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THE UNIVERSITY OF ALBERTA

PHYSIOLOGICAL AND METABOLIC RESPONSES OF SHEEP TO ACUTE AND CHRONIC COLD EXPOSURE

BY

JAMES HUBERT HETTMAN

A THESIS
SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Physiological and Metabolic Responses of Sheep to Acute and Chronic Cold Exposure" submitted by James Hubert Heitman, B.S., in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Animal Physiology.

Supervisor

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ABSTRACT

Experiments were conducted with sheep to investigate the nature and extent of changes in energy metabolism, some blood constituents, and skin temperatures during acute and chronic cold exposure. The chronic exposures were of two types, constant and fluctuating. The constant cold exposure was used to determine the effects of chronic cold per se while the fluctuating cold exposure was used in an attempt to simulate the fluctuating nature of the outdoor weather during winter. To study the influence of prior thermal history on response to acute, severe cold, groups of sheep were exposed chronically to either constant or fluctuating cold for 6 weeks prior to acute cold exposure. The thermal demands of the two chronic cold environments were calculated on the basis of a preliminary experiment to be approximately equal. Animals of both cold groups appeared to be able to adjust to their respective environments and, with the exception of plasma corticoid concentration, the changes in the measured blood parameters indicated that there were no differences between the responses of the animals to the two cold environments. The animals in the fluctuating cold group had higher plasma concentrations of the adrenal corticoids than the animals of the other cold group. Animals of both cold groups had significantly higher plasma concentrations of plasma protein bound iodine (PBI) ($p<0.01$) and higher blood hematocrits ($p<0.001$) than the control animals. There was no significant difference between the animals of the two cold groups for either measurement.

Significant increases ($p<0.01$) occurred in metabolic rate, hematocrits, and in the plasma concentrations of glucose, free-fatty acids (FFA), ketones, and corticoids during acute, severe cold exposure (-28°C). In general, all animals were able to re-establish thermal equilibrium after 1 to 3 hours in the cold. Of the blood

parameters measured, only glucose was significantly ($p<0.05$) increased when wind was imposed on the animals in addition to the cold. A small increase in metabolic rate was also noted. Propranolol, a beta-adrenergic blocking agent, reduced metabolic rate and the plasma concentration of glucose, FFA, and ketones when administered during one series of cold tests.

Prior exposure to chronic cold, whether constant or fluctuating, had no significant effect on the response of sheep to an acute, severe cold stress. The effects of phenoxybenzamine (PBA), an alpha-adrenergic blocking agent administered to each animal prior to one cold test, appeared to reveal a difference in cold adaptation between the animals of the constant cold and fluctuating cold groups. After PBA, only the constant cold animals were able to re-establish thermal equilibrium during acute, severe cold exposure and they did so with a metabolic rate higher than the metabolic rate established during similar cold tests without PBA. Both the fluctuating cold and control groups of animals suffered severe decreases in deep body temperature and their rate of heat production was 12 to 15% less than their rate of heat production during cold tests without PBA.

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I. INTRODUCTION

Adaptation to cold in mammals involves insulative and metabolic adjustments which reduce the intensity of the cold stimulus and enhance the capacity of the animal to respond to cold, respectively. Small mammals, such as the laboratory rat, have limited capacity to alter their thermal insulation. They do, however, exhibit a clearly defined pattern of metabolic acclimation to cold. This involves the gradual development of non-shivering thermogenesis which can be stimulated by exogenous noradrenaline and appears to depend on the presence of brown adipose tissue. Heroux (1963b), however, has drawn attention to differences between the pattern of metabolic adjustments to cold in the rat when induced by continuous exposure to controlled air temperatures in the laboratory or by natural exposure to winter conditions out of doors. These adjustments have been termed cold acclimation and winter acclimatization, respectively.

The sheep, in common with man and other animals of comparable size, does not possess brown adipose tissue, except in the first few days of life, and does not exhibit non-shivering thermogenesis to any significant extent. The extreme cold tolerance of the sheep is derived principally from the thermal insulation provided by the fleece. Adaptation in sheep to chronic cold exposure has previously been studied in this laboratory by exposing shorn animals to air temperatures below 10°C. Such studies showed that growth of the fleece had the effect of reducing the intensity of the cold stimulus and, thus, the magnitude of the metabolic response.

The present study was undertaken to investigate several physiological changes which occur in sheep following cold exposure under conditions whereby the effect of insulative changes on the intensity of the cold stimulus was

circumvented. The responses of the sheep were assessed in two ways. Firstly, the responses were assessed from the changes in the concentrations of certain blood constituents and from the general performance of the sheep measured during the course of a 6 week exposure period to either a constant or fluctuating cold environment. Secondly, the responses were assessed from the metabolic capacity of the animals to respond to cold as tested by acute exposures to a very severe cold condition (about -28°C) for periods of about 6 hours. These acute, severe challenges were augmented on occasion by selective pharmacological blockades of sympathetic alpha and beta receptors using phenoxybenzamine and propranolol, respectively.

The parameters measured and tests performed in this study were similar to measurements and tests which had previously been used in studies of metabolic adaptation to cold in small mammals that exhibit non-shivering thermogenesis. All parameters measured were related to the obvious need for the animals to produce more heat in the cold in order to maintain homeothermy. In the present study the measurements were used to indicate whether or not metabolic acclimation to cold can occur in large mammals and to compare, in a general way, such adaptation that might occur with the metabolic adaptation which is known to occur in small mammals. In order to establish the existence of adaptation per se, it is necessary at first to seek clues from a wide range of possible indices. Initially, it was necessary to examine many of these indices at a rather superficial level. More profound analysis of specific metabolic and endocrinological changes accompanying adaptation can only be justified if it can first be established that it is possible to achieve the necessary environmental conditions to induce metabolic adaptation, uncomplicated by insulative adaptation, and to find unequivocal evidence of a changed pattern of response that has adaptive significance.

II. LITERATURE REVIEW

A. Responses to Cold

1. Introduction

The early literature (prior to about 1954) on the subject of regulation of heat production in cold environments (cold thermoregulation) has been reviewed on several occasions (Burton and Edholm, 1955; von Euler, 1961; Gelineo, 1964; Hardy, 1961; Hart, 1957). Since 1960, there have been several reviews on thermoregulation (Brück, 1970; Carlson, 1960; Chaffee and Roberts, 1971; Davis, 1963, 1969; Hannon, 1963; Hart, 1963; Hemingway, 1963a, b; Heroux, 1969; Schonbaum, Steiner, and Sellers, 1970; Slonim, 1969). In addition, several recent reviews have dealt mainly with the neural control of thermoregulation (Benzinger, 1969; Bligh, 1966; Hammel, 1966, 1968). Himms-Hagen's (1967) review on the sympathetic control of metabolism deals, to some extent, with the control of cold thermogenesis. In view of the thoroughness of the above reviews, only the areas more relevant to the topic of this thesis will be considered herein.

Rubner, in 1902 (cited by Burton and Edholm, 1955), distinguished between physical thermoregulation and chemical thermoregulation. In more descriptive terms, these two types of thermoregulation can be called "control of heat loss" and "control of heat production", respectively. The lower critical temperature of an animal has been defined as the temperature below which metabolism must be increased in order to maintain thermal equilibrium (Scholander et al, 1950). Thus, when animals are exposed to environments with air temperatures below the lower critical temperature of the animal, heat production (Hp) must be increased and regulated according to the thermal demand of the cold environment which is defined as the amount of heat lost by

the animal in that environment (Burton and Edholm, 1955).

2. Control of Heat Production

The metabolic rate of a homeotherm in its normal, thermoneutral environment (hereafter called resting metabolic rate or RMR) is determined by its basal metabolic rate (BMR), metabolism due to muscular activity (minimal), and metabolism due to digesting and metabolizing the ingested food (heat increment of feeding) (Webster, 1967b). Basal metabolic rate is a term which describes the minimal metabolic rate of an animal. The conditions under which BMR is measured are that the animal be at rest in a thermoneutral environment and be in a post-absorptive state. It is difficult to fulfill all of these conditions in ruminants, especially in experiments where a 3 to 5 day fast would interfere with the results. It is convenient to use the term RMR when referring to metabolic rates determined on the fed animal (but not during, or immediately after, a meal) in a thermoneutral environment and with minimal activity.

a. Metabolic Response to Acute and Chronic Cold Exposure

Little information exists concerning the pattern of change in heat production during cold acclimation. Cottle and Carlson (1954) and Sellers and You (1950) described changes in the metabolic rates of rats during a period of cold acclimation. They found that after an initial increase in heat production, there was a period of slower increase that peaked during the first 30 to 40 days of cold exposure. After this time there was a tendency for a small decline in heat production, but for as long as 3 months in the cold, heat production was still much higher than that produced in a thermoneutral environment.

Metabolic Changes Associated with Cold Acclimation

In addition to the increased rate of heat production at the acclimation temperature, heat production at a thermoneutral temperature (previously defined as RMR) is also increased after cold acclimation. This is especially evident in smaller mammals such as rats (Sellers and You, 1950; Heroux et al, 1959; Heroux, 1963b; Hart and Jansky, 1963), hamsters (Adolph and Lawrow, 1951), rabbits (Kockova and Jansky, 1968; Heroux, 1967) and dogs (Gelineo, 1964). There is a great deal of variation in the literature regarding the percentage increase in RMR due to cold acclimation. This is probably due to a great deal of variation between species of animals. However, it appears that the increase in RMR is about 15 to 20%.

Evidence for changes in RMR in sheep during acclimation to cold is scanty. Webster, Hicks, and Hays (1969b) showed that RMR increased slightly in sheep kept in a refrigerated room at an air temperature that provided an intensity of cold comparable to that experienced by sheep kept outdoors during the Canadian winter. Part of this increase in RMR was undoubtedly due to an increased food intake. Sykes and Slee (1968) suggested that RMR may increase in sheep during cold acclimation, but they used measurements of heart rate as an indicator of metabolic rate. Webster (1967a) has shown there can be a close relationship between heart rate and heat production, but he has cautioned that there may be a great deal of variability between animals and that the relationship may hold true only within a certain range.

In most small mammals studied, the relationship between metabolic rate and environmental temperature differs between cold acclimated animals and control animals maintained in a thermoneutral environment. One difference is that cold acclimated animals have a greater heat production

than control animals over a wide range of temperatures. In his review, Hart (1957) found this to be true in all species studied except deer mice. Contrary to the findings of Hart (1957), it was found by Kockova and Jansky (1968) that cold acclimated rabbits had a lower metabolic rate than control animals at all temperatures below the critical temperature of the animals. This occurred in both shaved and unshaved animals and was interpreted by the authors as an indication that rabbits acclimated to cold by increasing both insulation and RMR. A second difference is that the metabolic capacity of cold acclimated animals is greater than that of control animals. This increased metabolic capacity can be demonstrated in two ways. The first is by an increase in the maximum metabolic rate that can be attained and the second is by the ability of an animal to maintain a high rate of metabolism.

Summit metabolism and peak metabolic effort are terms used by Alexander (1962b) and Depocas, Hart and Heroux (1957), respectively, to describe the maximum metabolic rate attained by animals before rectal temperature fell enough to lower metabolic rate. The peak metabolic rate of cold acclimated rats was 50% higher than that of control animals (Depocas et al, 1957). Summit metabolism was greater in cold acclimated lambs than in control animals, although the results were quite variable (Alexander, Bell, and Williams, 1970). Summit metabolism has not been measured in either cold acclimated or control adult sheep. Year-old cold acclimated sheep exposed to an air temperature of about -30°C did not reach summit metabolism (Webster et al, 1969b).

The ability to maintain a high rate of metabolism can be judged by several criteria, including (1) survival time during a severe cold test and (2) magnitude of the fall in deep body temperature during a severe cold test. Several species of cold acclimated animals have shown an increased

ability to maintain a high rate of metabolism compared to control animals as judged by one or both of the above criteria. These species include rabbits (Blair and Dimitroff, 1952; Heroux, 1967), rats (Heroux, 1963a; Sellers, Reichman and Thomas, 1951a), and mice (Hart, 1953; Sealander, 1953). Rats and mice, adopted to intermittent cold exposure, survived longer when tested at -20°C than control rats and mice (LeBlanc, 1967; LeBlanc et al, 1967).

Metabolic Changes Associated with Winter Acclimatization

Most reports indicate that RMR does not differ appreciably between summer and winter in animals living outdoors. Irving, Krog, and Monson (1955, cited by Hart, 1957) found this to be true for the red fox, porcupine, and red squirrel. The red fox and porcupine did show an increase in total insulation which was accompanied by a fall in critical temperature. White rats left outdoors during the winter were found to have a RMR similar to comparable animals left outdoors during the summer (Heroux et al, 1959). Hart and Heroux (1955) found a lower RMR in winter acclimatized lemmings compared to lemmings studied during the summer. However, a decrease in critical temperature of the winter acclimatized lemmings was accompanied by an increase in insulation. In 1953, Hart and Heroux found no difference in RMR between deer mice in the summer and deer mice in the winter, although they had lower critical temperatures in the winter than in the summer. The RMR of sheep kept outdoors during the winter steadily increased during the experimental period lasting from November to March (Webster et al, 1969b). Webster, Chlumecky and Young (1970) concluded that cattle exposed to the winter showed some adjustments in RMR which were attributable to the cold weather. Even though it appears that there is little or no adjustment in the RMR of several species of winter acclimatized mammals, increases in insulation enable

these animals to respond less, metabolically, to air temperatures below their lower critical temperatures.

There are very few reports indicating an increase in the metabolic capacity of winter acclimatized animals. Judging from their survival time at a very low temperature, mice (Hart and Heroux, 1953; Sealander, 1951), white rats (Heroux et al, 1959), and wild rats (Heroux, 1962) have been shown to be more cold resistant during the winter than during the summer.

b. Shivering and Non-Shivering Thermogenesis

The sources of cold thermogenesis are: (1) gross muscular activity, (2) shivering, and (3) non-shivering thermogenesis (NST). Energy expended in gross muscular activity results in the release of heat, and metabolic rate can be increased ten to twenty times during vigorous exercise (Hemingway, 1963a). Exercise thermogenesis can partially substitute for shivering thermogenesis in cold exposed rats. However, shivering alone is more efficient in maintaining deep body temperature (Hart and Jansky, 1963).

Shivering involves involuntary rhythmic contractions of skeletal muscles. It is a type of muscular activity in which no external work is done (Blaxter, 1967). Shivering has been found to increase oxygen consumption in armadillos by 500%. This appears to be the maximum increase in metabolism in homeotherms due to shivering (Iampietro et al, 1960).

Non-shivering thermogenesis can be defined as the increment in rate of heat production caused by cold exposure and not accounted for by shivering. Non-shivering cold thermogenesis is developed during chronic cold exposure and several tissues may contribute to it. When maximum heat production is needed in the cold acclimated white rat, NST

appears to be additive to shivering thermogenesis (Jansky, 1966).

For a long time there was no real proof that the increased heat production in the cold was due to anything but shivering thermogenesis. You and Sellers (1951) showed that there was an increased *in vitro* oxygen consumption by livers from cold acclimated rats compared to livers from control animals. In 1954, Sellers, Scott, and Thomas found that cold exposed, cold acclimated rats shivered much less than cold exposed control animals. These authors interpreted the results of both of the above reports to mean that metabolism of visceral tissues plays an important role in maintaining the increased heat production in cold acclimated rats. A reduction in shivering has since been noted in cold acclimated rats (Depocas, 1958; Hart and Jansky, 1963). A progressive decline in shivering during the acclimation period was noted in rats (Hart, Heroux, and Depocas, 1956) and recently in rabbits (Heroux, 1967). Winter acclimatized wild rats (Heroux, 1962) and winter acclimatized white rats (Heroux *et al*, 1959) have also been found to exhibit less shivering than summer acclimatized animals during severe cold tests.

Proof that the heat production of visceral tissues could be increased during cold exposure came when W. Cottle and Carlson (1956) first demonstrated that curarized, cold acclimated rats could increase their heat production enough to nearly maintain their deep body temperature during 2 hours of cold exposure. Curarized control animals could not increase their heat production during cold exposure and their deep body temperature dropped markedly. This observation has since been noted by Depocas (1958) and Davis *et al* (1960). In all of these experiments, curare inhibited muscle activity due to its action as a neuromuscular blocking agent.

An increased calorigenic response to noradrenaline by cold acclimated rats was first observed by Hsieh and Carlson (1957). Others have since confirmed this observation (Depocas, 1960; Evonuk and Hannon, 1963; Heroux, 1961, 1963a, 1967; Jansky, Bartunkova, and Zeisberger, 1967; Le Blanc et al, 1967). Noradrenaline induced thermogenesis in cold adapted animals has also been observed in winter populations of wild Norway rats (Heroux, 1962) and in white rats kept under outdoor conditions (Heroux, 1961). However, rats adapted to short-term cold exposure did not respond to noradrenaline, even though they did exhibit signs of cold acclimation as shown by increased survival time at very cold temperatures (LeBlanc, 1967).

Noradrenaline has a calorigenic action in cold acclimated adults of species other than rats. A response in rabbits has been found by several workers. Cottle (1963) and Judy (1966, cited by Hayward, 1968) used unshaved rabbits and the increases were small. Heroux (1967) and Kockova and Jansky (1968) used shaved rabbits and obtained a much larger increase in noradrenaline induced thermogenesis. Hemingway, Price, and Stuart (1964) demonstrated a moderate noradrenaline induced heat production in cold acclimated cats. Modest increases have also been noted in humans (Joy, 1963; Budd and Warhaft, 1966), guinea pigs (Jansky et al, 1969), and white mice (Jansky et al, 1969). Some other species respond to noradrenaline in both the warm and cold acclimated state. These include ground squirrels (Pohl and Hart, 1965), hedgehogs (Jansky et al, 1969), golden hamsters (Cassuto and Amit, 1968), and dogs (Nagasaka and Carlson, 1965). Noradrenaline induced NST was not found in adult sheep (Webster et al, 1969a), while in the new-born lamb it was found to occur (Alexander and Williams, 1968; Thompson and Jenkinson, 1969). The effect of noradrenaline on new-born mammals will be discussed later. Much

evidence exists for a link between NST and the sympathetic nervous system, besides the increased calorigenic response to noradrenaline.

W. Cottle and Carlson (1956) showed that adrenal demedullation caused a reduction in the metabolic rate of cold acclimated, curarized rats in the cold. In another investigation, Maikel et al (1967) showed a marked reduction in cold resistance of adrenal demedullated, warm acclimated rats. Mean survival time at 4°C was reduced from longer than 24 hours for intact animals to 11 hours for demedullated animals. The only other change in a measured parameter was a prevention of the increase in plasma glucose in the demedullated animals. An injection of adrenaline prior to cold exposure reversed the deleterious effects of adrenal demedullation (Maikel et al, 1967).

Use of sympathetic blocking agents has provided information regarding the necessity of the sympathetic nervous system in the response to cold. Hsieh, Carlson, and Gray (1957) found that hexamethonium, a ganglionic blocking drug, and piperoxan, an adrenolytic agent, markedly depressed the metabolic rate of cold acclimated rats during cold exposure. The effects of these drugs, which interfere with the normal functioning of the sympathetic nervous system and which were used in conjunction with adrenal demedullation, showed that a normal sympathetic nervous system is necessary in cold exposed rats (Maikel et al, 1967). These authors reported a reduction in mean survival time from 11 hours, with demedullation alone, to about 3 hours with a combination of demedullation and chemical sympathectomy. This last treatment also reduced glucose and free-fatty acid mobilization, as well as appearing to inhibit shivering and vasoconstriction (Maikel et al, 1967). In both of the above studies, administration of exogenous catecholamines partially or wholly reversed the effects of the sympathetic blocking

drugs. Propranolol and pronethalol, drugs which block the beta-adrenergic receptors, have been used to study the control of cold thermogenesis. These two drugs have been shown to reduce cold thermogenesis in mice (Estler and Ammon, 1969), rabbits (Heim and Hull, 1966), and guinea pigs (Bruck, 1970). Only a small reduction in cold thermogenesis occurred in adult sheep (Webster et al, 1969a). With the exception of Webster et al (1969a) and Alexander and Williams (1968), the above authors have assumed that propranolol and pronethalol influence thermogenesis by inhibiting the portion of total thermogenesis that is NST. However, propranolol did appear to depress shivering in the young lamb (Alexander and Williams, 1968). Administration of alpha-adrenergic blocking agents, such as phenoxybenzamine and phentolamine, has been shown to have deleterious effects on animals during cold exposure. Alexander and Williams (1968) found a reduction in the cold thermogenesis of young lambs after administration of either drug. Johnson and Sellers (1961) found that phenoxybenzamine reduced noradrenaline induced thermogenesis in rats. Leduc (1961) found that all rats died when exposed to cold after phenoxybenzamine was administered. Cold acclimated rats survived for several days, whereas warm acclimated rats survived for less than 1 day. In older sheep, which do not appear to respond to noradrenaline, phenoxybenzamine reduced the heat production of control animals in the cold (Webster et al, 1969a). Cold acclimated sheep responded with a higher metabolic rate in the cold after phenoxybenzamine was given, than in similar trials without the drug. A higher metabolic rate was apparently necessary to compensate for a reduction in tissue insulation due to the blockade of vasoconstriction by phenoxybenzamine and the subsequent vasodilation which occurred (judged from skin temperature changes).

Further evidence for the link between NST and the sympathetic nervous system is the increased urinary excretion

of catecholamines and their metabolites by cold stressed rats (Cottle, 1960; Johnson, Schonbaum and Sellers, 1966; Leduc, 1961; LeBlanc and Nadeau, 1961; Shum, Johnson and Flattery, 1969). Catecholamine excretion increased in cold stressed sheep (Webster et al, 1969a), and it appeared that there was a direct relationship between increases in excretion and increases in the intensity of the cold stress. This would suggest that catecholamines have a role in cold thermogenesis.

The relationship between the sympathetic nervous system and NST in rats is further demonstrated by the influence of temperature of adaptation, length of time in the cold, and ambient temperature during infusion of noradrenaline on the metabolic response of rats to noradrenaline. It has been demonstrated that the calorigenic response of cold acclimated rats to noradrenaline increases as the duration of cold exposure increases (Depocas, 1960; Himms-Hagen, 1969; Jansky et al, 1967). These authors found that a maximal response was obtained after 30 to 40 days of cold acclimation. The capacity of rats to respond to infused noradrenaline, which develops with time of exposure to cold, parallels the time course of disappearance of shivering and also the increase in food consumption, survival time in severe cold, and resistance to body cooling, all of which occur during cold acclimation (Depocas, 1960).

Jansky et al (1967) found that the metabolic response of cold acclimated rats to noradrenaline increased as the acclimation temperature decreased. It has also been shown that the metabolic response to noradrenaline by cold acclimated rats (Jansky et al, 1969) and rabbits (Kockova and Jansky, 1968) decreased as ambient temperature decreased. This implies that the thermogenic response to noradrenaline and to cold are the same phenomenon. Jansky (1969) concluded that the metabolic response to noradrenaline can be used as an

indication of the magnitude of NST in rats.

There has been a great deal of controversy over which tissues contribute to non-shivering thermogenesis. Functional evisceration did not prevent the immediate increase in oxygen consumption seen in the anaesthetized, cold acclimated rat during cold exposure (Depocas, 1958). Functional evisceration was accomplished by tying off the colon, esophagus, coeliac arteries, superior mesenteric arteries, and the portal vein. This procedure resulted in exclusion of the heat produced by the liver and other abdominal organs (excluding kidneys) from the total heat produced in the cold. Some shivering was recorded, but it was not nearly as much as the shivering exhibited by intact, warm acclimated animals at approximately the same metabolic rate. Curarization did not immediately abolish the metabolic response of eviscerated animals. Depocas (1960) showed that noradrenaline was nearly as effective in increasing the oxygen consumption of the eviscerated, cold acclimated rat as that of sham operated rats. These results tend to suggest that a good part of non-shivering heat production originates in muscle or in brown fat.

Jansky and Hart (1963) found an increase in the oxygen consumption of the leg, but not of the kidney, of curarized, cold acclimated rats during cold exposure. In the same study, noradrenaline caused an increase in the oxygen consumption of the leg, but not of the kidney, in cold acclimated rats. These results would tend to support the suggestion of Depocas (1960) that muscle is the principal site of non-shivering thermogenesis. However, muscle does not appear to account for all of the increased metabolism at very low temperatures (Jansky, 1966). The consensus of opinion appears to be that the contribution of tissues other than muscle and brown adipose tissue (BAT) to non-shivering thermogenesis is small (Jansky, 1966; Hayward, 1967).

Attention was first drawn to BAT as a source of

potential heat in cold exposed rats by Smith (1961). BAT has been found in many species, including the hedgehog, bat, rat, mouse, hamster, guinea pig, ground squirrel, golden-mantled squirrel, chipmunk, marmot, lemming, and in the newborn of the rabbit, cat, monkey, man, and sheep (Afzelius, 1970). In at least the rat and guinea pig, the amount of BAT decreases with age (Afzelius, 1970).

The responsiveness of different species to the calorigenic effects of noradrenaline is highly correlated with the amount of BAT present. Thus, in the new-born lamb there is a metabolic response to noradrenaline (Thompson and Jenkinson, 1969), while in the adult there is not (Webster et al, 1969a). The calorigenic effect of noradrenaline has been shown to decrease with age in rats (Himms-Hagen and Hagen, 1964) and in guinea pigs, humans, and cats (Bruck, 1970). Cold acclimation will cause an increase in the amount of BAT present and prolong the postnatal disappearance of BAT (Bruck 1970; Barnard and Skala, 1970).

Surgical removal of BAT from animals markedly reduces the response to noradrenaline. This has been shown in the rabbit (Hull and Segall, 1965) and in the rat (Leduc and Rivest, 1969, cited by Himms-Hagen, 1969). Leduc and Rivest (1969, cited by Himms-Hagen, 1969) also reported that removal of BAT from cold acclimated rats resulted in a decrease in cold resistance. Himms-Hagen (1969) has shown that removal of interscapular BAT from mice results in a progressive loss of response to noradrenaline and adrenaline. This suggests that BAT exerts its influence on NST indirectly, since the decreased metabolic response to noradrenaline cannot be accounted for by the loss in the heat production of BAT (Chaffee and Roberts, 1971). Himms-Hagen (1969) suggested that, although BAT is not present in sufficient mass to make a major contribution to the calorigenic response to noradrenaline in the cold acclimated rat, it serves as an endocrine

gland whose secretory product modifies the ability of other tissues to respond calorigenically to catecholamines. This hypothesis is highly speculative. Recent evidence found by Hayward and Davies (1972) supports the theory of a secretory function or some other mediatory role of BAT. Bruck (1970) suggested that BAT has an important local effect since it is situated around vital organs. He postulates that the intense heat produced by BAT suppresses shivering by warming thermal receptors in the spinal cord, which would then allow NST to develop.

Although there is controversy concerning sites of non-shivering heat production, it can probably be said that several tissues participate to some degree in NST. BAT confers a special type of NST as it appears to be necessary for the calorigenic response to noradrenaline by cold acclimated animals. The presence of BAT in the newborn of some species may improve their cold tolerance at birth compared to newborn without BAT.

Noradrenaline induced NST has not been demonstrated to any significant extent in species which do not possess brown adipose tissue. Thus, it is probably true that the quantitative importance of NST to adult sheep is very small, as they possess no BAT.

3. Endocrine Changes

a. Adrenal Cortical Hormones
Acute Cold Exposure

The importance of adrenal cortical hormones to animals during severe cold stress has been recognized for a long time. In 1942, Tyslowitz and Astwood observed that adrenalectomized rats were unable to maintain normal body temperature when exposed to a cold environment. This observation has since been confirmed by others (Fregly, 1960; Sellers, You, and Thomas, 1951b). Heroux (1955) demonstrated

that adrenalectomized rats did not survive as long in severe cold as intact animals.

