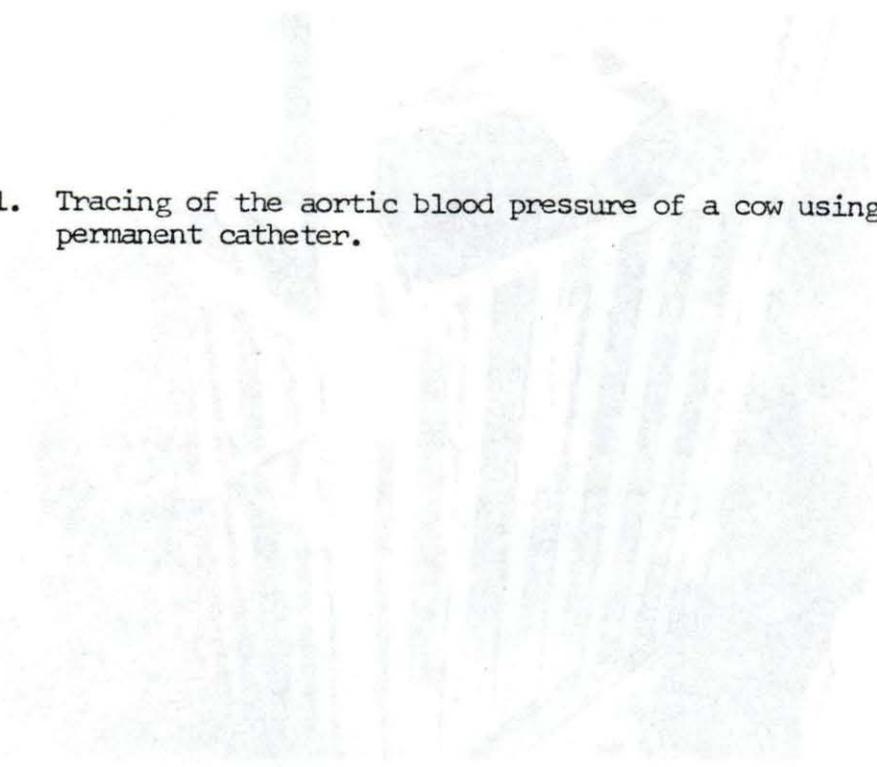


Figure 52. Electronic instruments used during the measurement and recording of physiological functions and temperature. A. Sanborn Model 769 Viso-scope. B. Sanborn Model 350 oscillographic recording system. C. Honeywell multipoint potentiometric recorder. D. Variac.