Many studies tend to indicate that adrenal cortical activity is increased during initial exposure to a cold stress. These studies have used a number of indices to indicate an increase in adrenal cortical activity. Most studies have been performed on white rats. These indices include: (1) increased plasma concentration of glucocorticoids (Boulouard, 1963, 1966), (2) increased adrenal gland weight (Heroux and Schonbaum, 1959; Straw and Frogly, 1967), (3) depletion of ascorbic acid from the adrenal gland (Booker, 1960; Hoijer, 1960; Schonbaum, Casselman, and Large, 1959), (4) a fall in blood eosinophil count (Heroux and Hart, 1954; Speirs and Meyer, 1949), (5) an increased in vitro production of glucocorticoids by rat adrenals (Schonbaum et al, 1959), and (6) an increased urinary excretion of glucocorticoid breakdown products (Munday and Blane, 1960).

Few studies have been made with large animals. An increase in the plasma concentration of glucocorticoids has been found in cold stressed sheep (Panaretto and Vickery, 1970; Reid, 1962). In addition, Panaretto and Vickery (1970) reported that entry rates of adrenal glucocorticoids into the circulation were increased three to fourfold in cold exposed sheep compared to entry rates determined on sheep at a thermoneutral temperature.

Cold Acclimation

While the evidence certainly suggests that there is increased adrenal cortical activity during the early stages of cold exposure, it appears that after prolonged exposure to cold, adrenal activity returns to normal. Several of the indices of adrenal activity which were previously found to indicate increased adrenal activity, have been found to return to normal after cold acclimation. These include: (1) eosinophil counts (Heroux and Hart, 1954), (2) plasma

concentration of glucocorticoids (Boulouard, 1966), and (3) *in vitro* secretion rate of glucocorticoids paralleled the disappearance of shivering. The above studies suggest that increased adrenal cortical activity is not necessary for the maintenance of non-shivering thermogenesis and that one criterion for the presence of NST might be the return to normal adrenal activity.

Winter Acclimatization

Although there is only a little evidence available to suggest it, winter acclimatization appears to involve a different adrenal response than cold acclimation. The *in vitro* secretion rates of adrenal glucocorticoids by adrenals from wild Norway rats (Willmer and Heroux, 1963) and white rats (Heroux and Schonbaum, 1959) were greater in winter acclimatized animals than in animals studied during the summer. Yousef, Cameron, and Luick (1971) found a higher plasma concentration of cortisol and a greater cortisol secretion rate in reindeer studied during the winter than when studied during the summer. These studies suggest that the stress caused by the fluctuating nature of winter weather is different than that caused by prolonged exposure to a steady cold.

Selye (1936) found a common pattern of response to several stressors, one aspect of the pattern being increased glucocorticoid secretion. In his studies of the general adaptation syndrome, as the pattern of response to these stressors was called, Selye (1936) found that cold was an ideal stressor. If it can be assumed that any stressful cold results in increased secretion of glucocorticoids, one possible explanation of the normal adrenal cortical activity following chronic cold exposure might be that after cold acclimation has developed, moderate cold exposure is no longer a stressor and more glucocorticoids are not needed. Exposure to cold winter climate might result in a continual stress because of the fluctuating nature of the weather and, thus, a greater secretion of glucocorticoids in winter acclimatized animals.

Since little information is available on the adrenal response to cold stress in large animals, one criterion of adrenal cortical activity, that of plasma concentration, was measured in the present study.

b. Thyroid Hormone

Cold Acclimation

A great volume of literature exists which suggests that one response to cold is an increase in thyroid gland activity. At least part of the increased metabolic rate of cold acclimated animals at a thermoneutral temperature has been ascribed to an increased secretion of thyroxine (Adolph, 1950; Sellers and You, 1950), because of the well known metabolic effect of thyroxine (Rall, Robbins, and Lewalen, 1964; Whaley, Hart, and Klitgaard, 1959).

Several indices have been studied which suggest an increased thyroid activity in cold acclimated small mammals. These indices include: (1) an increase in the thyroid gland weight of rats (Rand, Riggs, and Talbot, 1952; Straw and Fregly, 1967) and hamsters (Knigge, 1963), (2) an increase in the rate of uptake and release of labelled iodine by the thyroid gland of rats (Cottle, M. and Carlson, 1956; Straw and Fregly, 1967), (3) an increase in the amount of thyroxine needed for replacement therapy after thyroidectomy (Woods and Carlson, 1956), (4) an increase in the amount of exogenous thyroxine needed to block the release of labelled iodine from the thyroid gland of rats (Heroux, 1969), (5) an increase in the rate of disappearance of labelled thyroxine from the blood of rats (Cottle, M. and Carlson, 1956; Gregerman and Growder, 1963), (6) an increase in the thyroxine secretion rate of rats (Bauman and Turner, 1967), and (7) an increase in turnover of thyroxine in rats (Gregerman and Growder, 1963).

There are very few reports on thyroid activity in

cold acclimated large animals. Yousef, Kibler, and Johnson (1967) reported a faster rate of disappearance of labelled thyroxine from the plasma of cattle exposed to 1°C compared to animals exposed to 18°C.

Reports on changes in plasma level of protein bound iodine (PBI) in cold acclimated animals have been variable. Most reports indicate that PBI concentrations remain unchanged or are even lowered in cold acclimated animals (Cadot, Julien, and Chevillard, 1969; Freinkel and Lewis, 1957; Straw and Fregly, 1967). However, Halliday et al (1969) found a 10% increase in PBI levels in cold acclimated sheep compared to control animals and Yousef et al (1967) reported higher PBI levels in cattle exposed to 1°C compared to levels when exposed to 18°C.

There are a few reports in the literature which suggest that thyroid activity is unchanged or even reduced in cold acclimated animals. Heroux (1969) has reviewed these reports and concluded that in spite of these few reports, the great majority of the studies related to thyroid activity during cold exposure indicate that thyroid activity is increased in cold acclimated animals. However, there is some question as to the actual necessity for increased thyroid activity in cold acclimated animals. Sellers and You (1950) found that, although thyroidectomized rats could not survive in the cold for more than a few days, they could survive indefinitely in the cold if given minimal replacement doses of thyroxine. Furthermore, these animals were able to respond metabolically to cold. Sellers and You (1950) interpreted their results as showing that increased metabolism in the cold did not depend on increased amounts of thyroxine, but only on the presence of thyroxine. The report by Hsieh (1966, cited by Chaffee and Roberts, 1971) supports the findings of Sellers and You (1950). Hsieh (1966, cited by Chaffee and Roberts, 1971) found that thyroidectomized, cold acclimated

rats maintained on a small replacement dose of thyroxine, responded to cold exposure with an increase in non-shivering thermogenesis. He concluded that the thyroid gland does not actively control non-shivering thermogenesis.

Evidence is now beginning to accumulate which suggests that the changes in thyroid activity seen in cold exposed animals are diet dependent and are unnecessary to the animals once cold acclimation has taken place (Chaffee and Roberts, 1971). Rats fed commercial lab chows excreted about twice as much thyroxine in their feces as rats fed a synthetic, thyroxine free (T_4-free) diet (Magwood and Heroux, 1967; Sellers et al, 1971). Heroux (1968) found that rats fed a T_4-free diet during acclimation did not show an increased metabolic rate at a thermoneutral temperature and that thyroid weight remained normal. However, these animals increased their cold resistance (Heroux, 1968) and sensitivity to noradrenaline (Heroux and Petrovic, 1969). At this time, the effect of diet and its thyroxine content on the cold resistance of rats appears to be rather confusing. Heroux (1969) suggests that the increased thyroxine secretion may be the indirect result of a greater fecal excretion of thyroxine due to an increased metabolism of all tissue. However, he cautions that the reverse may also be true. In view of the fact that thyroidectomized rats receiving a constant, minimal dose of thyroxine can survive in the cold and even increase their resting metabolism, it seems questionable whether the increased thyroid activity usually found in the intact rat is necessary for the metabolic response to cold and the subsequent development of non-shivering thermogenesis.

Winter Acclimatization

Results from studies on thyroid activity in animals exposed to the outdoor climate during the winter suggest a basic difference between the processes of cold acclimation and winter acclimatization. This difference was previously

suggested several times. There appears to be no increase in thyroid activity of winter acclimatized small mammals. Heroux (1963b) found no increase in thyroid gland weight or in the amount of exogenous thyroxine needed to block the release of labelled thyroxine from the thyroid gland in winter acclimatized white rats or wild Norway rats compared to animals studied during the summer. There is some indication that thyroid activity may be increased in winter acclimatized large animals. Freinkel and Lewis (1957) found an increase in the rate of disappearance of labelled thyroxine from the plasma and an increase in turnover of thyroxine in winter acclimatized sheep compared to animals studied during the summer.

Summary

Most indices of thyroid activity that have been studied in cold exposed animals indicate that thyroid activity is increased in cold acclimated small animals and unchanged in winter acclimatized small animals. The thyroid activity of large mammals may be increased under both conditions, although little evidence is available. However, the question remains as to whether or not the increased thyroxine, which is apparently secreted, is necessary to the cold acclimated animal. It is known that some thyroxine is necessary, but evidence is mounting that more than normal amounts of thyroxine are not needed and that the increase in thyroid activity may be due to an increased fecal loss of thyroxine. Because of these doubts and because there is so little information on thyroid activity in cold exposed large animals, two possible indices of thyroid function were studied in the present experiment involving chronic exposure of sheep to constant and fluctuating cold.

B. Effect of Stress on the Plasma Concentration of Free-Fatty Acids, Ketones, and Glucose

The plasma concentrations of free-fatty acids (FFA), ketones, and glucose are known to change during stressful situations. Three types of stressful situations will be discussed: (1) cold stress, (2) psychological stress, and (3) stress of undernourishment.

Large increases in the plasma concentrations of FFA (Halliday et al, 1969; Slee and Halliday, 1968), ketones (Halliday et al, 1969), and glucose (Halliday et al, 1969; Reid, 1962) are known to occur in sheep during periods of acute, severe cold stress. Plasma glucose concentrations in non-pregnant ewes wintered outdoors were reported to be elevated during periods of extreme cold (Karihaloo et al, 1970).

Psychological stress would include any situation where an animal becomes excited. Various types of disturbances have been used to psychologically stress sheep. Reid and Mills (1962) found that plasma glucose levels were increased during and after transportation by truck. Slee and Halliday (1968) reported that plasma levels of FFA increased after animals were handled. It seems likely that in these types of disturbances mobilization of glucose and of free-fatty acids increases.

It has been suggested that plasma concentrations of the energy substrates, glucose, FFA, and ketones, are related to feed intake and can be used to assess the nutritional status of ruminants (Bowden, 1971; Reid and Hinks, 1962; Russel, Doney, and Reid, 1967). When feed intake of sheep is restricted, increases in the plasma concentrations of FFA and ketones occur, as well as a decrease in plasma glucose concentration (Annison and White, 1961; Karihaloo, Webster, and Combs, 1970; Reid and Hinks, 1962; Russel et al, 1967). Further demands of pregnancy in an undernourished animal

result in even greater changes in plasma levels of glucose, FFA, and ketones (Karihaloo et al, 1970; Russel et al, 1967). The effects of pregnancy were superimposed on the effects of winter in a study by Dietz (1971) using beef cows. During this study plasma FFA concentration increased steadily over a 4 month time period. It has been shown by some authors that during periods of undernourishment, the rates of utilization of glucose and FFA are directly proportional to their plasma concentrations (Annison and White, 1961; Armstrong et al, 1961; West and Annison, 1964). Ketone concentrations are usually elevated during instances when FFA concentrations are high (Krebs, 1966).

Increases in plasma concentration probably reflect increases in mobilization in an attempt to provide energy substrates during stressful situations that require increases in metabolism. If utilization also increases, the rate of mobilization must increase to a greater extent than the increase in the rate of utilization in order for an increase in plasma concentration to occur. During a period of feed restriction, decreases in plasma glucose levels reflect a decrease in availability of glucose and an increased dependence on fat as a source of energy.

It has not been shown whether there is a linear relationship between plasma concentration and rates of utilization of glucose or FFA during cold exposure of ruminants. It has not even been shown that rates of utilization of these energy substrates are increased during cold exposure, but it is reasonable to assume that they are, since metabolism is increased. It is not known how sensitive a measure of the degree of stress changes in concentration are or, for that matter, if changes in concentration mean anything at all. It is possible that the extent of any increase in plasma concentration of glucose or FFA could be proportional to the

severity of the cold stress which is being imposed on an animal. Consideration should be given to the possibility that excitement may be one response of animals to a severe cold stress. It is then possible that the effect of excitement on blood levels of the energy substrates might be superimposed on the response to the actual need for more energy substrates due to the increased thermal demand of a cold environment.

In the present study short term changes in the plasma concentrations of glucose and FFA were measured. Since ketones can be an important source of energy (Krebs, 1966), they, too, were measured. It was thought that plasma levels may reflect differences in the response of sheep with different thermal histories to severe cold stress. It is hoped that excitement of the animals was minimal due to thorough training.

III. MATERIALS AND METHODS

An investigation was made of some of the physiological changes which occur in sheep during exposure to cold. The study consisted of three series of experiments.

Experiment I. Measurement of physiological changes occurring in sheep exposed to acute, severe cold for 3 to 6 hours.

Experiment II. Measurement of physiological changes occurring in sheep exposed to constant or fluctuating moderate cold for 6 weeks.

Experiment III. Measurement of the effects of prior thermal history on the physiological responses of sheep to acute, severe cold.

A. Animals and Management

Some characteristics of the animals used are listed in Appendix I. Ages of the animals ranged from about 2 to 5 years for Experiment I and from 1.5 to 2 years for Experiments II and III. The animals used for Experiment II were also used for Experiment III.

Animals were kept in individual stalls during the experiments, except when used for acute cold tests. During any long periods between experiments, the animals were either kept indoors in small pens or outdoors in fields. Individual stalls were made of wood and had slotted floors. Fiberglass and metal stalls with screen floors replaced the wooden ones for the control animals used during Experiments II and III. All stalls measured approximately 0.6 x 1.5 meters. During acute cold tests, animals were held in a special stanchion (figure 1). The stanchion was on rubber casters to facilitate movement.

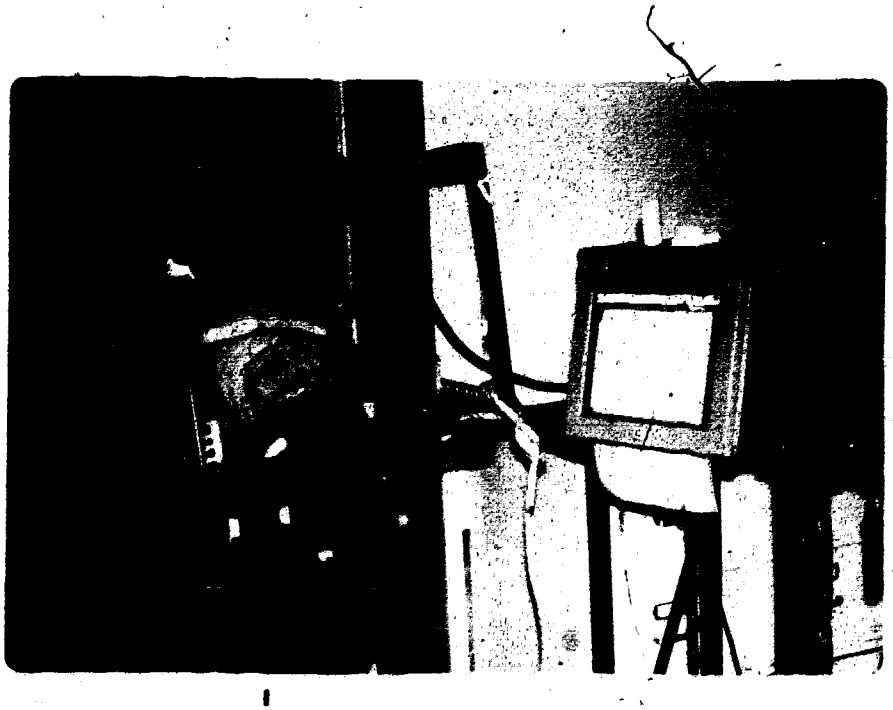

Figure 1. Sheep prepared for an acute cold exposure.

All acute cold tests and chronic cold trials were conducted in environmental chambers measuring 3 meters square. Air temperature could be controlled in one room over the range 10°C to -32°C and in the other rooms, 18°C to -20°C.

When several acute cold tests were run on one animal, the tests were spaced 19 to 21 days apart. At the beginning of each experiment the animals were assumed to have adjusted to their thermoneutral environment and will be called warm acclimated. It will be assumed that the animals remained warm acclimated unless exposed to one of the chronic cold environments. However, it is recognized that exposure to acute, severe cold may influence the response to subsequent cold tests.

Animals in Experiment I were fed good quality, chopped alfalfa-brome hay. Feeding was restricted to two 1 hour periods each day. The first period was from about 7:30 to 8:30 AM and the second period was from about 4:30 to 5:30 PM. Animals were allowed to eat as much as they wanted during the feeding periods. In Experiments II and III, alfalfa pellets were substituted for the chopped hay. Cobalt iodized salt and water were available at all times except during the acute cold tests.

The fleece of each sheep was shorn to about 3 mm in length 3 to 4 days before an acute cold test. Animals in Experiment II were shorn 3 to 4 days prior to the start of chronic cold exposure. The warm exposed (control) sheep were also clipped at this time. During the 6 weeks of the chronic cold trials the sheep were not clipped, but air temperature was progressively reduced each week in an attempt to compensate for wool growth and to keep the thermal demand of the cold environment relatively constant for the sheep. Further details of this are given later.

During all experiments, animals were kept in constant light. This was necessary because the large room, in which the control sheep were kept, held other animals requiring a constant light regime. Another reason for a constant light regime was to attempt to reduce seasonal variation in parameters, such as wool growth, which are under the influence of photoperiod.

All warm exposed sheep were kept in a large holding room at a temperature of 18°C to 21°C. This was considered to be above the lower critical temperature of these animals. An approximate critical temperature of these animals can be calculated, since the Suffolk sheep used in Experiment I had been used in the experiment of Webster et al (1969b). In that experiment, the control sheep had an average RMR of about 1.9 Mcal/m^2.24 hr and a tissue

insulation (I_T) of about $6.2^{o}C.m^2.24hr/Mcal$ (Webster et al, 1969b). Webster and Blaxter (1966) found an external insulation (I_E) of $8.4^{o}C.m^2.24$ hr/Mcal for very closely shorn Suffolk sheep. Using these values, critical temperature (T_{AC}) can be calculated using the formula of Webster et al (1969b),

$$T_{AC} = (T_R + 0.3I_E) - H_p (I_T + I_E),$$

where T_R is rectal temperature and H_p is RMR. A critical temperature of $13.8^{o}C$ is found at a rectal temperature of $39^{o}C$. This is below the temperature at which the sheep in the present experiment were held.

B. Experimental Procedures for Acute Cold Tests and Chronic Cold Trials

1. Experiments I and III

Responses of Sheep to Acute, Severe Cold Exposure

Objective

One criterion by which the cold tolerance of an animal may be assessed is by its ability to withstand a severe cold stress. In Experiment I, the changes in several physiological parameters were measured when shorn, warm acclimated sheep were exposed to an air temperature of about $-28^{o}C$ for 3 to 6 hours. In some tests the additional effects of wind and propranolol were measured. In Experiment III, changes in several physiological parameters were measured when cold exposed animals were exposed to a temperature of $-28^{o}C$ for 3 to 6 hours. This was done to assess the influence that prior exposure to chronic, moderate cold had on the response to acute, severe cold.

Procedure

The basic procedure used for all acute cold tests

is described below, followed by a brief description of the differences between the several series of acute cold tests.

1. An initial blood sample was taken from the animals through a jugular catheter while they were in their usual holding stalls, prior to the morning feeding on the day of the acute cold test. This sample, referred to as the pre-cold sample, was considered to give the best estimate of the normal values of the blood parameters under resting conditions. A sample was taken prior to feeding on the morning after the acute cold tests. This sample was taken to determine if the values of the parameters had returned to pre-test levels.

2. After the morning feeding, the animals were weighed and a jugular catheter containing a thermocouple for measurement of jugular blood temperature was implanted in animals used during Series 4 of Experiment I and during Experiment III. The animals were then placed in the special stanchion and prepared for the cold test. Thermocouples were fixed to various sites on the skin (see later) and a probe was placed in the rectum for measurement of rectal temperature during the tests of Experiment I, Series 1. A face mask, made of rubber and sheet metal, was placed over the nose and attached securely in place. Figure 1 shows an animal prepared for an acute cold test.

3. Skin and rectal or jugular blood temperatures were recorded for 30 minutes prior to the cold test. After the animal was placed in the cold room, air temperature usually reached -26°C to -28°C within an hour.

Table 1 gives the details of the procedures followed in the various acute cold tests. Table 2 gives the parameters which were measured in each series of acute cold tests listed in table 1.

Experiment I, Series 1, involved cold exposures of 3 hours duration. Thereafter, it was decided to increase the length of the cold test to 6 hours in order to increase the intensity of the cold stress. During the cold tests of Series 3, a fan was also switched on during the sixth hour to produce an air movement on the animals of about 110 meters/min and thereby increase the intensity of cold. At these times air temperatures were about -31°C. This was the maximum cold stress which could be obtained with the facilities available. Propranolol (1 mg/kg, i.v.) was given at the beginning of the sixth hour of Series 4 in an attempt to block any non-shivering thermogenesis. In this series wind was applied during the fifth hour and continued during the sixth.

The same procedure used for Series 4 of Experiment I was used for the acute cold tests of Experiment III. Animals used during Experiment III were the same as those used for Experiment II with the exception that two animals (8145, 8196) were added to the control group. The animals in the cold groups of Experiment II remained in their cold environments at all times except during the 2 acute cold tests run on each animal which make up Experiment III. The animals of the fluctuating cold group were taken out of their cold environment about 30 minutes after the start of the cold part of their temperature cycle. There was a 1.5 to 2 hour period of time between removal of the animals from their chronic cold environment and placement in the severe cold environment. After the 6 week chronic cold exposure, all animals were initially subjected to acute

TABLE 1

Summary of the Design of the Acute Cold Tests of Experiments I and III

Experimental groups	No. of sheep	No. of tests	Wind used	Propranolol used	Length of test (hr)
Experiment I					
Series 1	5	11	No	No	3
Series 2	4	13	No	No	6
Series 3	2	6	Yes	No	6
Series 4	4	6	Yes	Yes	6
Experiment III					
Controls	4				
Without PBA[1]		10	Yes	Yes	6
With PBA		4	No	No	4
Constant Cold	4				
Without PBA		4	Yes	Yes	6
With PBA		4	Yes	No	5
Fluctuating Cold	4				
Without PBA		4	Yes	Yes	6
With PBA		4	No	No	3

[1] Phenoxybenzamine

TABLE 2

Summary of Parameters Measured During Acute Cold Tests of Experiments I and III

Experimental groups	Hp[1]	Rectal/ jugular blood temperature	Skin temperature			Plasma concentration				
			Trunk	Legs	Ears	Hematocrit	Corticoids	Glucose	FFA[2]	Ketones
Experiment I										
Series 1	x[3]	x	x	x	x	x		x		x
Series 2	x					x	x	x	x	
Series 3	x					x	x	x	x	x
Series 4	x	x	x		x	x	x	x	x	x
Experiment III										
All Acute Cold Tests as Given in Table 1	x	x	x	x	x	x	x	x	x	x

1 Heat production.

2 Free-fatty acids.

3 Indicates parameter was measured.

cold for 6 hours in the way described above. Three weeks later this procedure was repeated after the animals had been given phenoxybenzamine (3 mg/kg, i.v.). The tests with phenoxybenzamine were of variable length. A combination of fall in deep body temperature and the general physical appearance determined how long the animals were allowed to remain in the cold (up to 6 hours). Deep body temperature was not allowed to fall below 31°C. Other characteristics of the tests and parameters measured are given in tables 1 and 2.

2. Experiment II

Metabolic Changes During Chronic Exposure to Constant and Fluctuating Cold.

a. Objective

Various physiological parameters were measured during exposure of sheep to 6 weeks of chronic cold. Since differences have been found between the responses of small mammals to constant cold and the responses to winter, sheep were exposed to fluctuating cold, as well as constant cold, in an attempt to simulate conditions more closely related to the naturally occurring stresses of winter.

b. Preliminary Experiment

Equating the thermal demand of the fluctuating and constant cold environments.

In order to make a valid comparison between metabolic responses induced in sheep by adaptation to constant and fluctuating cold environments, it is first necessary to ensure that the thermal demands of the two environments are approximately the same. In the present experiment the fluctuating cold consisted of 12 hours of cold, at the end of which the refrigeration units were turned off and the air allowed to gradually warm for 12 hours to about 18°C. The minimum temperature was reached about 30 minutes after the cold was turned on. The cold was turned on

at 8:00 AM and off at 8:00 PM. Air temperature, during the 12 hours of cold, was regulated so that the sheep in the fluctuating cold were exposed to twice the intensity of cold, but for half as long as those in the constant cold environment. Measurements made later of 24 hour metabolic rates of sheep in constant and fluctuating cold environments confirmed that the two environments did, in fact, constitute a comparable thermal demand.

Two shorn Suffolk wethers were exposed to each type of cold environment. Skin temperatures were measured continuously at six sites on the trunk. The temperature of the skin of the trunk (trunk skin temperature) of these animals which corresponds to the critical air temperature, at a tissue insulation of 6.2 $^{o}C \cdot m^2 \cdot 24$ hr/Mcal and at a thermoneutral metabolic rate of 1.9 Mcal/$m^2 \cdot 24$ hr, is 29.5^{o}C (about 30^{o}C); assuming the ratio of the area of trunk to extremities to be 9:1 and mean extremity temperature to be about 6^{o}C (Webster and Blaxter, 1966). The thermal demands of the two cold environments were regulated to be the same by controlling air temperature so that the number of degree-hours that the trunk skin temperature was below 30^{o}C was the same for both groups. It was found that an air temperature of 7^{o}C for the constant cold environment and 12 hours at -0.4^{o}C for the fluctuating cold environment produced equivalent thermal demands as assessed by number of degree-hours at which skin temperatures were below 30^{o}C (about 50 degree-hours).

Initially, an experiment was conducted in which six sheep were put into three groups of two sheep each, including a constant cold group (CC), a fluctuating cold group (FC), and a control group (Cont). Problems were encountered during the experiment which were probably associated with the chronic catheterizations. Animals from the FC and Cont groups were found to have signs of infection in the pulmonary arteries and jugular veins after 7 to 8 weeks

of experimentation. This, together with a continuous loss of body weight and low plasma protein bound iodine (PBI) concentration, suggested that the animals were abnormal, at least for part of the experiment. For this reason, much of the data was of little value and is not presented in detail in this thesis. Two observations were, however, of interest and justified repeating the experiment with appropriate changes in experimental procedure:

1. The CC animals gained weight and had a high feed intake, even though they were repeatedly catheterized like the other animals. This suggests that there were differences between the CC animals and the other two groups of animals. It is interesting to speculate that the CC group did adapt to the stress of cold and thereby acquired an increased resistance to non-specific stress leading to an increased resistance to infection as suggested by Webster (1970).

2. There appeared to be a significant difference between the response to acute cold of the CC animals and the animals in the other two groups after the alpha-adrenergic blocking agent, phenoxybenzamine (PBA), had been administered. These results have been reported elsewhere (Webster et al, 1969a). Briefly, after PBA was administered, the animals in the FC and Cont groups were unable to produce enough heat to maintain homeothermy; their rates of heat production were about 40% less than their rates of heat production at an equivalent time during trials without PBA. The animals in the CC group produced heat at a rate about 30% higher in trials with PBA than in trials without PBA. Abnormalities of the Cont and FC animals may, however, have been partially responsible for the difference in the response.

The average 24 hour heat production was 118.5 kcal/kg$^{3/4}$·24 hr for the CC animals and 117.1 kcal/kg$^{3/4}$·24hr for the FC animals. The average trunk skin temperatures were 27.8°C for the CC animals (over 24 hours) and 26.1°C for the FC animals (over 12 hours). Neither parameter varied significantly among the three successive measurements for either group of cold exposed animals. Air temperature was reduced 0.8°C each week to compensate for wool growth. This is obviously an over simplification since the same factor was used for both environments. However, the fact that heat production and skin temperatures did not alter between successive trials in either group suggests that the technique for equating the thermal demands of the two environments was valid.

c. Main Experiment

In this experiment, ten animals were divided into three groups: four into the CC group, four into the FC group, and two into the control group. All of these animals were obtained in the early fall of 1969 and kept indoors during the winter for use in the late summer of 1970. Animals in the CC and FC groups were cold exposed in pairs. Experiments on control animals were conducted at approximately even intervals over the 9 month period of duration of the experiment. Any seasonal effect on the parameters measured should have been present in all groups. The initial air temperatures used were the ones determined in the preliminary experiment.

Blood samples for the determination of hematocrit and the plasma concentration of PBI, glucose, adrenal corticoids, FFA, and ketones were taken before each feeding on 2 days during the middle of the first, second, fourth, and fifth weeks of cold exposure. Similar sets of samples

were taken from the control animals five times during the entire experiment. The blood samples from the FC animals were taken at the end of the warm period and after 8.5 hours of the cold period.

The plasma half-life of C^{14} labelled thyroxine was determined on one pair of animals in each of the cold groups during the first and the fourth weeks of cold exposure. Trials on the second pair of animals in each group were run on the second and fifth weeks. Similar trials were run three times during the entire experiment on the pair of control animals. The general procedure for these trials was modified from that used by Yousef and Johnson (1967); only half-life was measured in the present trials, whereas the technique of Yousef and Johnson (1967) was developed to measure thyroxine secretion rate. The night before a trial was to begin, a 25 ml sample of blood was taken trough a catheter implanted earlier. The blood was centrifuged for 30 minutes at 2050 x gravity. The separated plasma was measured and put into a 20 ml glass syringe with a cap over the luer-lok end. Enough 0.9% NaCl was added to make the final volume 20.5 ml. While the plasma was being gently stirred with a magnetic stirrer, 3 microcuries (μc) from the stock isotope solution[1] were added. The stirring was continued for 1 hour and then the syringe was stoppered and stored in a refrigerator overnight. The next morning the solution was allowed to warm to room temperature for at least 2 hours while being stirred again. Just prior to injection into the animal, two 50 μl aliquots were added to 15 ml of Bray's scintillation fluid for counting in a liquid scintillation system. The glass plunger was introduced into the barrel of the syringe and the isotope solution was injected through an 18 gauge hypodermic needle

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[1] DL-Thyroxine-$2^{14}C$, 3 μc/300 μl

into the jugular vein without the catheter. The injection was given 2 hours after the end of the morning feeding period. Samples of blood were withdrawn from the animals through the catheter at various intervals from 2 to 98 hours after injection. Plasma from these samples was frozen until analyzed. A description of the isotope preparation, isolation of thyroxine, and counting of the samples will follow later.

After the 6 week cold exposure period, acute cold tests, constituting Experiment III which was described earlier, were run on the animals. The animals were shorn on the last day of the 6 week period and the ambient temperature was raised to that of the beginning of the period. The first acute cold test was run 3 to 4 days later.

C. Measurement of Energy Expenditure

Energy expenditure was estimated from the measurement of O_2 consumption and, in the trials of Experiment III, from the measurement of both O_2 consumption and CO_2 production. The system used was the open-circuit respiratory system described by Webster and Hicks (1968). During the acute cold tests, a face mask was attached over the nose of the animal and air was drawn through it. Flow was kept constant and was monitored using a wet-test gas meter[2]. The ventilation rates used varied between 2400 and 3600 liters (at STP)/hour. This is well above the maximum volume of expired air of 800 liters/hour found for sheep during cold exposure (Joyce and Blaxter, 1964).

The O_2 content and the CO_2 content of a continuous

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[2] American Meter Company, Erie, Pennsylvania.

aliquot of the ventilating air stream were measured using a Beckman F-3[3] O_2 analyzer and an IR-215[3] CO_2 analyzer, respectively. The aliquot of air was dried by passage through silica gel before entering the analyzers.

Energy expenditure (heat production, Hp, kcal/hr) was calculated from O_2 consumption (liters/hr) according to the formula of Blaxter and Joyce (1963):

$$Hp = O_2 \text{ (liters/hr)} \times 4.68 \text{ (kcal/liter } O_2)$$

In trials where both O_2 consumption and CO_2 production were measured, Hp was calculated from the formula of Brouwer (1965), excluding the terms for nitrogen excretion and methane production:

$$Hp = O_2 \text{(liters/hr)} \times 3.866 \text{(kcal/liter)} + CO_2 \text{ (liters/hr)} \times 1.200 \text{(kcal/liter)}$$

D. Temperature Measurements

Various body temperatures were continuously measured during most of the acute cold tests. Two types of potentiometric recorders with internal reference junctions were used: a Speedomax-W[4] and a Honeywell Electronik 16[5]. Figure 2 shows the various sites on the animal which were used for temperature measurements.

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[3] Beckman Instruments, Inc., Fullerton, California.

[4] Leeds and Northrup (Canada) Ltd., Toronto 15, Ontario.

[5] Honeywell-Industrial Products Group, Wayne and Windrim Ave., Philadelphia 44, Pennsylvania.

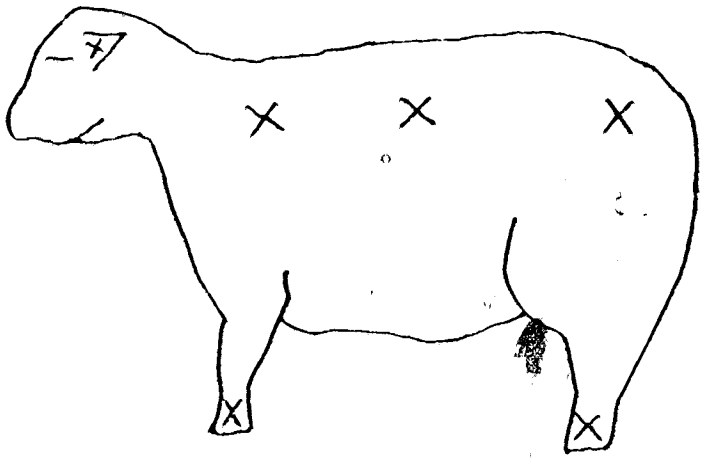

Figure 2. Placement of thermocouples for skin temperature measurements. All positions were duplicated on the right side of the animal as well.

Copper-constantan thermocouple wire(23' gauge) was used and one end was made into a junction. This was attached to the various sites on the animals and held in place by rubber cement and a patch of surgical tape. For the measurement of rectal temperature, a length of thermocouple wire was secured inside the metal jacket of a probe and inserted into the rectum approximately 8 cm. During trials when jugular blood temperature was used, instead of rectal temperature, as an index of deep body temperature, the thermocouple wire was threaded into a polyethylene catheter. The catheter was then put into the jugular vein in the same manner as for blood samples, which will be described in the next section.

In Experiment III, thermocouples from similar sites on the animal were first connected in parallel through a common wire that led to the recorder. This resulted in average temperatures being printed for the six sites on the trunk, the four legs, and the two ears. The wires to the

recorder passed through metal conduit which went through a port-hole in the wall of the cold room. Thus, the temperature recorder was kept outside the cold room.

E. Catheterization, Blood Sampling, and Drug Administration

1. Catheterization

Blood samples were withdrawn from and drugs were given through jugular vein catheters. The tip of the catheter was placed in the right side of the heart. This could be judged by the appearance of a pulse in an air bubble introduced into the catheter. A polyethylene intramedic catheter (PE-190/S36) [6] was used and was introduced into the jugular vein through a 13-gauge, 2 inch hypodermic needle. The catheter was held in place by a piece of surgical tape and a skin suture. A 3-way tap was connected to the free end of the catheter and attached to a collar around the animal's neck. The collar was wrapped with gauze to keep the catheter close to the neck so it would not freeze. When not in use, the catheter was filled with a 1% heparinized saline solution (100 U.S.P. units of sodium heparin[7]/ml of 0.9% NaCl).

Catheterization normally took place 18 to 24 hours before blood samples were taken. In a few cases when catheters came out during the night, a new catheter was put in the next morning prior to feeding.

2. Blood Sampling

Immediately prior to collection of a blood

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[6] Becton, Dickinson and Company, Parsippany, N.J.

[7] Riker Pharmaceutical Company Ltd., Cooksville, Ontario.

sample, the catheter was flushed with heparinized saline and the contents of the catheter, along with the first ml of blood, were drawn through the 3-way tap and discarded. The tap was then turned and 10 to 20 ml of blood were drawn into a disposable plastic syringe which contained a drop of sodium heparin[7]. The contents were expelled into a polypropylene test tube where the blood was gently mixed. Duplicate samples were then immediately withdrawn for the determination of blood hematocrit (Hct), which is the percentage of cells in the blood. The remainder was centrifuged for 30 minutes at 2050 x gravity. The separated plasma was then divided among several small glass test tubes and frozen at -26°C for subsequent analysis.

Duplicate hematocrit determinations were made on each blood sample using heparinized capillary tubes[8], a microcapillary centrifuge (Model MB)[8], and a microcapillary tube reader (cat. #2201)[8]. After the capillary tubes were filled, one end was sealed with Critoseal[9] and the tubes were then centrifuged for 15 minutes at 16,000 x gravity. Afterwards, the tubes were read on the tube reader.

3. Drug Administration

Propranolol[10] was given at a dosage of 1 mg/kg body weight (BW). The propranolol powder was dissolved in 10 ml of warm 0.9% NaCl. At the appropriate time it was injected quickly via the jugular catheter.

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8 Fisher Scientific Company, Ltd., Montreal, Quebec.

9 American Hospital Supply Corp., Evanston, Illinois.

10 Inderal (Propranolol Hydrochloride), Ayerst Laboratories, Montreal, Quebec.

Phenoxybenzamine [11], administered once to each animal in Experiment III, was prepared fresh for each trial and given at a dosage of 3 mg/kg BW. It was first dissolved in a volume of propylene glycol equal to 10% of the final volume. Acidified (pH 6.0) 0.9% NaCl was added to make the final concentration of drug 1.5 mg/ml. The drug was administered over 1 hour using a variable speed infusion - withdrawal pump (series 600-950V) [12].

F. Blood Analysis

The various chemical analyses on frozen plasma are described briefly below. Detailed descriptions of some analytical procedures, which differ markedly from published reports, are given in appendices.

1. Plasma Glucose:

Plasma glucose was determined using a Technicon automated procedure (N-9a or N-2a)[13] and a Technicon autoanalyzer[13]. The N-2a procedure was modified to dilute the samples and standards approximately 50% more than the procedure required. The extra dilution was used during the analysis of samples from the acute cold tests when high plasma glucose concentrations were expected. The dilution extended the analytical range of the procedure.

2. Plasma Protein-Bound Iodine (PBI):

Plasma PBI concentration was measured by the

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11 Dibenzyline (Phenoxybenzamine Hydrochloride), Smith Kline and French, Montreal, Quebec.

12 Harvard Apparatus Company, Dover, Mass.

13 Technicon Instruments Corp., Chauncey, New York.

Hycel cuvette PBI method[14]. The original procedure was modified by using 3.5% perchloric acid to precipitate the plasma proteins, rather than using an ion-exchange resin to remove the inorganic iodine. Using the protein precipitation, the inorganic iodine is left in the supernatant and the precipitate can be digested with the digestion reagent and the normal procedure followed from this stage.

3. Plasma Free-Fatty Acids (FFA):

FFA concentration was estimated using an adaptation of Dole's method (Mosinger, 1965). A slight modification was used to extend the range of standards during analysis of samples from the acute cold trials. Details of the procedure are in Appendix II.

4. Plasma Ketones:

Plasma ketone concentration was estimated using a slightly modified version of Baker and White's (1957) technique. Details of the modified technique are in Appendix III. The technique, as used, measures total ketones, expressed as acetone, and makes no attempt to separate acetone, beta-hydroxybutyrate, and acetoacetate.

5. Plasma Corticoids:

Plasma corticoid concentration was estimated using a fluorometric technique modified from Silber, Busch, and Oslapas (1958). The modified technique is outlined in Appendix IV. This technique measures mainly the glucocorticoids, cortisol and corticosterone,

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[14] Hycel, Inc., Houston, Texas. Manual No. 5004.

although a few other steroids are slight contaminants. For this reason the substances measured by this procedure have been given the general name corticoids. Since hydrocortisone (cortisol) is the predominant glucocorticoid in sheep (Bush and Ferguson, 1953; Coghlan, Wintour, and Scoggins, 1966), cortisol was used as the standard.

6. Thyroxine-^{14}C: Isotope Preparation, Thyroxine Isolation and Isotope Counting.

In the present studies, DL-Thyroxine-2-^{14}C was used (NEC-299)[15]. This isotope was obtained as a crystalline solid and had a specific activity of 17.9 mc/mM. The thyroxine was first dissolved in 1 ml of 0.01 M NaOH and then diluted to 5 ml with 0.9% NaCl. The final concentration was approximately 0.01 μc/μl.

The labelled thyroxine, from plasma, was isolated using the Hycel Butanol Extractable Iodine procedure[14] which was modified to use 3 to 4 ml of plasma. One volume of plasma was extracted with 4 volumes of butanol. The mixture was centrifuged for 10 minutes at 2050 x g gravity. A 0.5 ml aliquot from the supernatant, which contains the thyroxine, was then carried through the rest of the procedure for iodine determination. The rest of the recoverable supernatant was placed in a scintillation vial and the volume reduced by evaporation under a stream of air. Bray's scintillation fluid (15 ml, Bray, 1960) was added directly to the scintillation vials, which were then counted in a Nuclear Chicago Mark I Liquid Scintillation System[16] for 10 to 20 minutes.

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15 New England Nuclear, Boston, Mass. 02118.

16 Nuclear Chicago Corp., Chicago, Illinois.

Each sample was counted in triplicate. Counting efficiency was determined by the channels ratio method using quenched standards from Nuclear Chicago [16].

C. Statistical Analysis

The data was analyzed according to statistical procedures described by Steel and Torrie (1960). Tests of significance were done using the unpaired t-test. In some of the experiments the value of n was increased by using multiple animals and multiple trials on each animal. No attempt was made to separate sources of error.

IV Results

A. Experiment I

Some Responses of Warm Exposed Sheep to Acute, Severe Cold Stress; Effects of the Additional Stress of Wind and of the Sympatholytic Drug, Propranolol

Effects of Cold

Values for the parameters measured are illustrated in figures 3 to 7 (Refer to Appendices V-VIII for mean values.). In addition, some levels at 3 hours of cold exposure and maximum or plateau values are summarized in table 3 for all series of acute cold tests. All average increases referred to below are weighted to take into consideration the different numbers of cold tests in the different series.

1. Heat Production and Body Temperature

Heat production increased steadily during the first 1 to 2 hours of the cold tests and then stabilized (figures 3-6) at an average of about 3.9 Mcal/m^2.24 hr.[17] This is about 4.5 times the assumed fasting metabolic rate (0.85 Mcal/m^2.24 hr, Blaxter, 1967).

Rectal temperatures (figures 3 & 7) decreased an average of 0.4°C (39.5°C to 39.1°C) during the first 4 hours of cold exposure.

Skin temperatures of the trunk (figures 3 & 7), legs (figure 3), and ears (figures 3 & 7) decreased quickly at first, but all values tended to stabilize after about 1 hour. The skin temperature of the trunk stabilized at about 12°C, that of the legs about 2°C, and the ears about 7°C in Series 1 and 13°C in Series 4.

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[17] m^2 = surface area = $0.09 \times BW\ (kg)^{2/3}$

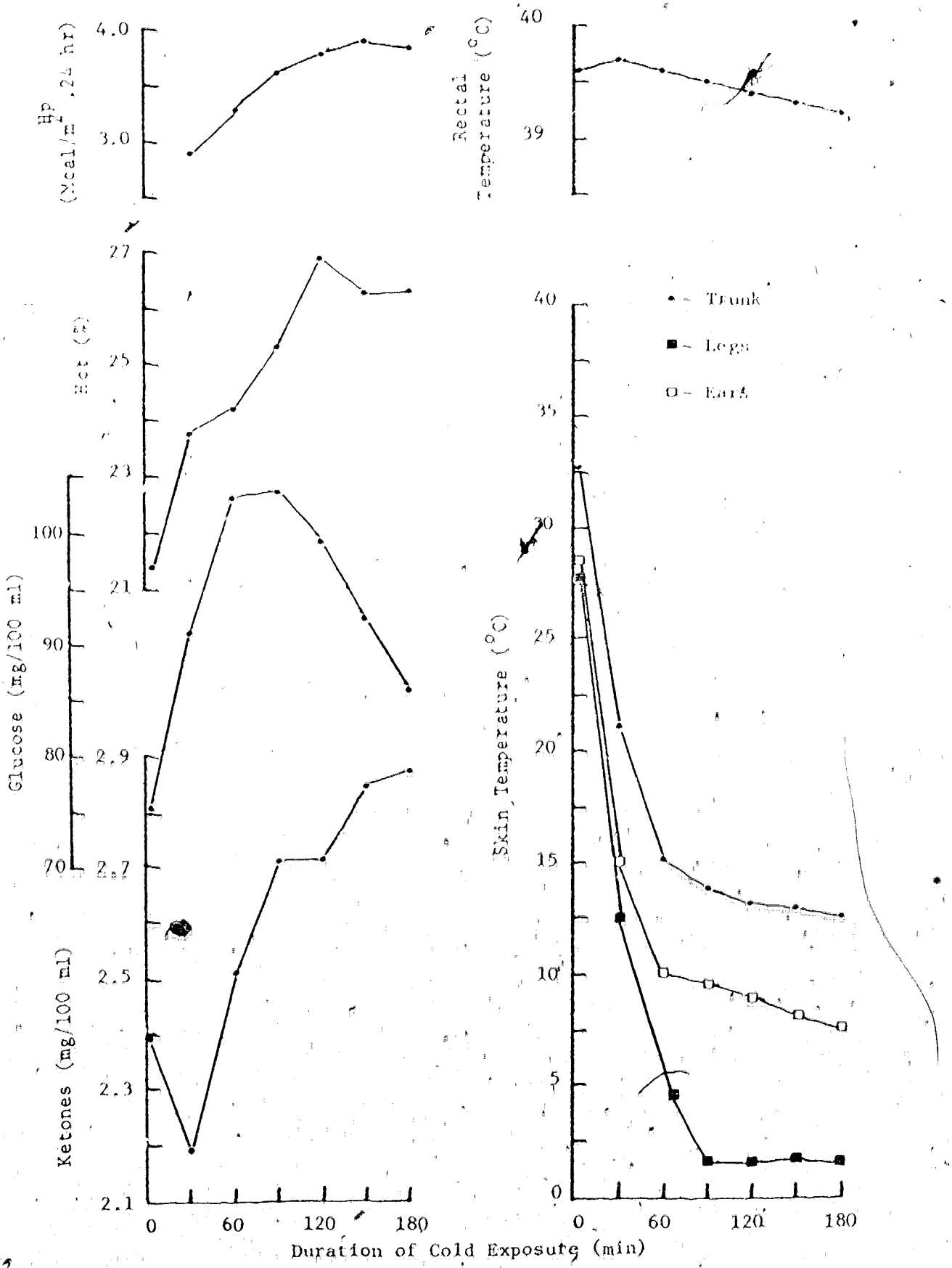


Figure 3. Values for heat production (Hp), hematocrit (Hct), glucose, ketones, rectal temperature, and skin temperatures during the acute cold tests (-28°C) of Experiment I, Series 1. (Each point is the mean of 11 tests)

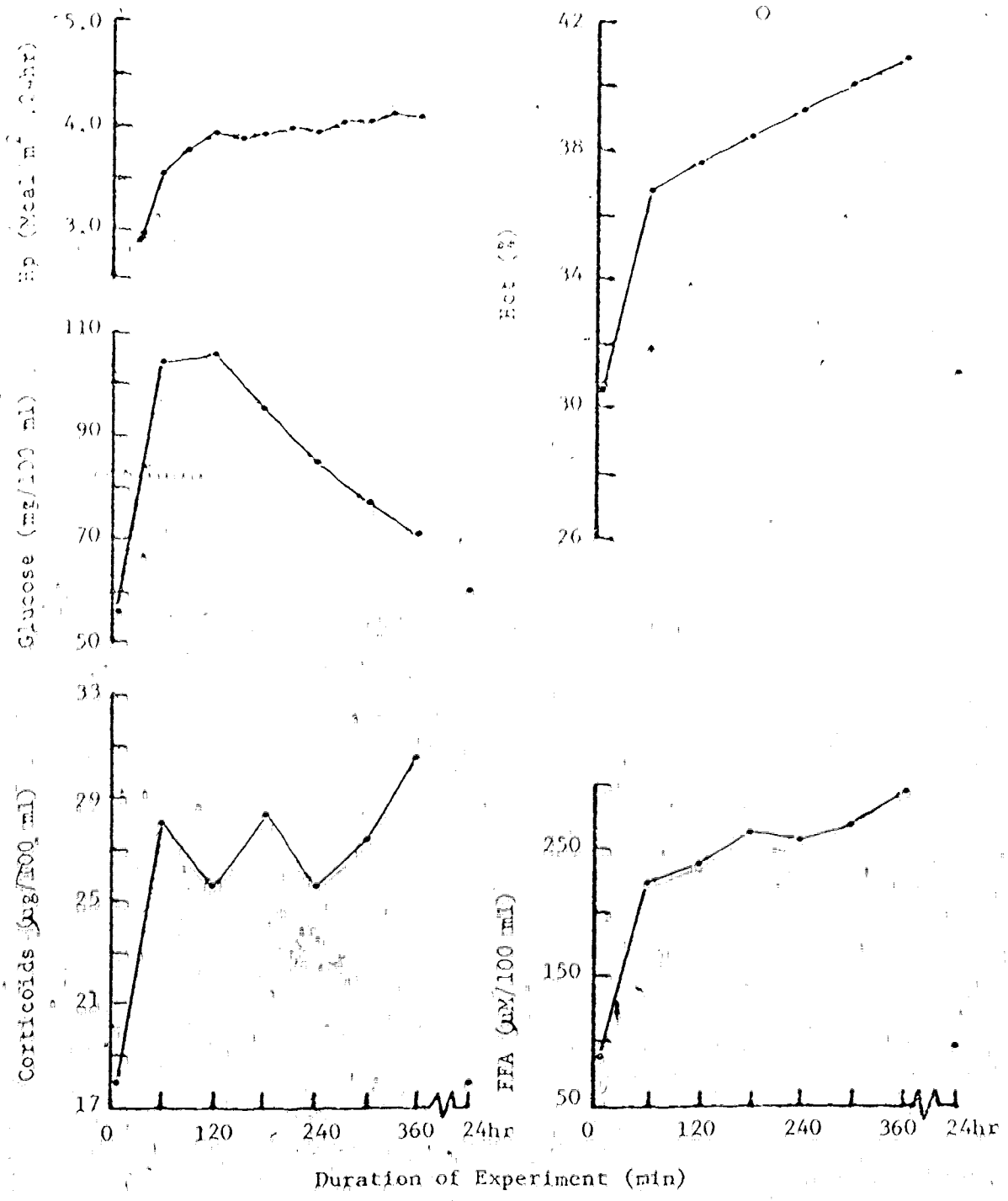


Figure 4. Values for heat production (Hp), glucose, corticoids, hematocrit (Hct), and free-fatty acids (FFA) during the acute cold tests (-28°C) of Experiment I, Series 2. (Each point is the mean of 13 tests. Cold exposure lasted 360 minutes)

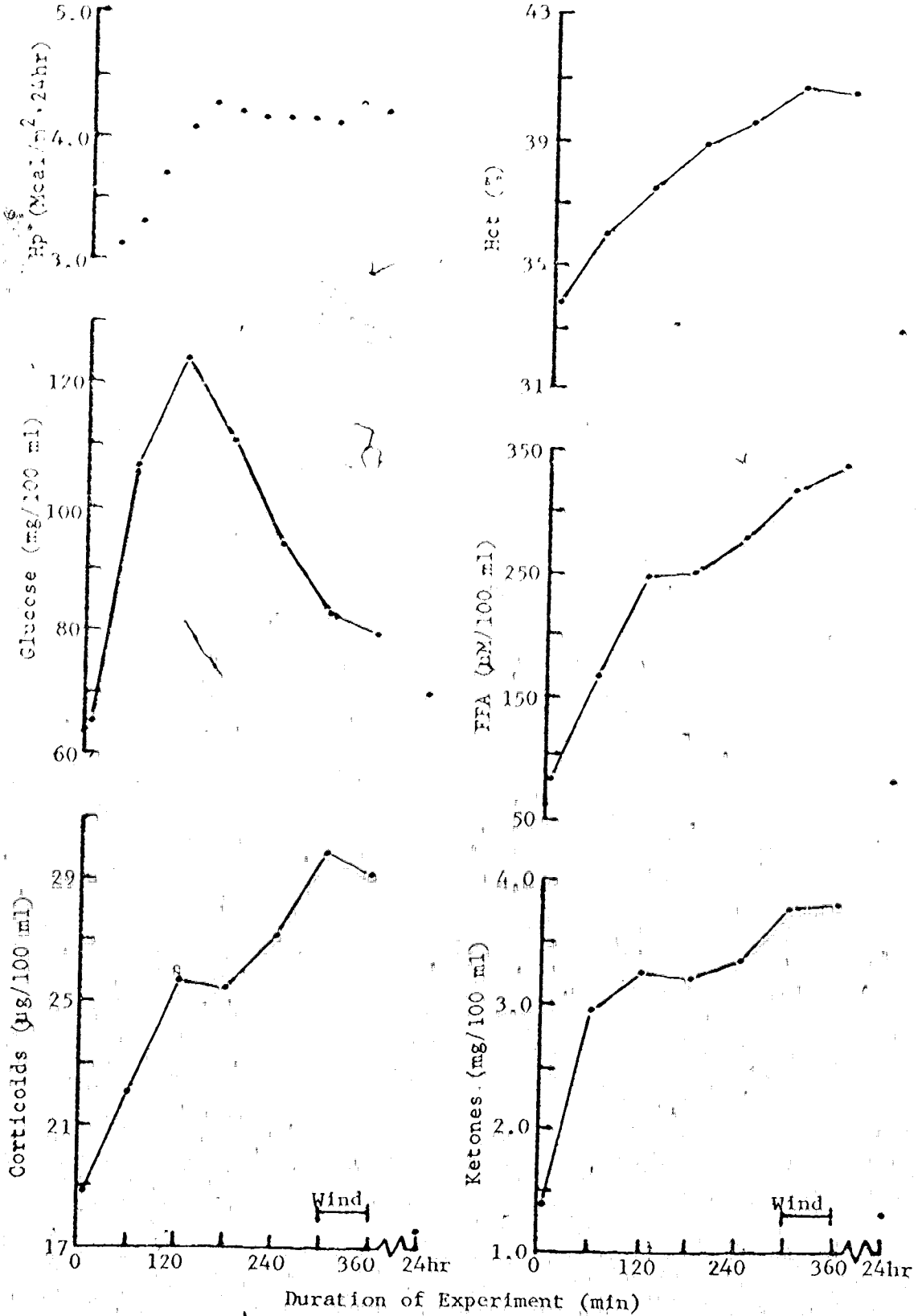


Figure 5. Values for heat production(Hp), glucose, corticoids, hematocrit(Hct), free-fatty acids(FFA), and ketones during the acute cold tests (-28°C) of Experiment I, Series 3. (Each point is the mean of 6 measurements. Cold exposure lasted 360 minutes.)

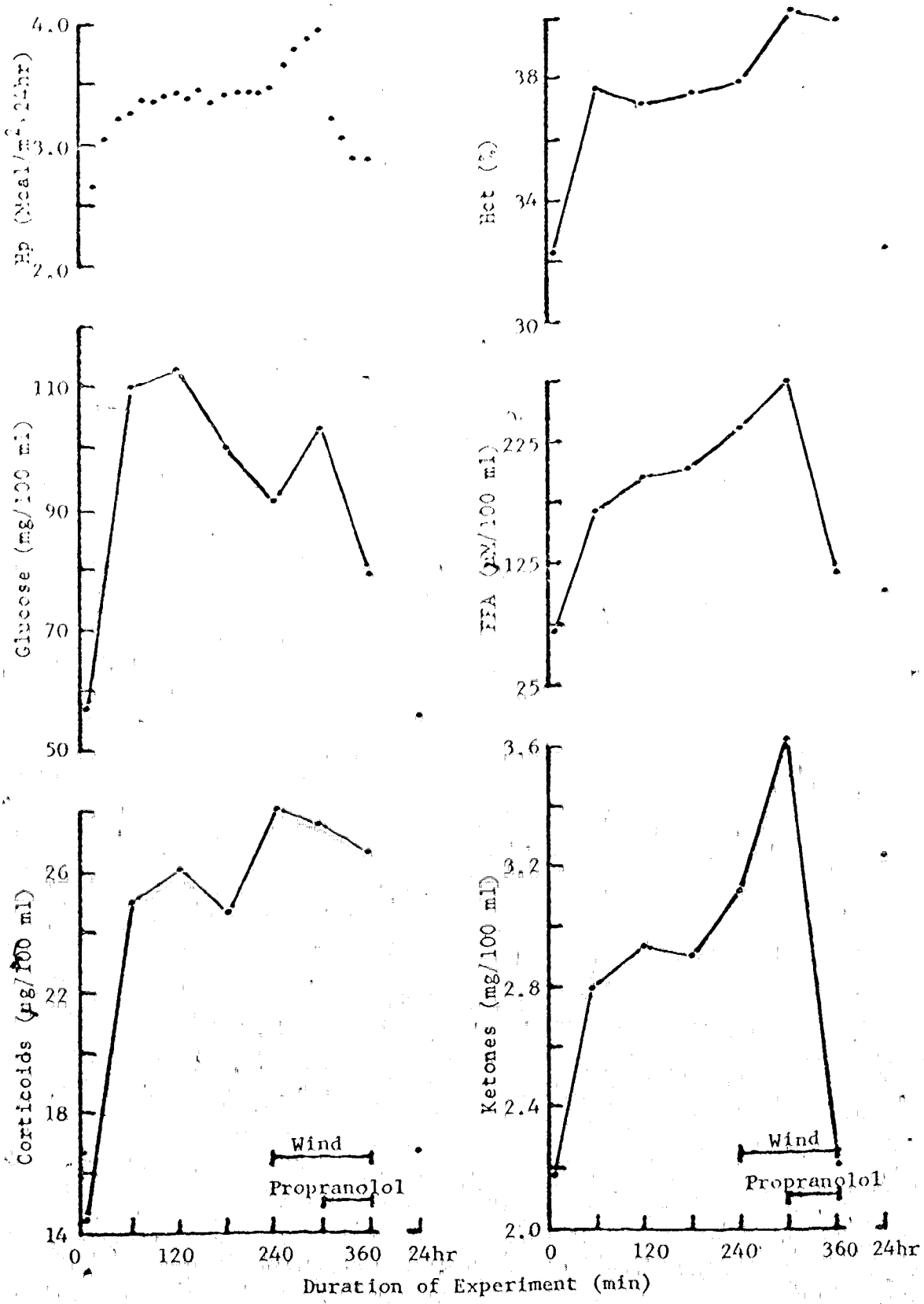


Figure 6. Values for heat production(Hp), glucose, corticoids, hematocrit(Hct), free-fatty acids(FFA), and ketones during the acute cold tests (-28°C) of Experiment I, Series 4. (Each point is the mean of 6 tests. Cold exposure lasted 360 minutes.)

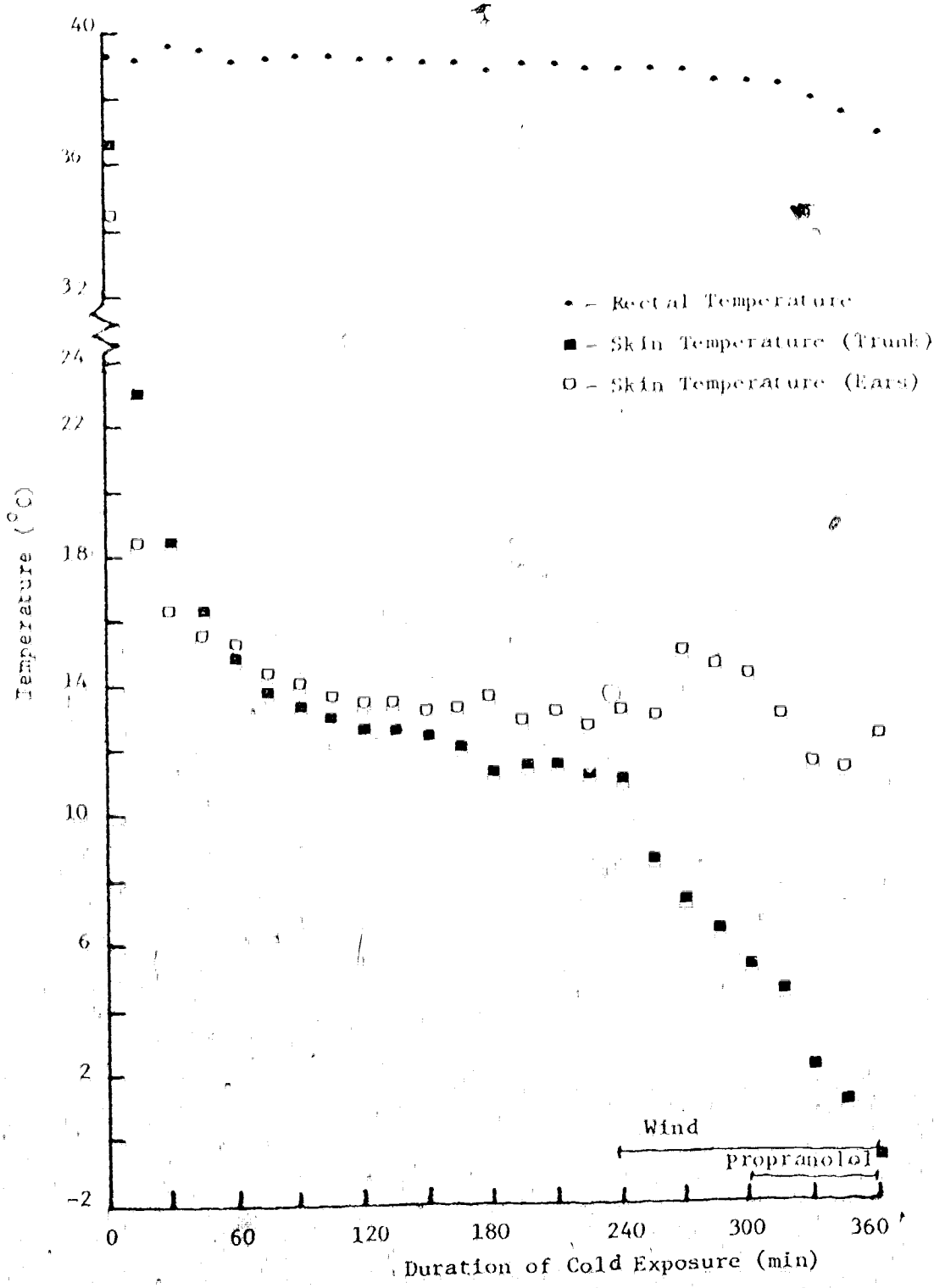


Figure 7. Values for rectal and skin temperatures during acute cold tests (-28°C) of Experiment I, Series 4. (Each point is the mean of 6 tests.)

TABLE 3

Summary of Some of the Values of Heat Production (Hp_t), Blood Hematocrit (Hct_t) and the Plasma Concentrations of Corticoids, Free Fatty Acids (FFA), Ketones, and Glucose During Acute Cold Exposure (T_a = 0°C) [All values are Means (± Standard Deviation) from Experiment 1, Series 1-4. Numbers in Parentheses after Series Number are Number of Tests (Observations) in Means of that Row.]

Measurement and Series	Pre-Cold	+3 Hours	Maximum or Plateau Value
Hp_t (Mcal/m^2·24 hr)			
Series 1 (11)	0.85[1]	3.81(±0.34) + 348%[c]	-
Series 2 (13)	0.85	3.98(±0.30) + 368%[c]	4.08(±0.39) + 380%[c]
Series 3 (6)	0.85	4.19(±0.31) + 393%[c]	4.18(±0.33) + 392%[c]
Series 4 (6)	0.85	3.41(±0.37) + 301%[c]	3.43(±0.42) + 304%[c]
Hct_t (%)			
Series 1 (11)	21.4(±1.2)	26.3(±2.9) + 23%[c]	-
Series 2 (13)	30.6(±1.8)	38.4(±3.0) + 25%[c]	40.8(±3.1) + 33%[c]
Series 3 (6)	33.8(±2.0)	38.9(±1.1) + 15%[c]	40.8(±2.2) + 21%[c]
Series 4 (6)	32.4(±1.5)	37.5(±2.4) + 16%[c]	37.9(±2.4) + 17%[c]
Corticoids (ug%)			
Series 2 (6)	17.9(±3.8)	28.3(±4.6) + 58%[b]	30.5(±2.9) + 70%[c]
Series 3 (6)	18.9(±3.2)	25.3(±2.2) + 34%[c]	29.8(±2.8) + 58%[c]
Series 4 (6)	14.5(±2.4)	24.5(±3.8) + 69%[c]	28.1(±2.9) + 94%[c]
Glucose (mg%)			
Series 1 (11)	74.8(±3.4)	-	103.4(±10.6)+40%[c]
Series 2 (13)	57.1(±4.8)	-	105.4(±15.3)+84%[c]
Series 3 (6)	64.8(±1.9)	-	123.6(±13.1)+91%[c]
Series 4 (6)	57.0(±5.3)	-	112.3(±14.9)+97%[c]
FFA (umoles/100 ml)			
Series 2 (13)	90(±22)	263(±39) + 193%[c]	295(±49) + 230%[c]
Series 3 (6)	83(±7)	250(±50) + 201%[c]	318(±58) + 283%[c]
Series 4 (6)	73(±13)	202(±67) + 178%[c]	238(±44) + 229%[c]
Ketones (mg%)			
Series 1 (11)	2.39(±0.26)	2.87(±0.50) + 20%[b]	-
Series 3 (6)	1.48(±0.44)	3.31(±0.45) + 123%[c]	3.90(±0.34) + 164%[c]
Series 4 (6)	2.18(±0.23)	2.90(±0.44) + 33%[b]	3.12(±0.56) + 43%[b]

Mean values followed by lettered superscripts differ significantly from the pre-cold mean.

[b] $p < 0.01$, [c] $p < 0.001$

[1] Estimated fasting heat production from Blaxter (1967).

Note: All percentages refer to percent increase over pre-cold mean.

2. Blood Hematocrit and Concentration of Plasma Constituents

Maximum increases in hematocrits amounted to, on the average, 25% of the pre-cold levels (table 3). This difference was highly significant ($p < 0.001$). In Series 2 (figure 4) and 4 (figure 6), a large part of the increases in hematocrit occurred during the first hour of cold exposure. Thereafter, moderate increases occurred. In Series 1 (figure 3) and 3 (figure 5), increases in hematocrit were slower, and tended to plateau near the end of the cold tests.

There was a marked rise in plasma adrenal corticoid concentrations (figures 4-6). The average maximum concentration was 74% above the average pre-cold concentration (table 3) and this was highly significant ($p < 0.001$). There was no indication that plasma adrenal corticoid levels stabilized during the acute cold tests.

Plasma glucose concentrations (figures 3-6) increased and reached a peak during the first 1 to 1.5 hours of the cold tests and then declined. The average maximum concentration was about 75% above the average pre-cold level (table 3) and this difference was highly significant ($p < 0.001$). Plasma glucose concentrations declined steadily after the peak concentration was reached.

There was a marked continuous rise in plasma FFA concentrations (figures 4-6) during the cold tests. The average level at the end of the cold exposure period was 240% above the average pre-cold concentration (table 3) and this difference was highly significant ($p < 0.001$).

Concentration of plasma ketones rose and showed no tendency to stabilize (figures 3,5, & 6). The average levels at the end of the cold exposures ranged from 20% to 164% above the average pre-cold levels (table 3), and these increases were also highly significant ($p < 0.01$). The percentage increase in Series 3 was much higher than in either Series 1 or

Series 4. When compared to Series 4, the high percentage increase in Series 3 is a reflection of a significantly lower pre-cold concentration in Series 3 ($p<0.01$).

Additional Effects of Wind

During the sixth hour of cold in Series 3 (figure 5) and the fifth hour of cold in Series 4 (figures 6 & 7) a 110 meter/min wind was directed at the animals and, in addition, room air temperature was lowered another 3 to 4°C. No significant changes occurred in any of the parameters measured in Series 3 which could be attributed to wind. In Series 4 (figure 6) a significant increase in glucose, from about 90 to 103 mg%, was found ($p<0.05$). Smaller, non-significant increases in heat production and hematocrit were observed. Also in Series 4 (figure 7), a continuous fall in skin temperature of the trunk, from about 11°C to 5°C, occurred during the wind period. Very little change was seen in the skin temperature of the ears or in rectal temperature.

Additional Effects of Propranolol

At the beginning of the sixth hour of the acute cold tests of Series 4, propranolol (1 mg/kg, i.v.) was injected into each animal. By the end of the sixth hour, significant ($p<0.05$) decreases from pre-propranolol levels had occurred in heat production and in the plasma concentration of glucose, free-fatty acids and ketones (figure 6). Comparisons of the levels after propranolol with levels prior to propranolol or prior to propranolol and cold are made in table 4. There were decreases from pre-propranolol values in heat production of 28%, in plasma glucose of 24%, in plasma FFA of 57%, and in plasma ketones of 39%. As heat production decreased, rectal temperature also declined (figure 7). The fall in rectal temperature was 1.6°C during the hour after propranolol was given. This is compared to

TABLE 4

Effect of Propranolol (1 mg/kg BW) on Heat Production(Hp) and the Plasma Concentration of Glucose, Free-Fatty Acids(FFA) and Ketones During Acute Cold Tests(-28°C)
All Values are Means (± Standard Deviation) from Experiment I, Series 4.
All Means are averages of 5 acute cold tests (n=5).

Sample Time	Hp (Mcal/m^2·24 hr)	Glucose (mg%)	FFA (umole/100 ml)	Ketones (mg%)
Pre-Cold	0.85[1,a]	57.0(±5.3)[a]	73(±13)[a]	2.18(±0.23)[a]
Pre-Propranolol (after 5 hr of cold exposure)	3.92(±0.59)[b]	102.8(±7.9)[b]	275(±33)[b]	3.62(±0.57)[b]
After Propranolol (after 6 hr of cold exposure)	2.84(±0.42)[c]	78.4(±8.9)[c]	118(±23)[c]	2.20(±0.28)[a]

1 Estimated basal heat production from Blaxter (1967).

Means in each column followed by a different lettered superscript differ significantly from other means in the column ($p < 0.01$).

a fall of 0.9°C during the 5 hours prior to injection of propranolol. By the end of the cold tests thermal equilibrium had not been re-established.

Recovery

The concentrations of all plasma constituents measured in Series 2 (figure 4) and Series 3 (figure 5) had returned to normal when the 24 hour samples were taken with the exception of glucose concentration in Series 3. In this series, glucose concentration 24 hours after the cold tests was slightly elevated (69.3 mg% vs. 64.8 mg%) and, statistically, this difference was highly significant ($p < 0.01$). In Series 4 (figure 6), both ketones and FFA remained elevated 24 hours after the acute cold tests ahd the differences were significant. Free-fatty acid concentration was 99 μmole/100 ml compared to 73 μmole/100 ml ($p < 0.05$) and ketone concentration was 3.23 mg% compared to 2.18 mg% ($p < 0.001$).

B. Experiment II

Some Responses of Sheep to Constant and Fluctuating Chronic Cold Exposure

1. Feed Consumption and Body Weight Changes

Feed consumption values and body weights for the 3 groups of animals are presented in figure 8. Feed consumption of the animals in the CC group increased during the first 4 weeks in the cold from 27.9 gm/kg BW to 35.8 gm/kg BW. A decline to 33.9 gm/kg BW occurred during the last 2 weeks. Feed consumption of the FC animals increased from 23.2 gm/kg BW to 27.9 gm/kg BW during the first 4 weeks of cold exposure and remained constant for the remaining 2 weeks. Feed consumption of the animals in the control group fluctuated markedly during the two 6 week periods shown, but the overall

averages for the 6 week periods were similar (24.8 gm/kg BW and 26.4 gm/kg BW).

The animals of the FC group had the lowest average weight gain, 5.3 kg. The average weight gain of the control animals differed between the two 6 week periods. During the first period they gained an average of 9 kg per animal and during the second period they gained only 5 kg per animal. The average of these, 7 kg, was similar to the average weight gain per animal (7.5 kg) for the CC group.

2. Blood Hematocrit and Plasma Constituents

Results of the measurements of the six measured blood and plasma constituents are presented in figure 9 (Refer to Appendix IX for mean values.).

Blood hematocrit values (figure 9A) for the animals of both cold groups were significantly higher than the values for the control animals at any time ($p < 0.001$) during the experimental period. There were no significant differences between the cold groups, nor were there any significant changes between any of the weekly averages for any of the groups of animals.

There were few differences in concentration of the plasma constituents measured between groups of animals. It appeared that chronic cold exposure may have resulted in higher ($p < 0.01$) plasma concentrations of PBI in the animals of both cold groups compared to the control animals (figure 9B). Fluctuating chronic cold exposure appeared to result in a higher concentration of plasma adrenal corticoids in the animals exposed to this type of environment compared to animals of the CC and control groups (figure 9C). Although there was some variation between corticoid levels

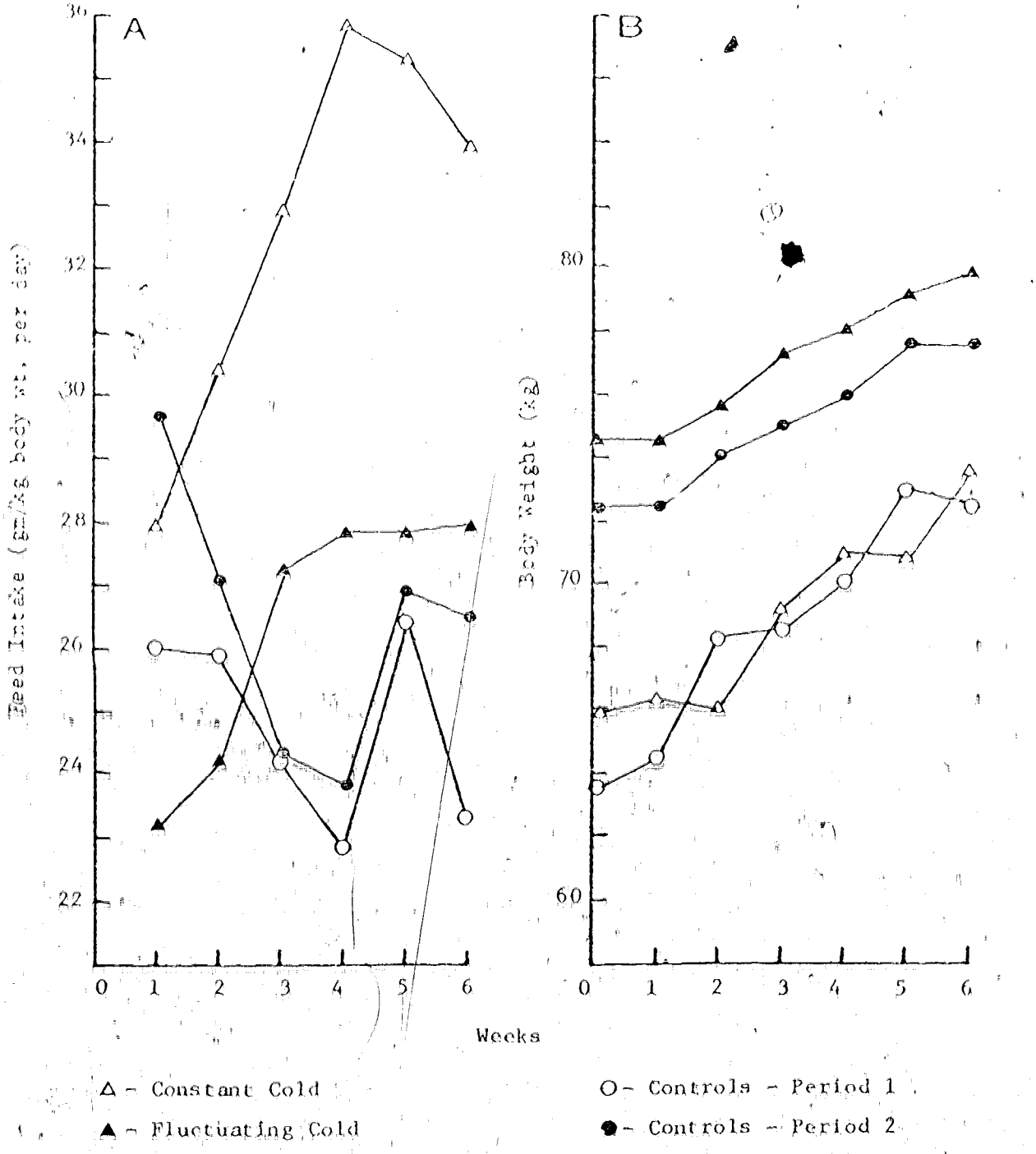
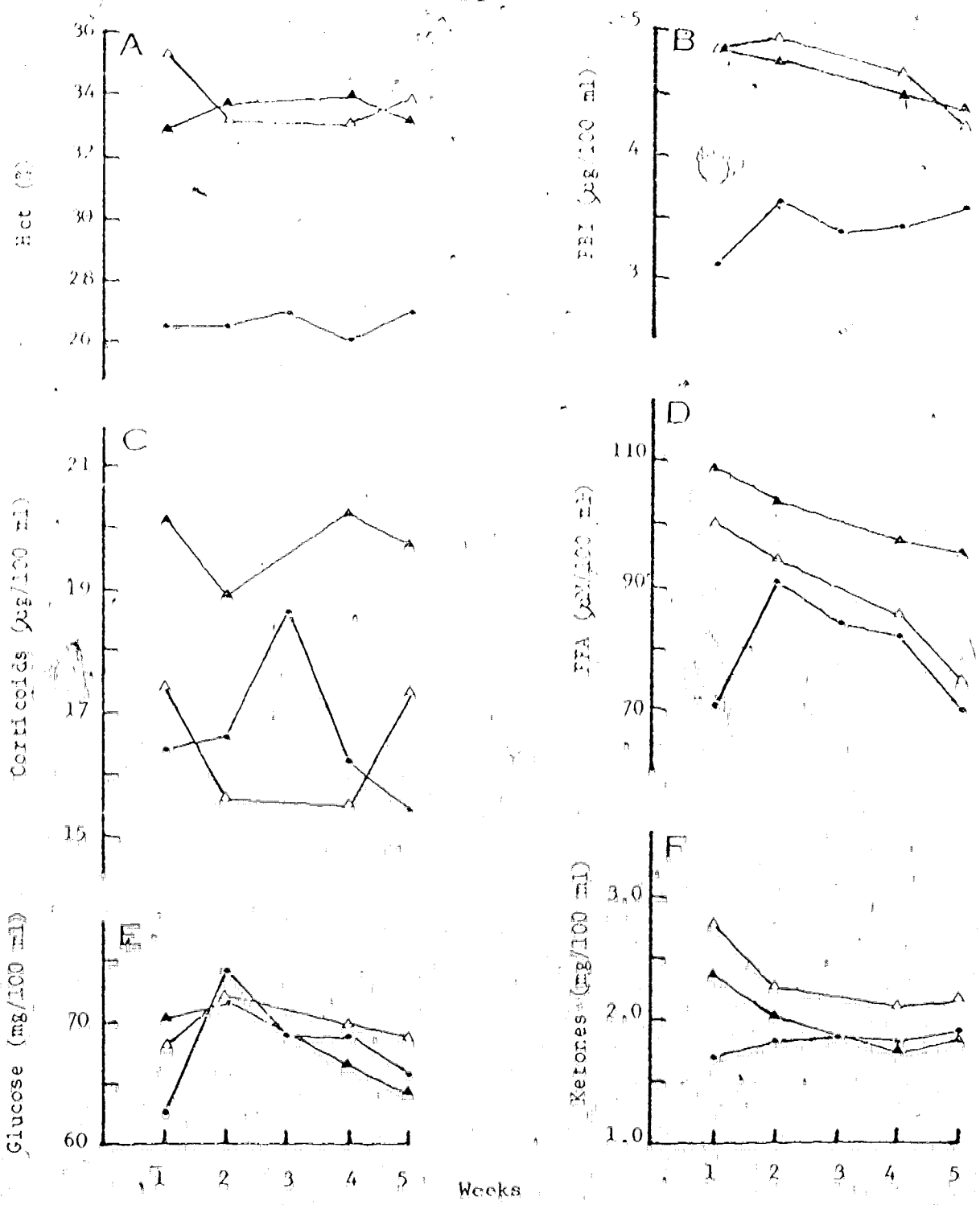


Figure 8. Values for feed consumption and body weight during the chronic cold trials of Experiment II.



△ – Constant Cold (n = 16)

▲ – Fluctuating Cold (n = 16)

• – Controls (n = 8)

Figure 9. Weekly values for hematocrit (Hct), corticoids, glucose, protein bound iodine (PBI), free-fatty acids (FFA), and ketones during the chronic cold trials of Experiment II.

in each group, the average concentration in the FC animals (19.7 µg%) was significantly ($p < 0.01$) higher than the average concentration for either the CC animals (16.5 µg%) or the control animals (16.6 µg%).

Smaller differences may have existed in the initial levels of plasma glucose (figure 9E), FFA (figure 9D), and ketones (figure 9F), between the animals of both cold groups and average levels in the control animals. However, the results are confounded by fluctuations in concentrations of the three plasma constituents in the control animals.

3. Half-Life of C^{14} Labelled Thyroxine

The half-lives of labelled thyroxine in plasma, determined during the chronic cold trials, are presented in table 5. All values were obtained graphically by plotting the logarithms of the plasma butanol extractable iodine specific activities (dpm/µg I) against time. Regression lines were then calculated and half-lives determined. All logs of specific activities can be found in Appendix X (a-c) and an example of one of the disappearance curves is illustrated in figure 10. Only points after 10 hours were considered for the calculation of the regression line. The half-life of thyroxine in the plasma was about 12% shorter in the animals of both cold groups compared to the control animals. However, there would appear to be larger differences between animals in the CC group than between animals in the FC group and control group. For both cold groups the trials on weeks 1 and 4 were run on 1 pair of animals and on weeks 2 and 5 on another pair.

TABLE 5

Thyroxine Half-Lives (hr)

Week	Control [1]	Constant Cold	Fluctuating Cold
Week 1	37.5	25.0	31.5
Week 2	34.5	35.5	30.0
Week 4	35.5	26.5	31.8
Week 5		37.5	31.8
Mean	35.8	31.1	31.3

Note: All values are means of 2 animals from Experiment II.

[1] Trials on control group were repeated at approximately equal intervals during entire experiment.

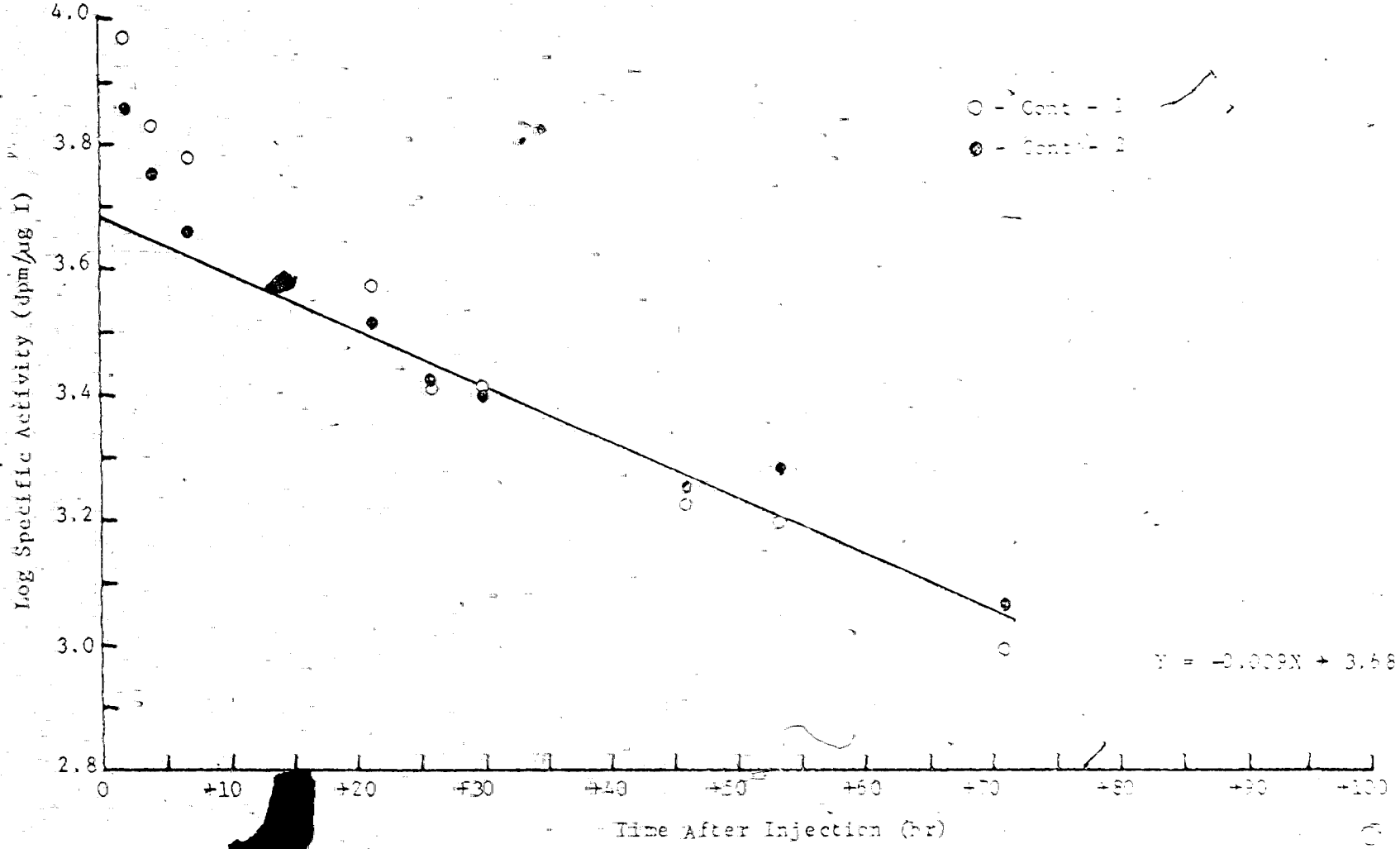


Figure 10. [redacted]roxine $-C^{14}$ disappearance during the chronic cold trials of Experiment II. (Each point is 1 measurment. Values are from the second set of measurements on this pair of sheep.)

C. Experiment III

Effects of Prior Chronic Cold Exposure on the Responses of Sheep to Acute, Severe Cold Stress

Responses to Cold Without Phenoxybenzamine

1. Heat Production and Body Temperatures

All mean values for these parameters can be found in Appendices XI and XII. The data is presented in figures 11A,C and 12A to 14A.

Prior chronic exposure to either a constant or a fluctuating cold environment appeared to have little effect on the metabolic response of sheep to a severe cold stress. Animals of all three groups had stable rates of heat production (figure 11A) and stable body temperatures (figures 11C, 12A-14A) during the last 1 to 2 hours before wind. This suggests that thermal equilibrium was re-established by this time.

Propranolol, given at the beginning of the sixth hour, resulted in a decrease in the heat production of all groups of animals. However, only the 19% decrease from pre-propranolol levels in the animals of the control group was statistically significant ($p < 0.01$).

2. Blood Hematocrits and Plasma Constituents

Means for all measured parameters can be found in Appendix XIII. The data is illustrated in figures 15A, 16A,C and 17A,C.

As with measurements of heat production and body temperature, prior chronic cold exposure of either type had little influence on changes in the blood parameters induced by acute, severe cold stress. This was true for both the patterns and extent of changes. Before beginning

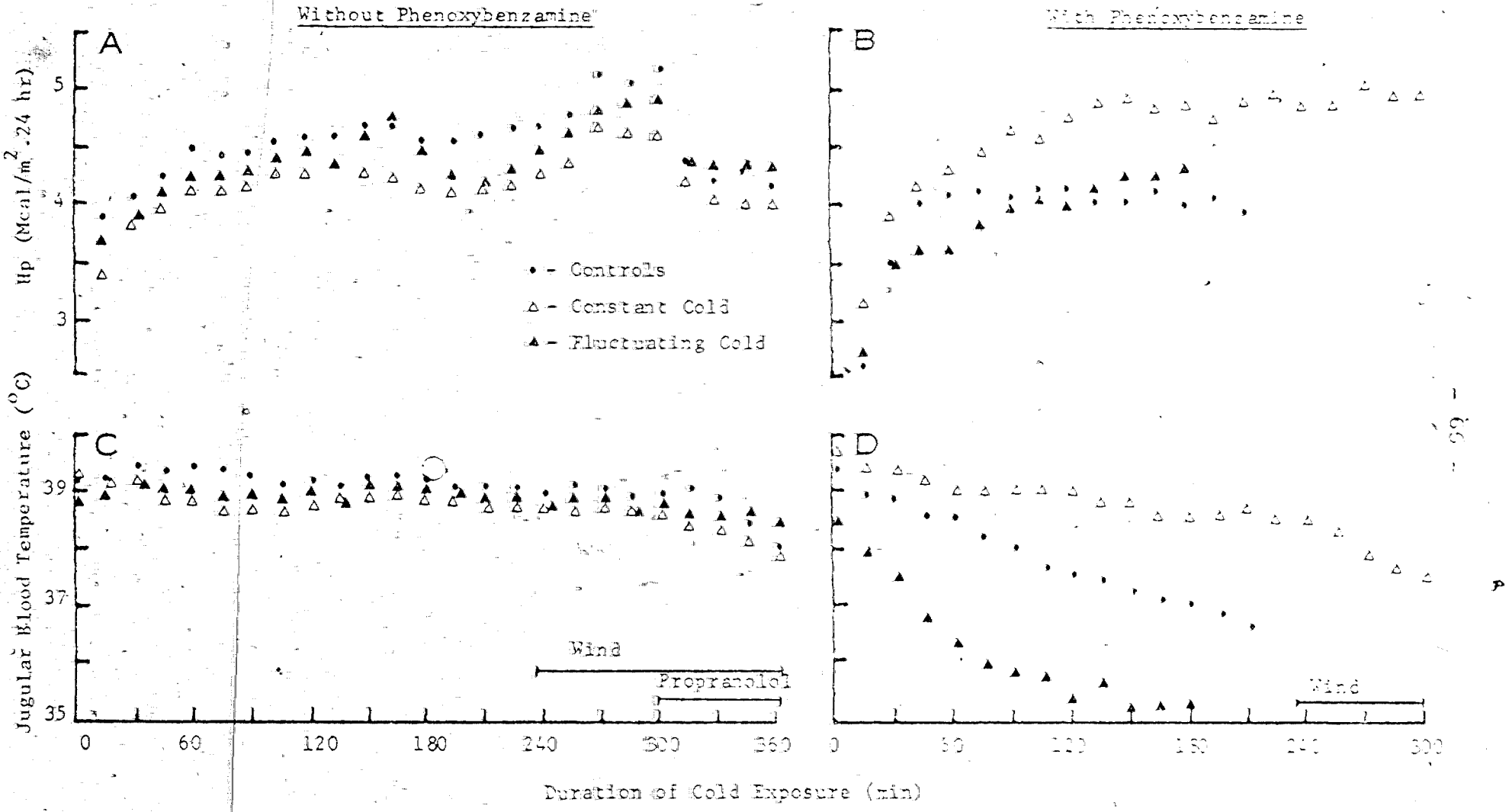


Figure 11. Values for heat production (Hp) and jugular vein temperature during the acute cold tests of Experiment III. (The values for the control animals without phenoxybenzamine are the means of 10 tests. The heat production values for the fluctuating cold animals in the tests with phenoxybenzamine are averages based on 3 tests. All other points are the means of 4 tests. Propranolol was given at a dosage of 1 mg/kg. Phenoxybenzamine was given at a dosage of 3 mg/kg.)

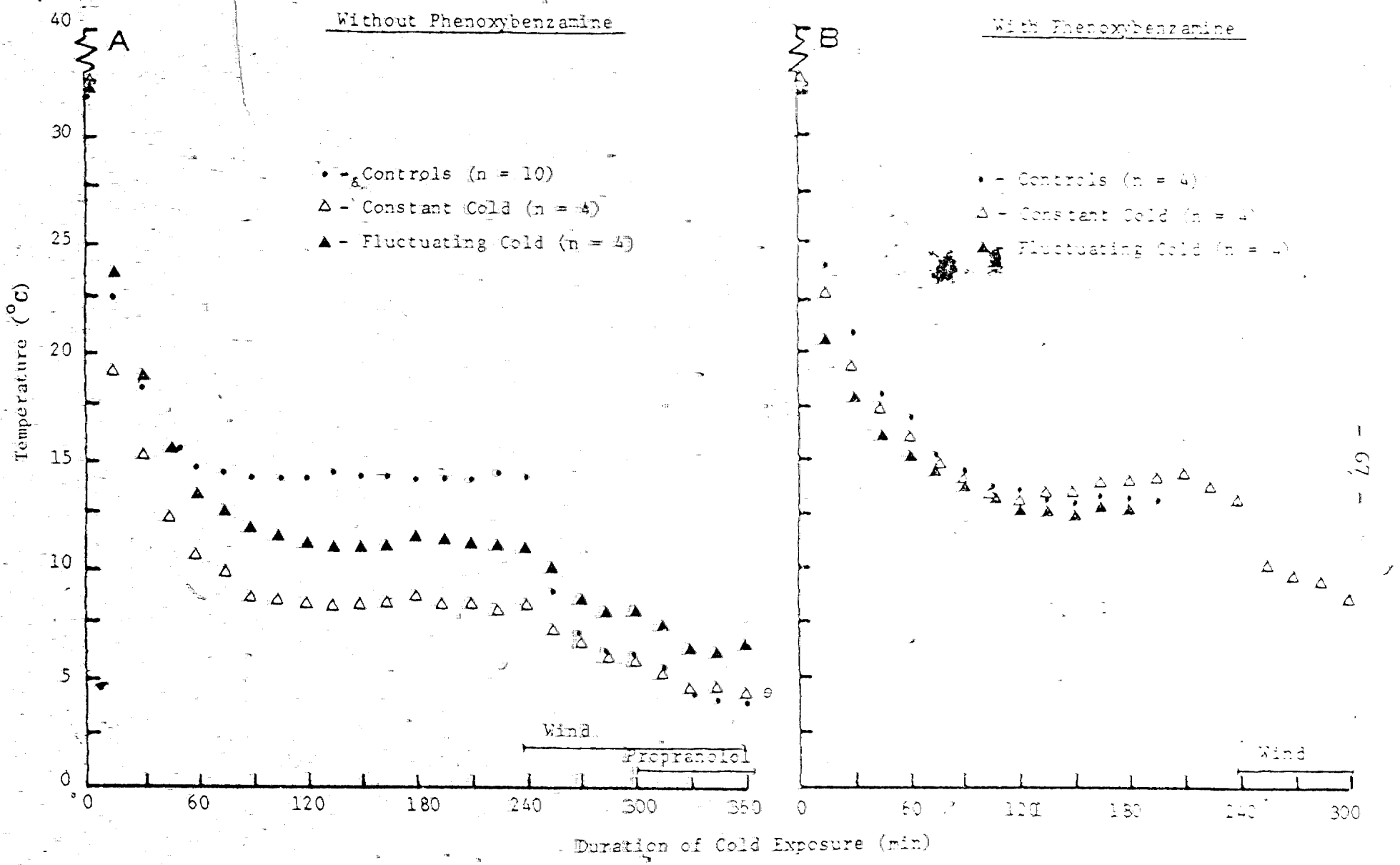


Figure 12. Values for the skin temperature of the trunk during acute cold tests (-25°C) of Experiment III. (Propranolol given at a dosage of 1 mg/kg. Phenoxybenzamine given at a dosage of 3 mg/kg.)

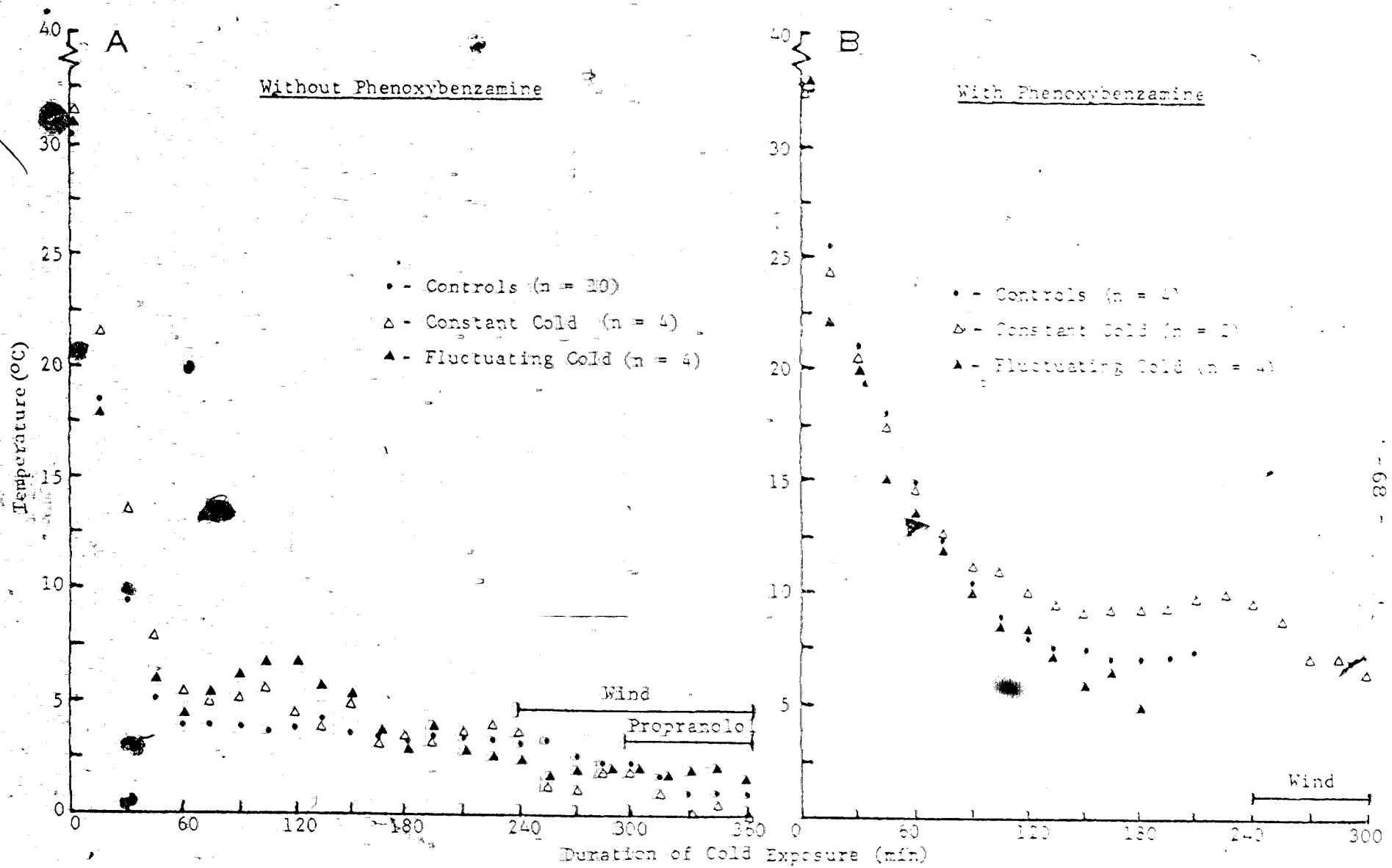


Figure 13. Values for the skin temperature of the legs during the acute cold tests (-28°C) of Experiment III. (Propranolol was given at a dosage of 1 mg/kg. Phenoxybenzamine was given at a dosage of 3 mg/kg.)

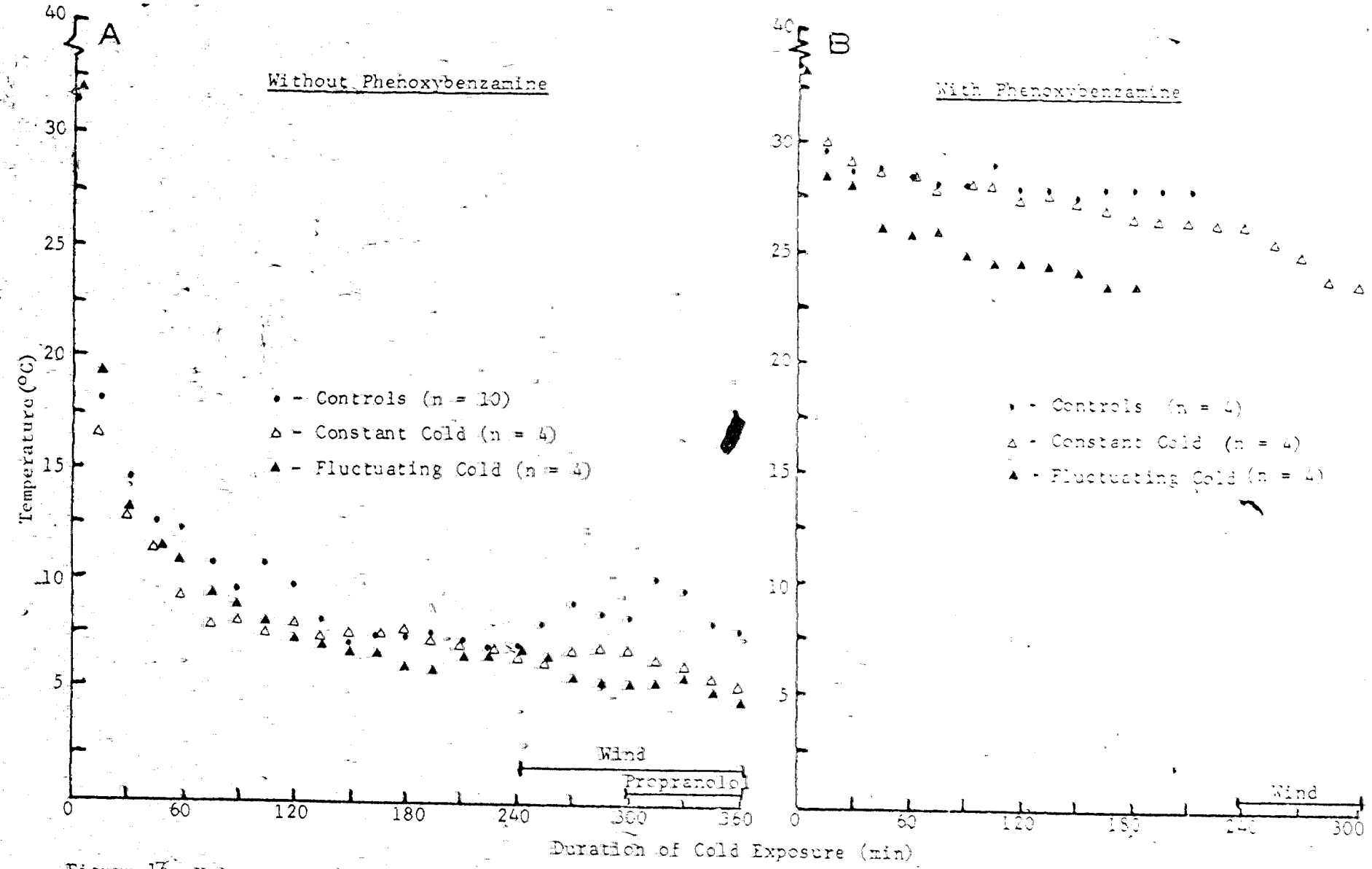


Figure 14. Values for skin temperature of the ears during the acute cold tests (-23°C) of Experiment III. (Propranolol was given at a dosage of 1 mg/kg. Phenoxybenzamine was given at a dosage of 3 mg/kg.)

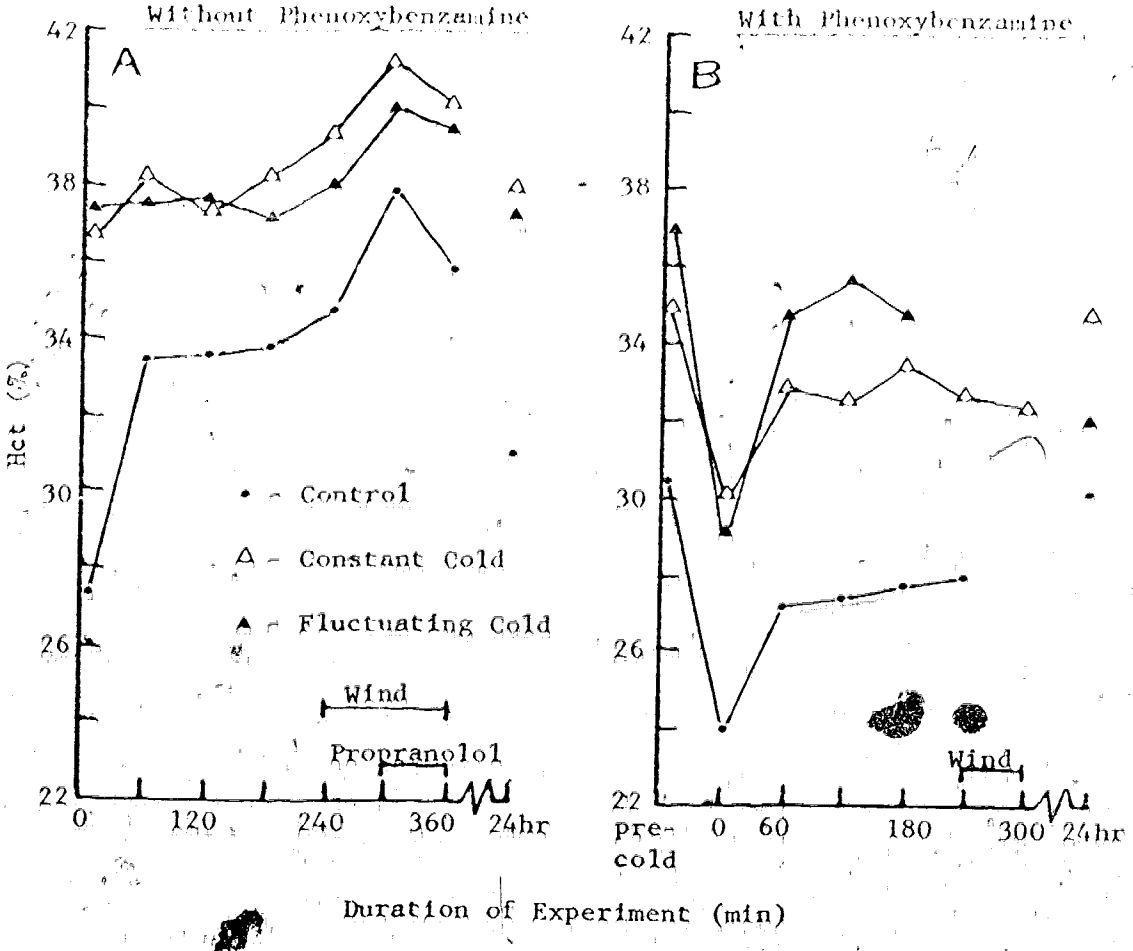


Figure 15. Values for hematocrit (Hct) during the acute cold tests (≈28°C) of Experiment III. (Points for controls without phenoxybenzamine are averages of 10 tests. All other points are averages of 4 tests. Propranolol was given at a dosage of 1 mg/kg. Phenoxybenzamine was given at a dosage of 3 mg/kg. Cold exposure lasted 180 to 360 minutes.)

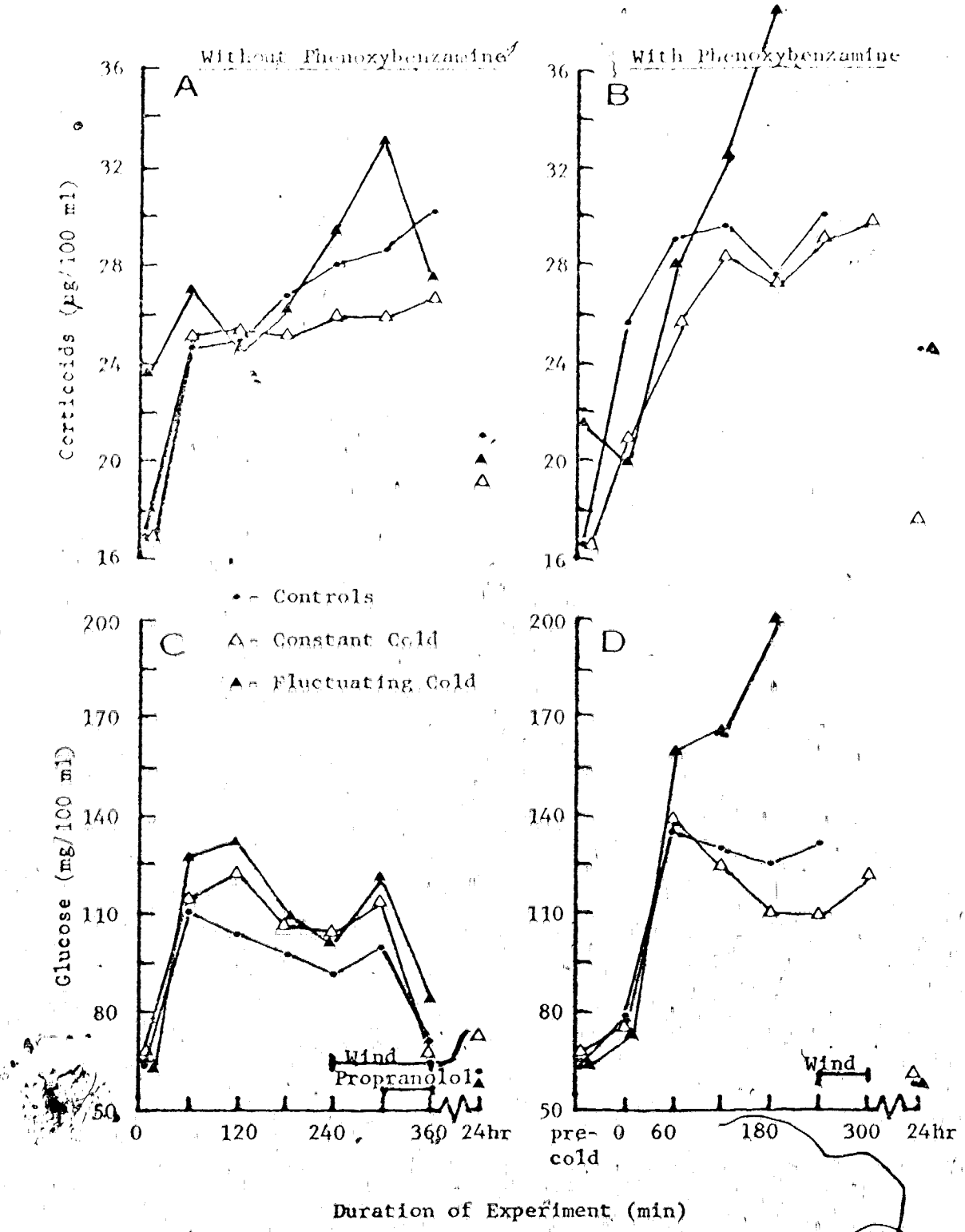


Figure 16. Values for plasma corticoids and glucose during the acute cold tests (-28°C) of Experiment III. (Points for controls without phenoxybenzamine are averages of 10 tests. All other points are averages of 4 tests. Propranolol was given at a dosage of 1 mg/kg. Phenoxybenzamine was given at a dosage of 3 mg/kg. Cold exposure lasted 180 to 360 minutes.)

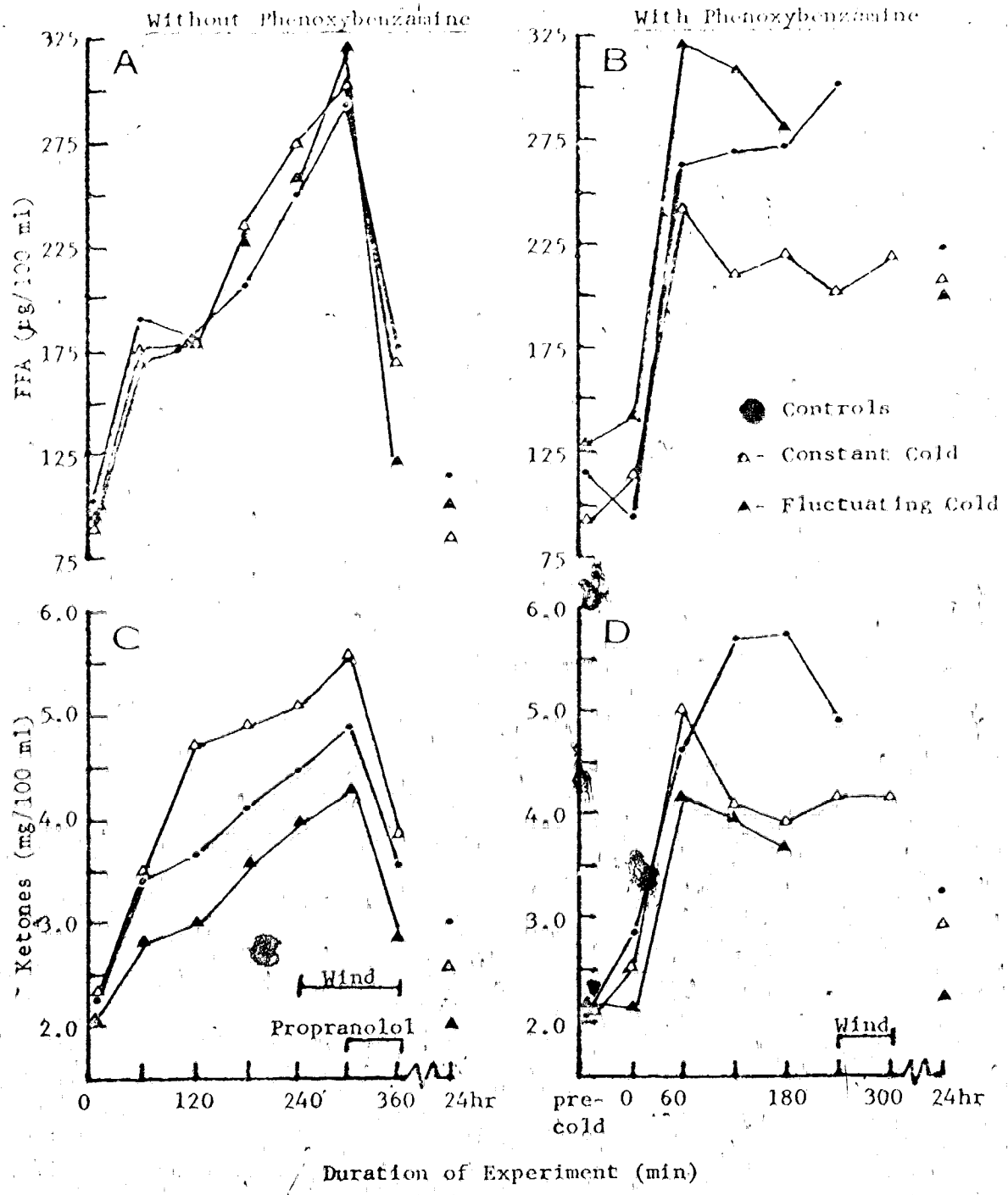


Figure 17. Values for plasma free-fatty acids (FFA) and ketones during the acute cold tests (-28°C) of Experiment III. (Points for controls without phenoxybenzamine are averages of 10 tests. All other points are averages of 4 tests. Propranolol was given at a dosage of 1 mg/kg. Phenoxybenzamine was given at a dosage of 3 mg/kg. Cold exposure lasted 180 to 360 minutes.)

the acute cold tests, the animals of both cold groups had significantly higher hematocrit values (figure 15A, $p<0.001$) than the animals of the control group. The animals of the FC group had significantly higher plasma levels of corticoids (figure 16A, $p<0.05$) than the animals of the CC and control groups. In both of these cases, the higher initial levels are a reflection of the higher levels seen throughout the acclimation period.

Responses to Cold After Phenoxybenzamine

All mean values for parameters measured in this section can be found in Appendices XIV to XVI.

1. Heat Production and Body Temperatures

Administration of phenoxybenzamine (3 mg/kg, i.v.) over a 60 minute period of time prior to cold exposure appeared to severely limit the ability of the FC and control animals to maintain thermal equilibrium during the subsequent acute cold tests. The animals of the CC group, on the other hand, seemed to be able to re-establish thermal equilibrium after a brief fall in deep body temperature (figure 11D). At the third hour of the cold tests, the rate of heat production by the CC animals was significantly higher than the heat production of either the FC or control animals (figure 11B, $p<0.05$). The apparent inability of the animals of these groups to produce more heat resulted in a rapid fall in deep body temperature (figure 11D).

2. Blood Hematocrit and Concentration of Plasma Constituents

Prior exposure to chronic cold appeared to influence, in only two instances, the changes in concentrations of the plasma constituents measured which were induced by severe, acute cold stress after treatment with phenoxybenzamine. Plasma corticoid concentration (figure 16B) continually

increased in the animals of the FC group during the entire (3 hr) cold test. At the third hour of exposure, plasma corticoids were significantly higher in these animals than in the animals of either the control or CC groups ($p<0.05$). A similar difference in plasma glucose concentration (figure 16D) was noticed. There was a continual increase in glucose concentration in the FC animals and at the third hour of exposure the concentration in these animals was significantly higher than the glucose concentration of the animals of the control and CC groups ($p<0.001$).

There were smaller differences in the plasma concentration of FFA between groups. However, there were large differences between animals as shown by the large standard deviations (Appendix XVI). In spite of the large variation between animals, the plasma concentration of FFA appeared to remain lower in the CC animals than in the control or FC animals (figure 17B).

V. DISCUSSION

A. Experiment II

Some Responses of Sheep to Constant and Fluctuating Chronic Cold Exposure

In view of the difficulties involved in the interpretation of blood or plasma concentration measurements alone, it might be difficult to place much biological significance on differences in levels of the blood parameters measured in the present experiments. One difficulty, that of the effects of psychological stress on some blood parameters, was discussed in the literature review. Even though the animals used in all experiments reported in this thesis were considered by this author to be well trained, the possibility exists that apparent changes within a group, or differences between groups, may have been due to excitability of one or more animals and not due to cold.

Changes in plasma volume could significantly influence the concentrations of plasma constituents. However, it has not been demonstrated in sheep whether or not changes in plasma volume do occur during acute or chronic cold exposure. Bass and Henschell (1956) have concluded, from their review of experiments involving mainly humans and dogs, that there are no changes in plasma volume during prolonged exposure to cold. Bass and Henschell (1956) did conclude that during acute cold exposure there is a small shift of water out of the circulatory system, probably amounting to no more than a 7 to 12% decrease in plasma volume. Thus, it seems likely that differences in concentrations of the plasma constituents measured in Experiment II of the present study cannot be attributed to a decrease in plasma volume. It is possible, however, that decreases in plasma volume may have contributed to some of the increases in

concentration measured in Experiments I and III. It is also possible that variation in the degree of change in plasma volume, if occurring at all, may partially account for some of the small differences in the concentrations of the measured plasma constituents between groups of animals in Experiment III. In view of the large changes in concentration which occurred in all groups of animals, it would be unlikely that a decrease in plasma volume could account for all of the changes which were observed.

The value of measurements of the concentrations of various plasma constituents is limited. There have been few instances where relationships between plasma levels of a substance and rate measurements, such as secretion rate and turnover rate, have been established for large animals exposed to cold. It cannot be definitely stated what causes a change in plasma levels unless rate measurements have been established. It is possible, for instance, that in a steady state the turnover rate of a substance such as glucose is the same in two animals with different plasma levels of glucose.

Measurements made of concentrations of plasma constituents in this study were not expected to yield precise, quantitative information concerning the rate of production or utilization of different metabolites in different environments. These measurements, as well as the other measurements made, have been of value in revealing that certain differences do exist between the changes induced in sheep during exposure to constant and fluctuating cold environments.

The feed intakes and weight gains of the sheep in the chronic cold trials (Experiment II) suggested that all groups of animals successfully adjusted to their environment. The constant cold animals ate and gained

more than the fluctuating cold animals. This is similar to what Webster et al (1969b) found. In their experiment sheep exposed to constant cold indoors gained weight almost as efficiently as control animals. Animals exposed to the winter weather were much less efficient.

In the present experiment there were no differences in plasma concentration measurements, with the exception of plasma corticoids, between animals exposed to constant cold and animals exposed to fluctuating cold. The greater plasma corticoid concentration in the animals of the FC group (figure 9C) may be an indication of greater adrenal cortical activity in that group. Adrenal cortical activity is known to increase, at least initially, during cold exposure (Boulouard, 1966). Several indices, including plasma concentration, have been interpreted as reflecting increased activity. This topic was reviewed in the literature review. If adrenal cortical activity was, in fact, higher in the animals of the FC group throughout the cold exposure period, the results are contrary to results found in studies with rats where adrenal activity returned to normal after 2 to 3 weeks in the cold (Boulouard, 1966; Strow and Freely, 1967). In both of these investigations, plasma concentrations and another index of activity were measured; in vitro secretion of adrenal corticoids was measured in the first, and response to a standard stress was measured in the second study. If one assumes that the fluctuating cold environment, to some extent, simulates the fluctuating stresses of winter, the results agree with those of Yousef et al (1971) who found adrenal cortical hyperactivity in reindeer during the winter in Alaska compared to the activity found in spring and summer. Secretion rate and plasma concentration were used by Yousef et al (1971) as indices of adrenal function in reindeer. It is possible that any adrenal hyperactivity in the CC animals may have been brief and

could have returned to normal by the time the first blood samples were taken during the first week of cold exposure. It is interesting to speculate that even though all plasma measurements, except corticoids, showed no difference between the FC and CC groups of animals, the higher corticoid level in the FC animals was an indication that the fluctuating cold environment was more stressful than the constant cold environment.

The differences in levels of some blood parameters between the control animals and the animals of both cold groups indicate that the animals exposed to cold did respond to the cold stress imposed on them.

The greater PBI concentrations of the animals of both cold groups (figure 9B) and the shorter plasma half-lives of labelled thyroxine (table 5) in 6 of 8 cold exposed animals may be indicative of increased turnover rates of thyroxine in these animals compared to the control animals. However, it must be recognized that secretion rate cannot be calculated without knowing the volume of distribution and that the measurements made in this experiment are only an indication of a greater secretion of thyroxine. Half-life of thyroxine in the plasma is only a measurement of the rate at which labelled thyroxine disappears from the blood.

The significance of the effect of cold on the half-lives of thyroxine in the animals of the CC group is questionable because of the very marked difference between the two pairs of animals: The pair on which thyroxine half-life was determined during the first and fourth weeks had an average half-life about 30% shorter than the pair of animals used during the second and fifth weeks. It would appear that this difference was due to animal variation. The average half-life of thyroxine for the

other pair of animals used during the second and fifth weeks was 36.5 hours compared to 35.8 hours for the control animals.

The results of the present experiment, except for the pair of CC animals used during the second and fifth weeks, are similar to the results found for cold exposed sheep by Frehkel and Lewis (1957). In their studies the half-life of thyroxine was about 25 hours for cold exposed animals and about 37 hours for control animals. Plasma PBI levels were slightly higher and the thyroxine distribution space was slightly lower in the cold exposed animals. It was calculated that the cold exposed sheep degraded about 70% more thyroxine per day than the controls. Yousef et al (1967) found increased plasma PBI levels and shorter half-lives of thyroxine and suggested that thyroid activity was increased in cold exposed cattle. Cold exposure in the study of Yousef et al (1967) lasted only 4.5 days which is probably not long enough for cold acclimation to develop (Gelineo, 1964). The results for the plasma PBI levels of the sheep in the present experiment differ from the results found for sheep by Halliday et al (1969), who found little change in PBI after 12 days of cold exposure. No other indices of thyroid function were measured in the study of Halliday et al (1969). Irvine (1967) found no significant difference in PBI levels and half-lives of thyroxine between cold acclimated and control horses. Thyroxine secretion rate was 17% higher in cold acclimated horses than in control animals. In the same study, horses exposed acutely to cold had a much higher PBI level, about the same thyroxine half-life, and a much greater (+65%) thyroxine secretion rate than control animals (Irvine, 1967).

Results from the literature are variable in the assessment of thyroid activity in large animals exposed to cold. Because of this, care must be taken in

the interpretation of the different indices of thyroid activity. The only conclusion that can be made from the measurement of thyroxine half-lives in the present study is that, under both cold conditions, thyroxine appeared to disappear faster from the blood, although this was only true for 6 of 8 animals. This result, together with the higher plasma PBI levels in these animals, could be indicative of an increase in thyroxine turnover.

The difference in blood hematocrit levels (figure 9A) between the animals of both cold groups and the control animals was probably due mainly to differences in the number of circulating red blood cells. Blood hematocrits would be expected to rise as the result of increased sympathetic activity found in cold exposed sheep (Webster et al, 1960a). It has been shown that both epinephrine and norepinephrine will cause splenic contractions, which will result in an increased number of circulating red blood cells (Milnor, 1968). The difference in hematocrits in the present experiment between the control animals and the cold exposed animals was a little greater than the increase measured by Mears (1966) in sheep exposed to a moderate cold stress and similar to the increase found by Gartner, Ryley, and Beattie (1965) in cattle subjected to visual stimulation. It is not known whether an increased hematocrit during moderate cold exposure serves a useful purpose or is just a coincidental response due to increased sympathetic activity.

B. Experiment I

Some Responses of Warm Exposed Sheep to Acute, Severe Cold Stress; Effects of the Additional Stress of Wind and of the Sympatholytic Drug, Propranolol

1. Heat Production and Body Temperature

The rate of heat production by the animals of

Experiment I, once thermal equilibrium was re-established during acute cold exposure, averaged about 4.5 times the estimated rate of basal heat production. Since there were no significant decreases in deep body temperature during severe cold exposure alone, it must be concluded that summit metabolism, as defined on page 6, was not reached. Giaja (cited by Gelineo, 1964) has defined the ratio of summit metabolism to basal metabolism as the metabolic quotient. Even though summit metabolism was not reached in the present experiment, metabolic quotient is still a useful value to compare the present results with results for sheep obtained by others under similar conditions. The present metabolic quotient of 4.5 compares favorably with that of 4.7 measured by Hays (1968). It is doubtful whether the warm acclimated sheep in the experiments of Hays (1968) had reached summit metabolism. Alexander (1962a) found a metabolic quotient of about 5 for young lambs which had reached summit metabolism. An estimated metabolic quotient of 5 was also found by Sykes and Slee (1968) for sheep. Heat production was estimated in the work of Sykes and Slee (1968) from heart rate and as a consequence care must be taken in interpreting the results. Greater decreases in rectal temperature were observed in the studies of Sykes and Slee (1968) than in the present experiments, but only after several hours of severe cold exposure when exhaustion could have occurred in the animals.

The purpose of the wind in Series 3 and 4 of Experiment I (figures 5 & 6) was to increase the intensity of the thermal demand at a time when the animals were already severely stressed by cold. This tested the capacity of the sheep to further elevate their heat production at a time when they might be expected to be approaching exhaustion. In Series 3 and 4 there were increases in heat production of about 2% and 12% respectively. Neither of the increases

was statistically significant. The drop in rectal temperature as a result of imposition of wind was not large, although it was a little greater than the drop over the previous 4 hours of cold exposure: 0.5°C compared to 0.4°C (figure 7). It appeared that the sheep tolerated a small fall in deep body temperature, although thermal equilibrium was not established during the hour with the wind. It is possible that summit metabolism had been reached and that rectal temperature would have continued to fall. The metabolic quotient at this time was about 5. No published reports were found on experiments with wind which were sufficiently similar to be compared with the present experiment.

The 28% decrease in heat production after the administration of propranolol to the sheep during the acute cold tests of series 4 (figure 6) appeared to be very significant, as rectal temperature fell 1.2°C during the last 30 minutes of cold exposure. Furthermore, there was no tendency for rectal temperature to begin to stabilize. The percentage decrease in heat production was about three times the percent decrease associated with propranolol during the acute cold trials of Webster and Hays (1968) and Webster et al (1969a) who observed no significant drop in rectal temperature during the cold exposures. In these two experiments propranolol (1 mg/kg) was infused over a 15 minute time period just prior to cold exposure. Since it was shown by Webster and Hays (1968) that propranolol is fully effective for only 1 hour, it is reasonable to assume that in this experiment the effectiveness of the drug was beginning to wear off some time during the first hour of cold exposure. This may account for the lower percentage reduction in heat production during the experiments of Webster and Hays (1968) and Webster et al (1969a) compared to the present experiment. Propranolol has been shown to reduce

heat production during cold exposure in such animals as mice (Estler and Ammon, 1969), newborn rabbits (Heim and Hull, 1966), and young lambs (Alexander and Williams, 1968). A reduction in cold induced thermogenesis has also been demonstrated in young guinea pigs after the administration of pronethalol, which, like propranolol, is a beta-adrenergic blocking agent (Bruck, 1970).

Some evidence exists which suggests that the proportion of heat production in the cold that is blocked by propranolol is the non-shivering component, and that propranolol can be used to assess amounts of cold induced non-shivering thermogenesis (NST) (Alexander and Williams, 1968; Bruck, 1970; Estler and Ammon, 1969; Heim and Hull, 1966). In mice (Estler and Ammon, 1969) and in guinea pigs (Bruck, 1970) propranolol did not appear to interfere with shivering, but the drug did appear to depress some shivering in young lambs (Alexander and Williams, 1968).

Warm exposed animals in the present study shivered vigorously during the acute cold tests. Because no attempt was made to actually measure shivering, it is very difficult to assess possible changes in the degree of shivering. It appeared that propranolol had no limiting effect on shivering, nor was shivering visibly increased to compensate for the decrease in thermogenesis. However, the sheep appeared to exhibit more voluntary movement, such as stamping of feet, after propranolol was given. It probably cannot be concluded from the above that cold induced NST accounted for 28% of the total heat production in the cold in the present experiments, since in Experiment III the effect of propranolol on the cold exposed animals was not different from the effect on the control animals. Thus, in sheep, there was no indication of the increased sensitivity to the effects of propranolol seen in animals that are known to exhibit NST.

There are two other important reasons why propranolol would be expected to reduce heat production in animals near summit metabolism. By blocking beta-adrenergic receptors, propranolol has been shown to limit cardiac acceleration during exercise (Cronin, 1967; Cumming and Carr, 1966) and cold exposure (Heitman, unpublished observations; Webster and Hays, 1968). By limiting heart rate, propranolol could also limit cardiac output and during times when a high rate of metabolism is necessary, the cardiac output may be insufficient to sustain a high metabolic rate. Propranolol has also been shown to have a limiting effect on substrate mobilization by limiting glycolysis and lipolysis (Ellis, 1967; Himms-Hagen, 1967). The results of the present study support this and will be discussed in more detail later.

2. Blood Hematocrit and Plasma Constituents

Large increases occurred in the concentrations of all plasma constituents measured and in hematocrit values (figures 3-6) during the exposures of the sheep in Experiment I to acute cold. Looked at as a whole, these changes are suggestive of a general mobilization of energy substrates to meet the metabolic demands of cold stress.

The average maximum plasma glucose concentrations in all of the series of acute cold trials with normal sheep were much lower than those found by Halliday et al (1969), although the patterns of response were very similar. The initial increases in glucose concentration were probably due to increased secretion of adrenaline and, to a lesser extent, noradrenaline as suggested by Bassett (1970), Himms-Hagen (1967) and Maickel et al (1967). The hyperglycemia caused by the catecholamines results primarily from a release of glucose from the liver and from a decreased peripheral

utilization of glucose caused by the inhibition of insulin secretion by adrenaline (Himms-Hagen, 1967). It might be expected that, after a delay, the increased concentration of the adrenal corticoids (Figures 4-6) would add to the glucose increase and help to maintain a higher plasma glucose concentration through their effect of increasing gluconeogenesis. It has been postulated that one purpose of increased adrenal activity is to help to increase the supply of glucose in the plasma from body reserves during periods of increased metabolism before feed intake is increased (Boulouard, 1966). The decreases in glucose concentration following peak levels may reflect a readjustment in the rates of glucose mobilization and utilization, and a glucose sparing effect of increased FFA metabolism. It is also possible that the decreases in plasma glucose could be due to a depletion of glycogen reserves. Information on glucose entry and utilization rates would be needed in order to fully explain the changes in plasma glucose concentrations. At the same time glucose concentrations were declining, plasma FFA and ketones were continually increasing. The patterns of changes for these were very similar to those found by Halliday et al (1969).

The steadily increasing plasma concentration of FFA during cold exposure is difficult to explain. From the conclusions of West and Annison (1964) and Armstrong et al (1961), increasing concentrations would imply that more and more FFA were being oxidized. In the present study, heat production had stabilized within 2 hours, but FFA concentration continued to increase for as long as 6 hours in Experiment I, Series 2 and 3, and 4 hours in Series 4. However, the experiments of West and Annison (1964) and Armstrong et al (1961) involved studies of fat metabolism in normal and undernourished animals, and it is possible their results cannot be compared to the results of the present study. An impairment

of utilization, along with continual mobilization, could account for the rise. It might also be possible that there was a change in the relationship between FFA concentration and FFA oxidation. Another explanation might be that, in order to conserve glucose, comparatively greater amounts of FFA were oxidized. If less and less glucose was being oxidized and was reflected by constantly decreasing plasma glucose concentrations after the initial peak, proportionately more FFA would have to be oxidized and this might be reflected by continually increasing plasma concentrations of FFA. Trials involving the determination of utilization rates of these energy substrates would have to be carried out to fully explain the changes in plasma levels observed in the present study.

The percentage increases in plasma ketones were much smaller in the present studies than in the study of Halliday et al (1969) who only measured blood acetone. In the present study total plasma ketones were measured, but they were expressed in terms of acetone. Although the increases in plasma ketones in the present study might be large enough to indicate some impairment of glucose metabolism, the levels are certainly not high enough to indicate ketosis (Karihaloo et al, 1970). Ketones themselves can be an important source of energy (Krebs, 1966) and it is likely that the increases in plasma concentration of ketones in the present experiment are due to an increase in FFA oxidation. This will occur especially if glucose is in limited supply (Krebs, 1966). However, in the present experiment ketones increased despite ample plasma glucose levels (figures 3, 5, & 6).

As previously mentioned, propranolol is known to have a limiting effect on energy substrate mobilization. When propranolol was given to young lambs during summit metabolism, plasma FFA concentrations fell drastically,

while smaller and more variable decreases in plasma glucose occurred (Alexander, Mills, and Scott, 1968). Propranolol has also been found to interfere with substrate mobilization in other species including rats, dogs, and man (see review, Himms-Hagen, 1967). A recent study by Werrbach et al (1970) has shown that propranolol lowers both plasma glucose and FFA concentrations in baboons at thermoneutral temperatures. In the present experiments, the greatest effect was on plasma FFA concentrations. In Experiment I, Series 4, FFA decreased by 57% from pre-propranolol levels. Results from the other series of cold tests would indicate that the levels should have increased, or at least stayed the same, during the sixth hour of exposure. There appeared to be a greater than normal decrease in plasma glucose after propranolol was given. However, the results are very difficult to interpret because plasma glucose concentrations were normally declining at this time as can be seen from the trials without propranolol.

C. Experiment III

Effect of Prior, Chronic Cold Exposure on Responses of Sheep to Acute, Severe Cold Stress

1. Cold Tests Without Phenoxybenzamine

There were no instances where the responses of the different groups of animals to an acute cold test (-28°C) appeared to be significantly different from each other. It is possible that differences, especially in summit metabolism, might have become apparent at even lower air temperatures since summit metabolism was not reached by any of the animals in the present experiment.

2. Cold Tests with Phenoxybenzamine

Even though the results previously discussed have failed to reveal any large differences between groups of sheep with different thermal histories, results for the cold tests after phenoxybenzamine administration suggest the existence of some type of metabolic adaptation in the animals of the CC group. This was not apparent during severe cold exposure ($-28^{\circ}C$) alone.

Phenoxybenzamine (PBA) is a long acting, alpha-adrenergic blocking agent and one effect of this drug is a blockade of the vasoconstrictor action of noradrenaline (Nickerson, 1949). Normally during severe cold exposure, maximum vasoconstriction should occur which will be especially noticeable in the appendages where skin temperatures fall to only a few degrees above freezing (Meyer and Webster, 1971; Webster and Blaxter, 1966). After PBA is administered, higher skin temperatures might be expected during cold exposure due to the blockade of vasoconstriction. In the present study, all skin temperature measurements of the legs (figure 13) and ears (figure 14) were warmer during the cold tests with PBA compared to cold tests without PBA. Ear temperatures were especially warm. Because of the warmer appendages during the tests with PBA, more heat would be lost to the environment, and in order for thermal equilibrium to be established more heat would have to be produced during the cold tests with PBA compared to the cold tests without PBA. Skin temperatures of the trunk were not markedly affected.

Phenoxybenzamine appears to have significantly reduced the capacity for cold thermogenesis in the animals of the control and FC groups. Neither of these groups of animals was able to offset the increased heat loss, as rates of heat production were the same or lower during the cold

tests with PBA than during the cold tests without PBA (figure 11). The opposite was true for the animals of the CC group. At the third hour of the cold tests with PBA, the rate of heat production of the animals of the CC group was significantly higher than the rates of heat production of the FC and control animals ($p<0.05$) and higher than the heat production at an equivalent time during the cold tests without PBA. There are no other reports of any effects of phenoxybenzamine on metabolism of animals other than small mammals and young lambs. After administration of phenoxybenzamine, a reduction in rate of heat production during cold exposure was found for young lambs by Alexander and Williams (1968). Leduc (1961) found that all rats died when exposed to cold after adrenergic blockade produced by PBA. However, Leduc (1961) found that cold acclimated rats survived for several days, whereas warm acclimated rats survived for less than 1 day. There was a very large increase in urinary excretion of catecholamines by rats after administration of PBA (Leduc, 1961). It has also been shown that PBA reduces noradrenaline induced thermogenesis in rats (Johnson and Sellers, 1961). It appears that in animals that respond to noradrenaline with an increase in heat production, PBA may reduce the rate of heat production by interfering with the stimulation of thermogenesis by noradrenaline. In older sheep, which show no increase in heat production during noradrenaline infusion (Webster et al, 1969a), it would appear that PBA affects heat production in a different manner.

Some recent reports might suggest a possible mechanism of action of phenoxybenzamine which could account for the observed effects. In 1970, Gale et al suggested that alpha-adrenergic blockade might inhibit shivering. Mejsnar and Jansky (1971) showed that the heat production of muscle was increased when noradrenaline was infused into the muscle. In 1972, Maxwell and Sumptor suggested that noradrenaline

receptors (presumably alpha-receptors) in the spinal cord control muscle spindle activity. These studies might suggest that phenoxybenzamine acts by blocking a direct effect of noradrenaline on shivering. Thus, the reduced heat production of the phenoxybenzamine treated FC and control animals of Experiment III might have been caused by reducing the heat production of muscle. This might also tend to cause an accumulation in the plasma of substrates of energy metabolism unless there was a reduction in their mobilization. These few reports from the literature suggest that PBA inhibits shivering and energy metabolism in muscle. In the present study an effect of PBA on shivering was not observed, but glucose, particularly in the FC group of animals (figure 16C), and FFA (figure 17B) did appear to accumulate more rapidly in the plasma of acutely cold exposed sheep treated with PBA than in the trials without PBA treatment. Such accumulation might have been a result of interference with energy metabolism in muscle, but it could have also been caused by the catecholamine releasing effect of PBA which was suggested by Scopes and Tizzard (1963). A transient increase in plasma catecholamine levels would result in an increased mobilization of FFA and glucose. This effect of catecholamines is known to occur in rats (Himms-Hagen, 1967; Wenke, 1966), in adult sheep (Bassett, 1970), and in lambs (Alexander et al, 1968). It would be expected that such increases in mobilization would result in increased blood levels of FFA and glucose since the mobilization was caused by a drug and not necessarily by a need for increased amounts of these two energy substrates. An increased need would increase utilization and tend to, at least partially, offset an increased mobilization and subsequent increased plasma levels.

The precise mechanism of action of PBA during acute cold exposure cannot be explained from the results of this study. Clearly, there was a marked difference in effect of PBA on the animals of the constant cold group

compared to the animals of the other two groups. It is not known whether heat production was reduced in the control and FC groups because of a reduced cellular uptake of energy substrates or because of a direct limitation on the utilization of the substrates or a combination of both. As a first step in obtaining an indication of the mechanism of action of PBA, FFA and glucose turnover rates and flow rates should be measured in animals undergoing acute cold exposures and treated with PBA.

VI SUMMARY AND CONCLUSIONS

In this study, three aspects of the response of sheep to cold have been considered. The first aspect was the effect on sheep of chronic cold exposure. Two types of chronic exposure were studied. In the first, the exposure to cold was continuous and in the second the sheep were exposed for 12 hours each day to a greater intensity of cold than those exposed to cold continuously for 24 hours. Both environments were calculated to impose approximately equal 24 hour thermal demands. During the chronic cold study, lasting 6 weeks, the animals of both cold groups ate more than the animals of the control group. Animals of all groups appeared to adapt to their environments. Few differences existed in concentrations of blood parameters between any of the groups. Blood hematocrit values were significantly higher in both cold groups compared to the controls. From measurements of plasma concentrations of protein bound iodine (PBI) and the half-lives of thyroxine in plasma, there was some suggestion that thyroid activity was increased in 6 of the 8 animals in the cold groups. Plasma glucose, free-fatty acids (FFA), and ketones appeared to be elevated in the animals of both cold groups during the first week of cold acclimation which might be an indication of increased mobilization of glucose and lipids. Plasma corticoid concentration was highest in the animals of the fluctuating cold group. Although somewhat variable, plasma corticoid concentrations were similar in the constant cold and control groups.

The second aspect of cold stress studied was the response of warm exposed sheep to acute, severe cold ($-28^{\circ}C$). All parameters measured were related to the obvious need to produce more heat to maintain thermal

equilibrium. Heat production was calculated to be approximately 4.5 times the estimated fasting heat production of adult sheep. All measurements of temperature stabilized, along with heat production, indicating all animals were able to re-establish thermal equilibrium during the acute cold tests. The exact explanation for the patterns of changes of glucose and FFA cannot be given. However, from the large increases in plasma corticoid concentrations in these animals and the known increase in the secretion of catecholamines during cold exposure in small mammals, it might be concluded that, at least initially, there were large increases in mobilization of glucose and FFA. Subsequent decreases in plasma glucose suggest that either mobilization decreased or utilization increased relatively more than mobilization. The continued increase in the FFA concentration may have been due to a continued high rate of FFA mobilization. There were also large increases in plasma corticoid concentrations.

The third aspect investigated was the influence of prior thermal history on the response to acute, severe cold (-28°C). There were only small differences in the changes in metabolic response and in blood measurements between the groups when they were exposed to cold without blockade of the sympatho-adrenal system. The effects of the alpha-adrenergic blocking agent, phenoxybenzamine (PBA), administered during one set of cold tests to all sheep in Experiment III, appear to have demonstrated a clear difference between the constant cold animals and the animals of the other two groups. The control and fluctuating cold animals were unable to re-establish thermal equilibrium at any time during the cold tests with PBA and their heat production was lower than it was in the acute cold without PBA. The constant cold group was able to re-establish thermal

equilibrium about 1 hour after the start of the cold exposure. Their rate of heat production was higher at this time than during the cold tests without PBA and significantly higher than the heat production of the other two groups. Plasma glucose and corticoid levels were extremely high in the fluctuating cold group. It can be concluded that PBA had a limiting effect on the maximum heat production of the control and fluctuating cold animals, but not of the constant cold animals. The mechanism of this effect is not known.

Under the conditions of these experiments the following general conclusions can be drawn:

1. Shorn sheep were able to adapt to living in two types of cold environments with approximately equal 24 hour thermal demands. There were some differences between the adjustments made to the two environments. However, it is impossible to draw definite conclusions from measurements of concentrations of the plasma constituents studied.

2. Warm and cold exposed sheep were able to re-establish thermal equilibrium when exposed for up to 6 hours to a severe cold stress (-28°C). Large increases in heat production and a lowering of skin temperatures, indicative of vasoconstriction, occurred. Large increases in concentration of the various plasma constituents were suggestive of increases in mobilization of lipids and glucose. Reasons for subsequent decreases in glucose are not known. It is possible that even lower air temperatures

may have resulted in differences between warm and cold exposed animals.

3. Changes in the response to severe cold stress after administration of phenoxybenzamine to warm and cold acclimated sheep suggested the existence of some type of metabolic adaptation in animals exposed to a continuous cold environment that was not present in the animals acclimated to fluctuating cold and that was not apparent in the constant cold animals during acute, severe cold stress alone. There appeared to be a limitation of heat production in the animals of the control and fluctuating cold groups as a result of phenoxybenzamine.

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APPENDIX I

Description of Sheep Used in the Experiments

Experiment	Sheep No.	Group	Breed	Sex	B.W. (kg)[1] I	II	III
I- Series 1	12y		S[2]	W[4]	73.5		
	59y		S	W	86		
	27y		S	W	85.3		
	65y		S	W	79.2		
	88y		S	W	81		
I-Series 2	48		L[3]	E[5]	81.5		
	400		L	E	86		
	308		L	E	75.5		
	487		L	E	86		
I-Series 3	65y		S	W	96.5		
	88y		S	W	98		
I-Series 4	48		L	E	89.5		
	308		L	E	70		
	1028		L	E	83		
	400		L	E	78		
II & III	8230	CC[6]-1	S	W		74.5	77
	8240	CC -2	S	W		72	73
	8281	CC -3	S	W		58	59
	8284	CC -4	S	W		72	72
	8287	FC[7]-1	S	W		83	84.5
	8292	FC -2	S	W		79.5	80.5
	8286	FC -3	S	W		77.5	73
	8288	FC -4	S	W		77.5	77
	8289	Cont[8]-1	S	W		68.5	73.5
	8285	Cont -2	S	W		72	75.5
	8145	Cont -3	S	W			44
	8196	Cont -4	S	W			67.5

[1] Body Weight [3] Lincoln [5] Ewes [7] Fluctuating Cold

[2] Suffolk [4] Wether [6] Constant Cold [8] Control

APPENDIX II

Photometric Adaptation of Dole's Microdetermination of Free Fatty Acids [1]

From: Mosinger, E. J. Lipid Res. 6: 157-159.

Reagents and Equipment

1. Extraction Mixture

   40 parts Isopropanol
   10 parts Heptane
   1 part 1 N H_2SO_4

2. Stock Buffer

   0.5% Phenol Red in 0.12M Sodium Barbital

3. Color Reagent

   99 ml absolute Ethanol
   200 ml Heptane
   1 ml stock buffer

4. Standard Palmitate Solution

   0, 0.5, 1.0, 1.5, and 2.0 μmoles/ml in heptane

5. Matched set of cuvettes and a set of screw capped test tubes - 19 x 150 mm.

6. Spectronic "20" colorimeter(Bausch and Lomb).

Procedure

1. Place 0.5 ml of plasma, blank heptane, or standard in a screw-capped test tube and add 6 ml of extraction mixture. Shake for 1 to 2 minutes.

2. Add 3.6 ml of heptane and 2.4 ml of distilled water. Shake for 1 to 2 minutes and allow phases to separate.

3. Pipette 4 ml of the upper phase into a cuvette.

4. Bubble N_2 through the solution for 10 seconds and add 6 ml of color reagent while continuing to bubble N_2 through the solution for a further 15 seconds. Immediately stopper the cuvette.

5. Allow each sample to stand for 40 minutes and then read all cuvettes at 560 mμ on the colorimeter. Expand the scale by setting the blank at 10% transmission and the 2.0 μmole standard at 80% transmission.

6. A wider range of concentration can be analyzed by increasing the concentration of sodium barbital in the stock buffer from 0.12M to 0.15M. Standards as high as 5 μmole/ml can be analyzed. The highest standard used should be set on 80% transmission.

APPENDIX III

Procedure for the Determination of Ketone Bodies in Plasma

From: Baker, N. and R. White. 1957. Simplified micro-method for the colorimetric determination of total ketone bodies in blood. New Zealand J. Sci. Tech. 38: 1001-1008. Also in Chem. Abstr. 54: 14345. 1960.

Reagents and Equipment

1. 11.7 N H_2SO_4
2. 0.34% $K_2Cr_2O_7$
3. 7.6 N NaOH
4. Alcoholic salicylaldehyde - 20% vol/vol in 95% ethanol.
5. An acetone standard containing 0.01 mg/ml in a dark bottle and stored in a refrigerator.
6. Teflon tape.
7. Drying oven and autoclave.
8. 19 x 150 mm Kimax tubes with teflon lined screw caps.
9. Small tubes approximately 60 x 8 mm.
10. Spectronic "20" colorimeter (Bausch and Lomb).
11. Protein-free blood filtrate solutions:

    5% Zinc sulfate
    0.3 N Barium Hydroxide

    When titrated against each other these two reagents must be of equal molar concentration. The final pH of the solution in plasma should be slightly acidic, approximately pH 6.5, to obtain a clear filtrate.

Procedure

1. Prepare a protein-free blood filtrate. Measure 1 ml of plasma and 4 ml of distilled water into a test tube and mix. Add 2.5 ml $Ba(OH)_2$ and mix. Then add 2.5 ml zinc sulfate and mix. Next, centrifuge for about 10 minutes at 1450 x gravity.
2. Measure 2 ml of 11.7 N H_2SO_4 into the large test tubes.

3. To the above add 2 ml of protein-free filtrate and 2 ml of water and mix.

4. Measure 1 ml of 0.34% $K_2Cr_2O_7$ into small tubes and lower one carefully into each of the large tubes so as to keep the solutions separate.

5. Cover the threaded portion of the Kimax tubes with teflon tape (2 turns) and then tighten the screw caps firmly.

6. Heat the tubes for 15 minutes in the drying oven at 110-120°C.

7. Remove the tubes from the oven and invert them to mix the solutions. Thoroughly mix the solutions by inverting and shaking several times. Reheat for 30 minutes in the autoclave at about 120°C and 15 lbs of pressure.

8. Prepare standards by pipetting 3 ml, 2 ml, 1 ml, and 0 ml of the standard acetone solution(0.01 mg/ml) into tubes containing 1 ml, 2 ml, 3 ml, and 4 ml, of water respectively. Add to each, 1 ml of 11.7 N H_2SO_4 and 1 ml of 0.34% $K_2Cr_2O_7$. Mix by swirling, but do not heat.

Color Development

1. To the unknowns, standards, and blanks add 6.0 ml of 7.6 N NaOH and mix thoroughly using a vortex mixer.

2. Add 1 ml of alcoholic salicylaldehyde, cap the tube, and quickly mix thoroughly using the vortex mixer.

3. Heat in a water bath at 45-50°C for 40 minutes.

4. Allow the tubes and solutions to cool for 40 minutes to develop the color and then read at 490 mμ in the Spectronic "20".

APPENDIX IV

Spectrofluorometric Method for Corticosterone or Hydrocortisone(Cortisol) Determination in Sheep Plasma

Note: Both corticosterone and cortisol fluoresce in sulphuric acid and are not separated by the procedure. However, cortisol is the predominant steroid in sheep.

Bush, I.E. and K.A. Ferguson. 1953. J. Endocrinol. 10: 1-8.

Coghlan, J.P., M. Wintour, and B.A. Scoggins. 1966. Aust. J. Exp. Biol. Med. Sci. 44: 639-664.

Method Adapted From:

Silber, R.H., R.D. Busch, and P. Oslapas. 1958. Clin. Chem. 4: 278-285.

Martin, M.M. and A.L.A. Martin. 1968. J. Clin. Endocrinol. 28: 137-145.

Reagents and Equipment

1. 0.9% saline solution.
2. H_2SO_4 - Ethanol(ratio of 4 vol. H_2SO_4 to 1 vol. of Ethanol). The sulfuric acid was 95-98%. The ethanol was 98%. This solution was prepared fresh each day.
3. Methylene chloride - purified by passing it through a column of activated silica-gel(grade 12, mesh size-28-200).
4. 19 x 150 mm test tubes with teflon lined screw caps.
5. Farrand Manual Spectroflurometer. Farrand Optical Co., Inc., Mount Vernon, New York.
6. Standard cortisol solutions of 0.1, 0.2, 0.3, 0.4, and 0.5 µg/ml and a blank were used. Standards were made up in absolute ethanol.

Procedure

1. To 1 ml of plasma in a screw capped test tube add 1 ml of 98% ethanol. Mix thoroughly by shaking and then centrifuge for 10 minutes at 900 x gravity.

2. One ml of the supernatant is placed in a clean screw capped tube and diluted to 5 ml with 0.9% NaCl.

3. Fifteen ml of methylene chloride are added and the tube is rotated at 30 rpm for 10 minutes and then centrifuged for 10 minutes at 900 x gravity.

4. The aqueous phase (top) is removed and discarded.

5. The methylene chloride is washed with 1 ml of water by shaking by hand for 15-20 seconds and centrifuging for 5 minutes at 900 x gravity. The aqueous phase and any precipitate between the two phases are removed and discarded.

6. Ten ml of the remaining methylene chloride are placed in a clean test tube and 2 ml of the H_2SO_4 - ethanol solution are added. The tubes are shaken by hand for three, 30 second periods at 30 second intervals. The tubes are allowed to stand until the phases are separate. The methylene chloride (top) is then removed and discarded.

7. One hour after the addition of the acid-ethanol solution, the fluorescence is read at 470 mμ excitation, and 520 mμ fluorescence, wavelengths.

APPENDIX V

Values of Heat Production (Hp), Blood Hematocrit (Hct), Plasma Glucose and Ketone Concentrations, Rectal Temperatures, and the Skin Temperatures of the Trunk, Ears, and Legs During the Acute Cold Tests (-28°C) of Experiment I, Series 1. [Values are Means (±Standard Deviation) of 11 Tests.)]

	Pre-cold	+30 min, [1]	+ 60 min.	+ 90 min.	+120 min.	+150 min.	+180 min.
Hp (Mcal/m^2.24 hr.)		2.89(±0.41)	3.27(±0.43)	3.59(±0.46)	3.76(±0.41)	3.89(±0.36)	3.81(±0.34)
Hct (%)	21.4(±1.3)	23.8(±2.3)	24.2(±2.6)	25.3(±2.9)	26.9(±3.2)	26.3(±2.7)	26.3(±3.1)
Glucose (mg%)	74.8(±3.4)	90.8(±10.8)	102.9(±9.5)	103.4(±10.6)	99.1(±12.0)	92.0(±12.7)	85.5(±7.7)
Ketones (mg%)	2.39(±0.26)	2.19(±0.19)	2.51(±0.28)	2.71(±0.21)	2.71(±0.50)	2.84(±0.61)	2.87(±0.50)
Rectal Temperature (°C)	39.6(±0.4)	39.7(±0.4)	39.6(±0.4)	39.5(±0.4)	39.4(±0.4)	39.3(±0.4)	39.2(±0.4)
Trunk Temperature (°C)	32.6(±2.1)	21.2(±2.8)	15.2(±4.1)	13.8(±4.4)	13.6(±3.8)	13.4(±4.0)	13.3(±3.9)
Ear Temperature (°C)	28.5(±5.6)	15.0(±3.1)	10.1(±5.8)	9.6(±7.3)	8.9(±6.8)	8.1(±6.8)	7.5(±5.9)
Leg Temperature (°C)	27.9(±4.8)	12.7(±4.4)	4.4(±2.7)	1.6(±2.4)	1.5(±2.4)	1.6(±2.3)	1.4(±2.4)

[1] Times after start of cold exposure.

APPENDIX VI

Values of Heat Production (Hp), Blood Hematocrit (Hct), and the Plasma Concentrations of Glucose, Corticoids, and Free-Fatty Acids (FFA) During the Acute Cold Tests (-28°C) of Experiment I, Series 2. (Values are Mean (±Standard Deviation) of 13 Tests.)

	Pre-cold	+24 hrs.	+30[1]	+60	+90	+120	+150	+180	+210	+240	+270	+300	+330	+360
Hp(Mcal/m^2·24 hr.)			2.85 (±0.26)	3.53 (±0.34)	3.74 (±0.38)	3.91 (±0.37)	3.86 (±0.23)	3.93 (±0.34)	3.97 (±0.22)	3.95 (±0.31)	4.02 (±0.29)	4.02 (±0.31)	4.10 (±0.37)	4.08 (±0.39)
Hct (%)	30.6 (±1.8)	31.2 (±1.2)		36.8 (±2.7)		37.6 (±2.7)		38.4 (±3.0)		39.2 (±2.8)		40.0 (±2.7)		40.8 (±3.1)
Glucose (mg%)	57.1 (±4.8)	59.9 (±4.3)		104.1 (±13.0)		105.4 (±15.3)		95.5 (±15.0)		84.6 (±12.0)		76.8 (±9.9)		71.6 (±8.2)
"Corticoids" (µg%)	17.9 (±3.8)	17.9 (±2.7)		28.0 (±6.3)		25.6 (±6.0)		28.3 (±4.6)		25.6 (±4.6)		27.4 (±4.6)		30.5 (±2.9)
FFA (µM/100 ml)	89.5 (±21.9)	97.4 (±21.1)		222.5 (±30.3)		238.4 (±25.0)		262.6 (±38.6)		258.2 (±46.9)		270.6 (±38.9)		295.2 (±48.5)

[1] Times (min.) after start of cold exposure

APPENDIX VII

Values of Heat Production (Hp), Blood Hematocrit, and the Plasma Concentrations of Glucose, Corticoids, Free-Fatty Acids (FFA), and Ketones During the Acute Cold Tests (-28°C) of Experiment 1, Series 3. [Values are Means (±Standard Deviation) of 6 tests.]

	Pre-Cold	+24 hrs.	+30[1]	+60	+90	+120	+150	+180	+210	+240	+270	+300[2]	+330	+360
Hp(Mcal/m^2·24 hr)	-	-	3.13 (±0.35)	3.30 (±0.25)	3.70 (±0.43)	4.03 (±0.35)	4.27 (±0.32)	4.19 (±0.31)	4.18 (±0.33)	4.15 (±0.34)	4.17 (±0.35)	4.13 (±0.34)	4.28 (±0.29)	4.21 (±0.26)
HCT (%)	33.8 (±2.0)	32.8 (±1.9)		36.0 (±1.9)		37.5 (±1.8)		38.9 (±1.1)		39.6 (±1.5)		40.8 (±2.2)		40.6 (±0.9)
Glucose (mg%)	64.8 (±1.9)	69.3 (±1.3)		106.6 (±11.3)		123.6 (±13.1)		110.3 (±14.3)		94.0 (±17.8)		83.3 (±18.9)		79.8 (±17.1)
"Corticoids" (µg%)	18.9 (±3.2)	17.5 (±2.7)		22.0 (±2.6)		25.6 (±3.4)		25.3 (±2.2)		27.1 (±3.0)		29.8 (±2.8)		29.0 (±1.9)
FFA(µM/100 ml)	83 (±7)	82 (±14)		167 (±42)		245 (±51)		250 (±50)		278 (±67)		318 (±58)		337 (±57)
Ketones (mg%)	1.48 (±0.44)	1.41 (±0.17)		3.05 (±0.33)		3.37 (±0.24)		3.31 (±0.45)		3.47 (±0.37)		3.90 (±0.34)		3.91 (±0.46)

1 Times (min.) after start of cold exposure

2 Wind on after 300 min.

APPENDIX VIII

Values of Heat Production (Hp), Rectal Temperature, Skin Temperatures of the Trunk and Ears, Blood Hematocrit, and the Plasma Concentrations of Glucose, Corticoids, Free-Fatty Acids (FFA), and Ketones During the Acute Cold Tests (-28°C) of Experiment 1, Series 4. [Values are Means (±Standard Deviation) of 6 Tests.)]

Time (Min.)	Hp (Mcal/m^2·24 hr)	Rectal Temp. (°C)	Trunk Temp. (°C)	Ear Temp. (°C)	Hct (%)	Glucose (mg%)	"Corticoids" (µg%)	FFA (µM/100ml)	Ketones (mg%)
Pre-Cold	-	39.3(±0.6)	36.6(±0.4)	34.4(±2.5)	32.4(±1.3)	57.0(±5.3)	14.5(±2.4)	73(±13)	2.18(±0.23)
+24 hr.	-	-	-	-	32.3(±1.1)	55.2(±6.6)	16.6(±4.7)	99(±19)	3.23(±0.29)
+15[1]	2.66(±0.27)	39.2(±0.5)	23.0(±2.9)	18.3(±3.3)					
+30	3.04(±0.41)	39.7(±0.6)	18.5(±4.1)	16.5(±1.7)					
+45	3.20(±0.48)	39.5(±0.5)	16.4(±4.8)	15.7(±3.6)					
+60	3.24(±0.47)	39.2(±0.6)	14.9(±5.3)	15.3(±4.0)	37.7(±1.9)	110.2(±16.6)	25.0(±3.3)	170(±49)	2.80(±0.60)
+75	3.37(±0.58)	39.3(±0.4)	13.8(±5.4)	14.3(±2.9)					
+90	3.36(±0.56)	39.3(±0.5)	13.3(±5.3)	13.9(±2.3)					
+105	3.39(±0.45)	39.3(±0.3)	13.0(±5.2)	13.7(±2.2)					
+120	3.45(±0.44)	39.2(±0.4)	12.6(±5.1)	13.4(±2.2	37.2(±2.9)	112.3(±14.9)	26.0(±4.7)	198(±57)	2.94(±0.79)
+135	3.39(±0.37)	39.2(±0.4)	12.6(±5.3)	13.4(±1.9)					
+150	3.43(±0.35)	39.1(±0.4)	12.4(±5.4)	13.2(±2.0)					
+165	3.37(±0.33)	39.1(±0.5)	12.0(±5.4)	13.3(±1.7)					
+180	3.41(±0.37)	38.9(±0.6)	11.3(±5.3)	13.5(±1.7)	37.5(±2.4)	99.8(±9.8)	24.5(±3.8)	202(±67)	2.90(±0.44)
+195	3.43(±0.38)	39.0(±0.6)	11.5(±5.6)	12.9(±1.6)					
+210	3.43(±0.39)	39.0(±0.5)	11.5(±6.0)	13.1(±1.8)					
+225	3.42(±0.36	38.9(±0.5)	11.3(±6.1)	12.7(±2.3)					
+240[2]	3.43(±0.42)	38.9(±0.5)	11.1(±6.2)	13.1(±2.5)	37.9(±2.4)	90.2(±7.3)	28.1(±2.9)	238(±44)	3.12(±0.56)
+255	3.63(±0.43)	38.8(±0.7)	8.6(±6.5)	13.0(±2.2)					
+270	3.72(±0.56)	38.8(±0.6)	7.4(±6.5)	15.0(±3.5)					
+285	3.85(±0.58)	38.5(±0.7)	6.3(±5.8)	14.6(±3.2)					
+300[3]	3.92(±0.59)	38.4(±0.6)	5.4(±4.8)	14.2(±2.9)	40.2(±2.1)	102.8(±7.9)	27.5(±1.2)	275(±33)	3.62(±0.57)
+315	3.19(±0.48)	38.3(±0.6)	4.6(±4.1)	13.0(±3.1)					
+330	3.03(±0.40)	38.0(±0.6)	2.2(±2.3)	11.6(±3.4)					
+345	2.87(±0.40)	37.4(±0.8)	1.1(±1.5)	11.4(±3.5)					
+360	2.84(±0.42)	36.8(±1.1)	-0.6(±1.7)	12.3(±2.9)	39.9(±2.4)	78.4(±8.9)	26.6(±3.8)	118(±23)	2.20(±0.28)

[1] Time after start of cold exposure

[2] Wind on at +240 min.

[3] Propranolol (1 mg/kg) given at +300 min.

APPENDIX IX.

Values of Blood Hematocrit (Hct) and the Plasma Concentration of Protein Bound Iodine (PBI), Glucose, Corticoids, Free-Fatty Acids (FFA), and Ketones During Constant Cold (CC), Fluctuating Cold (FC), and Warm (Cont.) Acclimation Trials of Experiment II. [Values are Means (±Standard Deviation) of 16 Observations for the CC and FC Groups and 8 Observations for the Cont. Group.]

Weeks of Cold Exposure	Cont.	CC	FC	Cont.	CC	FC
		PBI (μg%)			Hct (%)	
1	3.11(±0.13)	4.86(±0.56)	4.85(±0.92)	26.5(±3.1)	35.2(±3.2)	32.8(±3.2)
2	3.58(±0.33)	4.91(±0.96)	4.77(±0.59)	26.4(±3.0)	33.1(±2.6)	33.6(±2.5)
3	3.36(±0.24)	-	-	26.9(±2.8)	-	-
4	3.40(±0.24	4.66(±0.60)	4.45(±0.59)	25.9(±3.3)	33.0(±2.3)	33.9(±1.8)
5	3.56(±0.26)	4.2(±0.58)	4.38(±0.70)	26.9(±3.0)	33.8(±2.2)	33.1(±2.6)
		Glucose (mg%)			Corticoids (μg%)	
1	62.4(±2.9)	68.0(±8.4)	70.1(±7.0)	16.4(±2.0)	17.4(±2.5)	20.1(±3.3)
2	73.8(±3.8)	71.8(±5.3)	71.4(±6.8)	16.6(±2.6)	15.6(±2.4)	18.9(±2.3)
3	68.9(±3.3)	-	-	18.6(±2.2)	-	-
4	68.7(±5.0)	69.6(±4.6)	66.3(±5.4)	16.2(±2.3)	15.5(±1.9)	20.2(±3.9)
5	65.5(±4.4)	68.5(±4.1)	64.1(±4.8)	15.4(±2.6)	17.3(±2.3)	19.7(±4.4)
		FFA (μM/100ml)			Ketones (mg%)	
1	71(±13)	100(±15)	109(±14)	1.70(±0.15)	2.78(±0.41)	2.37(±0.31)
2	91(±16)	94(±15)	104(±13)	1.83(±0.08)	2.27(±0.34)	2.03(±0.24)
3	84(±13)	-	-	1.86(±0.19)	-	-
4	82(±19)	86(±14)	98(±15)	1.80(±0.14)	2.11(±0.24)	1.77(±0.33)
5	70(±9)	75(±12)	96(±11)	1.90(±0.13)	2.17(±0.28	1.83(±0.50)

APPENDIX Xa

Thyroxine-^{14}C Disappearance from the Plasma of the Warm Acclimated Control Sheep of Experiment II. All Values are Logs of the Specific Activity(dpm/ug I).

	Control		Control		Control	
Time(hr)	1	2	1	2	1	2
2-3	4.17	4.20	3.97	3.86	3.83	4.01
4-4.5	4.09	4.10	3.83	3.75	3.87	3.96
7-7.5	3.95	4.00	3.78	3.66	-	-
20.5-21.5	3.78	3.74	3.57	3.51	3.72	3.82
24-26	-	-	3.41	3.42	3.66	-
28-30	3.70	3.63	3.41	3.40	3.57	3.57
44-44.5	3.56	3.60	-	-	3.43	3.40
46-48	-	-	3.22	3.25	3.40	3.45
52-53.5	3.50	3.46	3.19	3.28	3.37	3.50
71	-	-	2.99	3.06	-	-
73.5-75	3.43	3.29	-	-	3.18	3.33
98.5	-	-	-	-	3.02	-

APPENDIX Xb

Thyroxine-^{14}C Disappearance from the Plasma of the Constant Cold (CC) Acclimated Sheep of Experiment II. All Values are Logs of the Specific Activity(dpm/µg I).

	Week 1		Week 2		Week 4		Week-5	
Time(hr)	CC-3	CC-4	CC-1	CC-2	CC-3	CC-4	CC-1	CC-2
2	4.09	3.90	3.63	4.10	4.02	4.04	4.13	4.14
4-6	3.98	3.81	3.75	3.92	3.99	4.02	4.00	4.10
7-8	3.91	-	-	-	3.92	-	3.83	3.86
20-22	-	3.54	3.73	3.61	-	3.62	3.62	3.61
23-25	3.54	3.42	-	-	3.54	3.64	-	-
27.5-28	-	3.48	-	-	3.45	3.71	-	-
30-32.5	3.38	3.38	3.69	3.47	3.42	-	3.53	3.61
43.5	-	-	-	-	-	3.45	-	-
46-47.5	3.16	3.21	3.44	3.39	3.15	3.30	3.41	3.46
51.5-53.5	-	-	3.40	3.26	-	3.21	-	-
54-55.5	3.07	3.07	-	-	3.14	-	3.36	3.36
71.5-73	2.93	-	3.37	3.18	2.87	3.14	-	-
76	-	-	-	-	-	-	3.14	-
95-97	-	-	-	-	-	2.92	-	-

APPENDIX Xc

Thyroxine-^{14}C Disappearance from the Plasma of the Fluctuating Cold(FC) Acclimated Sheep of Experiment II. All Values are Logs of the Specific Activity(dpm/μg I).

	Week-1		Week-2		Week-4		Week-5	
Time(hr)	FC-3	FC-4	FC-1	FC-2	FC-3	FC-4	FC-1	FC-2
2-4	3.98	3.43	3.92	3.98	3.72	4.24	3.79	3.76
4	-	-	3.88	3.91	-	-	3.71	3.72
6-7.5	-	3.62	3.81	3.71	3.79	4.11	3.63	3.77
20-22	3.50	-	3.50	3.61	3.78	3.81	3.46	3.67
24-26	3.41	3.66	3.46	3.44	-	-	3.36	3.52
27-29	3.36	3.60	-	-	3.76	-	-	-
29.5-30.5	-	-	3.48	3.37	3.63	3.71	-	-
39.5	3.28	-	-	-	-	-	-	-
45.5-46.5	-	-	3.25	3.31	3.43	-	3.18	3.38
49.5-50.5	3.17	-	-	-	-	-	-	-
53.5-54.5	3.14	3.34	3.13	3.13	3.42	3.54	3.09	-
71-71.5	-	3.19	2.99	3.03	-	-	2.85	3.20
74.5-75	2.92	-	-	-	3.20	3.29	-	-
78	-	-	-	-	-	-	2.84	3.05
95.5-98	2.68	-	-	-	3.03	3.07	2.72	2.93

APPENDIX XI

Values of Heat Production (Hp), Jugular Blood Temperature, and Skin Temperatures of the Trunk for the Constant Cold (CC), Fluctuating Cold (FC), and Warm (Cont.) Acclimated Animals During the Acute Cold Tests (-28°C) of Experiment III. [Values are Means (±Standard Deviation).]

	Hp(Mcal/m^2·24 hr)			Jugular Blood Temperature (°C)			Trunk Skin Temperature (°C)		
Time (min.)	Cont. (10)[3]	CC (4)	FC (3)	Cont. (10)	CC (4)	FC (4)	Cont. (10)	CC (4)	FC (4)
0				39.1(±1.1)	39.2(±0.6)	38.8(±0.7)	33.8(±2.3)	35.0(±0.9)	34.5(±1.2)
+15	3.90(±0.41)	3.38(±0.40)	3.66(±0.26)	39.2(±1.1)	39.2(±0.5)	38.9(±0.8)	22.6(±1.9)	19.1(±2.3)	23.7(±0.6)
+30	4.07(±0.50)	3.82(±0.54)	3.86(±0.37)	39.4(±1.2)	39.1(±0.6)	39.1(±0.7)	15.3(±2.4)	15.2(±2.3)	18.8(±1.0)
+45	4.23(±0.43)	3.90(±0.55)	3.97(±0.52)	39.3(±1.2)	38.8(±0.8)	39.0(±0.7)	15.5(±1.7)	12.3(±2.5)	15.5(±2.1)
+60	4.43(±0.42)	4.07(±0.56)	4.12(±0.56)	39.4(±1.4)	38.8(±0.7)	39.0(±0.8)	14.6(±2.0)	10.6(±2.9)	13.5(±1.4)
+75	4.40(±0.33)	4.11(±0.49)	4.20(±0.44)	39.3(±1.4)	38.7(±0.6)	38.8(±0.7)	14.4(±1.7)	9.8(±3.0)	12.7(±1.4)
+90	4.43(±0.38)	4.14(±0.48)	4.23(±0.52)	39.2(±1.4)	38.6(±0.5)	38.9(±0.8)	14.2(±1.4)	8.6(±2.6)	11.8(±1.6)
+105	4.52(±0.25)	4.26(±0.45)	4.34(±0.55)	39.1(±1.4)	38.6(±0.5)	38.8(±0.6)	14.2(±1.5)	8.3(±2.3)	11.5(±1.9)
+120	4.56(±0.37)	4.29(±0.43)	4.42(±0.65)	39.1(±1.5)	38.7(±0.5)	39.0(±0.7)	14.2(±1.4)	8.4(±2.2)	11.1(±1.6)
+135	4.59(±0.40)	4.24(±0.32)	4.26(±0.89)	39.0(±1.4)	38.8(±0.5)	38.8(±0.7)	14.4(±1.4)	8.3(±2.0)	10.9(±2.0)
+150	4.69(±0.41)	4.29(±0.28)	4.62(±0.50)	39.2(±1.5)	39.0(±0.5)	38.9(±0.7)	14.3(±1.6)	8.3(±2.2)	11.0(±2.2)
+165	4.69(±0.25)	4.23(±0.27)	4.71(±0.35)	39.2(±1.6)	38.9(±0.6)	39.0(±0.7)	14.3(±1.5)	8.4(±2.0)	11.0(±2.4)
+180	4.51(±0.28)	4.16(±0.38)	4.48(±0.70)	39.1(±1.5)	38.9(±0.5)	39.0(±0.8)	14.1(±1.5)	8.7(±1.8)	11.3(±2.4)
+195	4.53(±0.35)	4.11(±0.30)	4.22(±0.70)	39.0(±1.6)	38.8(±0.5)	38.9(±0.6)	14.2(±1.7)	8.3(±1.7)	11.3(±2.5)
+210	4.61(±0.35)	4.12(±0.23)	4.15(±0.64)	39.0(±1.5)	38.7(±0.5)	38.8(±0.8)	14.1(±1.5)	8.4(±1.7)	11.3(±2.2)
+225	4.65(±0.32)	4.19(±0.22)	4.29(±0.52)	39.0(±1.6)	38.7(±0.5)	38.9(±0.9)	14.3(±1.7)	8.3(±1.7)	11.0(±1.8)
+240[1]	4.67(±0.34)	4.27(±0.23)	4.45(±0.37)	38.9(±1.5)	38.7(±0.5)	38.7(±0.8)	14.1(±1.7)	8.3(±1.5)	10.8(±2.0)
+255	4.79(±0.43)	4.38(±0.47)	4.61(±0.69)	39.0(±1.5)	38.6(±0.6)	38.8(±0.8)	8.9(±1.9)	7.2(±1.7)	10.0(±2.1)
+270	5.10(±0.44)	4.67(±0.53)	4.78(±0.80)	39.0(±1.5)	38.7(±0.6)	38.8(±0.9)	7.0(±2.1)	6.6(±1.6)	8.6(±1.2)
+285	5.07(±0.50	4.60(±0.51)	4.87(±0.79)	38.8(±1.6)	38.6(±0.5)	38.6(±1.1)	6.2(±2.3)	6.0(±1.6)	7.8(±0.8)
+300[2]	5.15(±0.51)	4.60(±0.56)	4.90(±0.79)	38.8(±1.6)	38.6(±0.5)	38.7(±1.0)	5.8(±2.4)	5.8(±1.5)	8.1(±0.8)
+315	4.38(±0.33	4.21(±0.45)	4.39(±0.65)	39.0(±1.5)	38.4(±0.6)	38.5(±1.0)	5.5(±2.3)	5.3(±1.3)	7.3(±0.9)
+330	4.22(±0.38)	4.07(±0.48)	4.35(±0.49)	38.8(±1.4)	38.3(±0.5)	38.5(±0.9)	4.4(±2.3)	4.5(±1.4)	6.3(±1.3)
+345	4.31(±0.41)	4.00(±0.43)	4.32(±0.45)	38.4(±1.4)	38.1(±0.5)	38.5(±1.1)	4.0(±2.2)	4.3(±1.4)	6.1(±1.0)
+360	4.17(±0.58)	4.02(±0.44)	4.32(±0.52)	38.0(±1.3)	37.9(±0.4)	38.4(±1.1)	3.9(±2.1)	4.2(±1.3)	6.5(±1.7)

[1] Wind on

[2] Propranolol given (1 mg/kg)

[3] Numbers in parentheses are number of tests in each average value.

APPENDIX XII

Values of the Skin Temperatures of the Legs and Ears for the Constant Cold (CC), Fluctuating Cold (FC), and Warm (Cont.) Acclimated Animals During the Acute Cold Tests (-28°C) of Experiment III. [Values are Means (±Standard Deviation)]

	Leg Skin Temperature (°C)			Ear Skin Temperature (°C)		
Time (min)	Cont. (10)[3]	CC (4)	FC (4)	Cont. (10)	CC (4)	FC (4)
0	30.5(±4.8)	33.9(±2.6)	31.8(±1.9)	33.4(±4.1)	34.2(±3.6)	32.4(±1.6)
+15	18.5(±4.0)	21.5(±2.6)	17.9(±3.6)	18.2(±4.4)	16.7(±2.8)	19.3(±1.2)
+30	9.6(±3.8)	13.6(±2.2)	9.7(±3.0)	14.6(±3.4)	13.0(±2.9)	13.2(±2.9)
+45	5.2(±3.8)	7.9(±1.5)	5.8(±1.2)	12.7(±2.2)	11.4(±2.5)	11.3(±2.7)
+60	3.9(±4.0)	5.4(±0.5)	4.4(±1.5)	12.3(±3.0)	9.3(±1.8)	10.8(±1.9)
+75	3.9(±3.6)	5.0(±1.2)	5.1(±1.0)	10.8(±4.0)	8.0(±1.7)	9.4(±2.8)
+90	3.9(±3.8)	5.2(±0.5)	6.2(±1.3)	9.7(±2.9)	8.2(±2.4)	9.0(±2.2)
+105	3.7(±3.1)	5.6(±0.9)	6.7(±3.3)	10.7(±4.4)	7.7(±2.3)	8.1(±2.2)
+120	3.8(±3.4)	4.5(±0.8)	6.7(±3.3)	9.8(±3.6)	8.2(±2.2)	7.4(±2.1)
+135	4.2(±3.4)	4.0(±0.8)	5.7(±1.9)	8.3(±3.0)	7.5(±1.7)	7.2(±1.6)
+150	3.6(±3.0)	5.0(±1.9)	5.1(±2.3)	7.2(±2.0)	7.6(±1.8)	6.9(±2.5)
+165	3.4(±3.2)	3.2(±0.8)	3.6(±1.4)	7.6(±2.8)	7.7(±1.9)	6.7(±2.8)
+180	3.0(±2.8)	3.5(±0.9)	3.3(±1.4)	7.5(±3.0)	7.7(±2.3)	6.1(±2.8)
+195	3.3(±3.0)	3.4(±1.5)	4.0(±2.4)	7.7(±2.7)	7.3(±1.7)	6.0(±2.3)
+210	3.5(±3.0)	3.6(±1.1)	2.8(±1.4)	7.4(±2.5)	7.0(±2.0)	6.7(±0.9)
+225	3.3(±2.4)	3.9(±0.9)	2.5(±0.9)	7.1(±2.5)	7.0(±2.1)	6.8(±2.4)
+240[1]	3.1(±1.9)	3.6(±1.3)	2.3(±0.4)	7.2(±2.4)	6.5(±2.0)	6.9(±1.7)
+255	3.2(±2.8)	1.2(±0.7)	1.6(±1.0)	8.1(±2.8)	6.3(±2.4)	6.5(±2.6)
+270	2.6(±2.8)	1.1(±0.8)	1.9(±1.7)	9.0(±3.3)	6.9(±2.0)	5.7(±2.1)
+285	2.4(±3.1)	1.8(±1.7)	2.0(±1.5)	8.6(±3.6)	7.0(±2.1)	5.5(±2.3)
+300[2]	-2.2(±3.3)	-0.3(±0.6)	2.0(±1.9)	8.4(±3.4)	6.9(±2.4)	5.4(±2.3)
+315	1.7(±3.0)	0.8(±1.0)	1.7(±2.5)	10.2(±4.1)	6.5(±2.2)	5.4(±1.9)
+330	0.9(±2.6)	0(±1.6)	1.9(±3.6)	9.6(±3.6)	6.0(±2.5)	5.8(±1.4)
+345	1.0(±2.2)	0.3(±2.1)	2.1(±1.8)	8.3(±2.2)	5.7(±2.2)	5.1(±1.4)
+360	0.9(±2.2)	-0.2(±0.6)	1.6(±2.5)	7.9(±1.7)	5.3(±1.7)	4.5(±1.8)

[1] Wind on

[2] Propranolol given (1 mg/kg)

[3] Numbers in parentheses are number of tests in each average value.

APPENDIX XIII

Values of Blood Hematocrit (Hct) and the Plasma Concentration of Glucose, Corticoids, Free-Fatty Acids (FFA), and Ketones for the Constant Cold (CC), Fluctuating Cold (FC), and Warm (Cont.) Acclimated Animals During the Acute Cold Tests (-28°C) of Experiment III. [Values are Means (±Standard Deviation.)]

	Corticoids (µg%)			Glucose (mg%)			Hct (%)		
Time (hr)	CC (4)[1]	FC (4)	Cont. (10)	Cont. (10)	CC (4)	FC (4)	Cont. (10)	CC (4)	FC (4)
Pre-cold	16.9(±3.7)	23.5(±2.9)	16.8(±2.5)	64.4(±6.3)	69.0(±4.3)	60.9(±3.1)	27.4(±1.7)	36.8(±0.7)	37.4(±2.6)
+24	19.0(±2.8)	19.9(±4.1)	21.0(±3.9)	62.4(±5.6)	71.2(±5.2)	59.3(±5.8)	31.1(±2.8)	38.0(±2.3)	37.3(±2.3)
+1	24.9(±5.6)	27.0(±4.7)	24.7(±4.6)	111.0(±10.4)	114.3(±16.5)	127.0(±20.0)	33.5(±2.6)	38.2(±1.6)	37.5(±2.8)
+2	25.2(±5.1)	25.2(±3.7)	24.9(±5.8)	104.3(±17.1)	123.5(±16.8)	131.9(±26.1)	33.7(±2.1)	37.4(±1.0)	37.7(±4.9)
+3	25.0(±3.2)	26.2(±3.5)	26.7(±4.5)	97.2(±14.0)	108.5(±14.4)	108.1(±18.1)	33.8(±2.1)	38.2(±1.7)	37.2(±4.2)
+4	25.8(±3.4)	29.4(±2.7)	27.9(±4.8)	91.8(±14.0)	104.3(±14.6)	102.1(±13.3)	34.8(±2.1)	39.4(±2.7)	38.0(±3.9)
+5	25.7(±4.1)	33.0(±2.8)	28.6(±4.8)	103.4(±12.2)	117.2(±11.2)	122.1(±10.8)	37.9(±3.2)	41.3(±2.5)	40.1(±4.7)
+6	26.6(±1.8)	27.4(±2.4)	30.0(±3.9)	71.3(±14.2)	68.7(±13.6)	84.5(±14.3)	35.9(±2.3)	40.2(±2.5)	39.5(±5.8)

	FFA (µM/100 ml)			Ketones (mg%)		
Time (hr)	Cont. (10)	CC (4)	FC (4)	Cont. (10)	CC (4)	FC (4)
Pre-cold	101(±24)	89(±17)	94(±21)	2.28(±0.36)	2.33(±0.24)	2.04(±0.50
+24	112(±29)	86(±17)	101(±13)	3.00(±1.29)	2.55(±0.55)	1.99(±0.22)
+1	190(±44)	167(±12)	175(±31)	3.42(±1.05)	3.50(±0.50)	2.85(±0.61)
+2	181(±36)	180(±34)	178(±57)	3.66(±1.24)	4.73(±1.11)	3.01(±0.87)
+3	205(±39)	234(±64)	225(±61)	4.21(±1.13	4.87(±0.82)	3.55(±0.58)
+4	250(±37)	275(±70)	257(±59)	4.48(±1.06)	5.11(±0.69)	3.96(±0.71)
+5	293(±39)	302(±56)	321(±42)	4.91(±0.95)	5.59(±0.80)	4.27(±0.74)
+6	173(±31)	167(±27)	116(±49)	3.57(±1.00)	3.83(±0.43)	2.83(±0.49)

[1] Numbers in parentheses are number of tests in each average value.

APPENDIX XIV

Values of Heat Production (Hp), Jugular Blood Temperature, and Skin Temperatures of the Trunk for the Constant Cold (CC), Fluctuating Cold (FC), and Warm (Cont.) Acclimated Animals During the Acute Cold Tests (-28°C) with Phenoxybenzamine (3mg/kg) of Experiment III. [Values are Means (±Standard Deviation).]

Time (min.)	Hp (Mcal/m^2·24 hr)			Jugular Blood Temperature (°C)			Trunk Skin Temperature (°C)		
	Cont. (4)[2]	CC (4)	FC (3)	Cont. (4)	CC (4)	FC (4)	Cont. (4)	CC (4)	FC (4)
0				39.3(±2.0)	39.5(±0.5)	38.4(±1.6)	35.3(±0.9)	35.6(±0.7)	34.4(±1.8)
+15	2.62(±0.28)	3.17(±0.53)	2.73(±0.15)	38.9(±2.4)	39.3(±0.6)	37.9(±2.2)	24.0(±1.0)	22.7(±4.4)	20.6(±1.3)
+30	3.53(±0.40)	3.91(±0.42)	3.52(±0.18)	38.8(±2.1)	39.2(±0.5)	37.5(±2.2)	20.8(±1.0)	19.3(±3.7)	17.8(±0.9)
+45	4.08(±0.34)	4.16(±0.32)	3.62(±0.10)	38.5(±2.0)	39.1(±0.6)	36.7(±2.3)	18.1(±1.0)	17.4(±3.3)	16.1(±1.1)
+60	4.13(±0.30)	4.31(±0.36)	3.63(±0.08)	38.5(±2.1)	38.9(±0.5)	36.3(±2.3)	17.0(±1.4)	16.1(±2.8)	15.2(±1.2)
+75	4.13(±0.28)	4.46(±0.17)	3.83(±0.09)	38.1(±1.8)	38.9(±0.5)	35.9(±2.4)	15.2(±0.9)	15.2(±2.3)	14.5(±0.7)
+90	4.09(±0.20)	4.62(±0.18)	3.98(±0.12)	38.0(±1.8)	38.9(±0.4)	35.8(±2.3)	14.4(±0.8)	14.2(±2.1)	13.9(±0.4)
+105	4.14(±0.19)	4.59(±0.18)	4.03(±0.14)	37.6(±1.3)	38.9(±0.5)	35.7(±2.3)	13.7(±1.0)	13.5(±2.2)	13.4(±0.5)
+120	4.16(±0.16)	4.74(±0.21)	3.99(±0.24)	37.5(±1.1)	38.8(±0.5)	35.3(±2.7)	13.6(±1.1)	13.1(±2.6)	12.7(±0.3)
+135	4.09(±0.23)	4.88(±0.20)	4.13(±0.21)	37.4(±1.1)	38.7(±0.5)	35.6(±2.7)	13.4(±1.6)	13.3(±2.3)	12.7(±0.6)
+150	4.03(±0.39)	4.91(±0.15)	4.27(±0.27)	37.2(±1.3)	38.7(±0.5)	35.2(±3.5)	13.1(±1.9)	13.5(±2.1)	12.5(±1.0)
+165	4.16(±0.31)	4.85(±0.18)	4.25(±0.31)	37.1(±1.3)	38.5(±0.7)	35.2(±3.5)	13.3(±1.2)	13.8(±1.9)	12.8(±1.0)
+180	4.03(±0.37)	4.83(±0.22)	4.30(±0.34)	37.0(±1.4)	38.5(±0.6)	35.1(±3.7)	13.0(±1.5)	13.9(±2.1)	12.8(±1.3)
+195	4.07(±0.34)	4.77(±0.25)		36.8(±1.1)	38.5(±0.6)		13.0(±1.7)	14.1(±2.0)	
+210	3.95(±0.43)	4.88(±0.30)		36.6(±1.3)	38.6(±0.7)		12.5(±1.8)	14.3(±2.0)	
+225		4.93(±0.28)			38.5(±0.7)			13.7(±1.9)	
+240[1]		4.88(±0.27)			38.4(±0.7)			13.0(±2.6)	
+255		4.88(±0.14)			38.2(±0.7)			10.1(±2.0)	
+270		5.02(±0.20)			37.8(±0.8)			9.6(±0.8)	
+285		4.93(±0.24)			37.6(±0.9)			9.3(±1.6)	
+300		4.96(±0.27			37.4(±1.0)			8.7(±2.0)	

[1] Wind on

[2] Numbers in parentheses are number of tests in each average value.

APPENDIX XV

Values of the Skin Temperature of the Legs and Ears for the Constant Cold (CC), Fluctuating Cold (FC), and Warm (Cont.) Acclimated Animals During the Acute Cold Tests (-28°C) with Phenoxybenzamine (3 mg/kg) of Experiment III. [Values are Means (±Standard Deviation.)]

	Leg Skin Temperature (°C)			Ear Skin Temperature (°C)		
Time (min)	Cont.(4)[2]	CC(2)	FC (4)	Cont. (4)	CC(4)	FC (4)
0	35.5(±1.8)	34.9	35.3(±2.4)	36.8(±1.0)	36.7(±0.5)	36.3(±1.7)
+15	25.6(±1.8)	24.3	21.9(±3.0)	29.5(±1.6)	29.9(±1.8)	28.4(±2.9)
+30	21.1(±1.9)	20.5	20.0(±5.6)	28.6(±1.7)	29.0(±2.0)	28.0(±3.5)
+45	18.1(±0.9)	17.3	15.1(±1.4)	28.9(±1.5)	28.7(±1.7)	26.1(±4.8)
+60	14.8(±2.1)	14.6	13.6(±0.8)	28.5(±1.5)	28.5(±1.7)	25.9(±4.7)
+75	12.3(±1.9)	12.5	11.8(±0.6)	28.2(±1.7)	27.8(±2.1)	25.9(±4.0)
+90	10.4(±3.1)	11.2	10.1(±1.3)	28.1(±1.8)	28.1(±1.8)	24.9(±5.9)
+105	8.9(±2.8)	10.8	8.7(±1.8)	28.9(±1.2)	27.8(±1.9)	24.6(±6.1)
+120	8.1(±3.6)	9.9	8.1(±2.1)	28.1(±2.0)	27.4(±2.4)	24.6(±6.2)
+135	7.5(±3.1)	9.3	7.4(±1.3)	28.0(±2.4)	27.6(±2.1)	24.5(±6.9)
+150	7.4(±2.6)	9.0	5.9(±1.0)	27.6(±2.5)	27.3(±2.0)	24.2(±5.5)
+165	6.9(±1.7)	9.2	6.4(±1.0)	28.0(±2.4)	26.9(±2.0)	23.5(±6.1)
+180	7.0(±2.2)	9.2	5.0(±1.3)	28.1(±2.2)	26.6(±2.2)	23.5(±6.1)
+195	7.1(±1.6)	9.2		28.0(±2.1)	26.4(±1.8)	
+210	7.3(±2.0)	9.5		27.8(±2.2)	26.4(±2.0)	
+225		9.8			26.3(±2.3)	
+240[1]		9.5			26.2(±2.2)	
+255		8.7			25.5(±2.2)	
+270		7.1			24.8(±1.9)	
+285		7.1			23.9(±2.9)	
+300		6.4			23.7(±2.6)	

[1] Wind on

[2] Numbers in parentheses are number of tests in each average value.

APPENDIX XVI

Values of Blood Hematocrit (Hct) and the Plasma Concentrations of Glucose, Corticoids, Free-Fatty Acids (FFA), and Ketones for the Constant Cold (CC) Fluctuating Cold (FC), and Warm (Cont.) Acclimated Animals During the Acute Cold Tests (-28°C) with Phenoxybenzamine (3 mg/kg) of Experiment III.

[Values are Means (±Standard Deviation) of 4 Tests.]

Time (hr)	Corticoids (µg%)			Glucose (mg%)			HCT (%)		
	CC	FC [2]	Cont.	CC	FC [2]	Cont.	CC	FC [2]	Cont.
Pre-Phenoxy.	16.5(±3.2)	21.5(±3.5)	16.5(±2.0)	65.1(±2.5)	64.0(±3.8)	63.5(±3.6)	35.0(±1.8)	37.0(±4.1)	30.5(±2.0)
+24	17.7(±1.9)	24.5(±5.2)	24.5(±3.3)	61.9(±7.9)	57.8(±7.5)	59.0(±9.7)	34.8(±2.3)	32.1(±3.4)	30.2(±3.1)
0	20.8(±1.0)	20.0(±2.8)	25.7(±5.6)	76.1(±8.7)	74.2(±5.5)	79.8(±9.7)	30.1(±1.8)	29.2(±4.2)	23.9(±5.0)
+1	25.7(±2.5)	27.9(±3.6)	29.2(±5.6)	138.5(±24.6)	159.7(±30.0)	135.4(±35.7)	32.9(±1.2)	34.7(±4.1)	27.3(±5.2)
+2	28.4(±3.2)	32.3(±10.3)	29.7(±5.5)	125.4(±6.8)	165.7(±20.7)	130.5(±24.2)	32.6(±0.6)	35.5(±6.2)	27.5(±5.6)
+3	27.3(±1.7)	37.3(±6.9)	27.6(±1.5)	111.3(±14.0)	199.7(±16.0)	124.9(±7.4)	33.4(±0.3)	34.8(±4.1)	27.8(±5.0)
+4 [1]	29.1(±1.0)	-	30.0(±2.5)	110.6(±10.0)	-	132.3(±21.1)	32.7(±2.2)	-	27.9(±4.8)
+5	29.8(±3.2)	-	-	122.4(±13.6)	-	-	32.5(±2.7)	-	

Time (hr)	FFA (µM/100 ml)			Ketones (mg%)		
	CC	FC [2]	Cont.	CC	FC [2]	Cont.
Pre-Phenoxy.	93(±19)	126(±28)	114(±30)	2.06(±0.32)	2.14(±0.57)	2.02(±0.18)
+24	207(±24)	198	222(±18)	2.93(±0.82)	2.23	3.23(±0.83)
0	112(±26)	141(±55)	91(±10)	2.54(±0.24)	2.11(±0.32)	2.86(±0.71)
+1	241(±69)	319(±74)	260(±33)	5.02(±0.78)	4.19(±0.48)	4.64(±1.08)
+2	209(±75)	307(±72)	267(±50)	4.10(±0.52)	3.95(±0.49)	5.70(±1.03)
+3	218(±59)	277(±28)	273(±53)	3.95(±0.30)	3.70(±0.63)	5.75(±0.74)
+4 [1]	200(±56)	-	302(±53)	4.15(±0.87)	-	4.90(±1.40)
+5	217(±46)	-	-	4.13(±0.51)	-	-

[1] Wind on at end of fourth hour.

[2] Values are averages of 3 tests. All others are averages of 4 tests.