SOME EFFECTS OF COLD EXPOSURE ON MILK SECRETION IN THE GOAT

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by

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SUMMARY

The work described in this thesis was carried out in order to determine the effects of certain short-term changes in climatic conditions on mammary gland function, notably milk yield and composition in the goat.

The technique involved transferring lactating British Saanen goats from an insulated byre to a precision climatic chamber where they were exposed for 2 or 3 days to a controlled cold environment. Whilst under this climatic stress the animals were fed and milked as in the byre. Milk yield and composition were determined over this period and certain plasma constituents were also monitored.

In Chapter 3, Section 1, the effects of a range of environments on milk yield and composition are described. The thermoneutral environment (22°C) had a slight negative effect on milk yield after 3 days exposure but the 'mild cold' (0°C ± 1°C, still air) and the 'moderate cold' (0°C ± 1°C, wind speed 5.6 m/s) environments lead to a decline in milk yield to 86% and 81% of their previous yields respectively. The 'cold and wet' environment (as for 'moderate' but with cold water sponged onto the animal's back) did not produce any significant effects on milk yield. All the test environments lead to small changes in the ionic composition of milk, but there was no suggestion of disruption of the mammary epithelium. Milk lactose concentration rose under all the test climatic conditions; there were no significant changes in milk citrate levels and milk fat content rose only in the 'mild cold' conditions.

The results of Section 2 show that the effects of the 'moderate cold' environment on milk yield were most marked in mid lactation and that composition was most affected in early lactation.

Section 3 examined the relationship of energy balance and the response of rate of milk secretion to the 'moderate cold' environment. Food intake was restricted, thus lowering the calculated energy intake, and output of milk energy was determined by calculation. The results indicate that the response in terms of reduction in milk secretion was more closely related to stage of lactation than initial energy balance.

In Section 4 the response of denervated and intact mammary glands to moderate cold stress was found to be equal.

Section 5 examined the concentration of intracellular metabolites in milk during exposure to cold environments. Phosphoenol pyruvate levels were found to decline during exposure of goats in mid-lactation to the moderate cold environment. Concentration of other metabolites was unaffected by the cold conditions.

The results of plasma analysis shown in Section 6 indicate that plasma cortisol concentration was not affected by the cold environments, but that the cold and wet conditions lead to increases in free fatty acid concentration. It is suggested that the animals exposed to the cold and wet conditions were the most stressed, but that this stress was not reflected in mammary gland function.

In the final chapter, the response of the lactating goat to cold environments is discussed, in relation to effects on milk secretion.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Aims of the Project

The primary aim of this project was to examine the effects of cold exposure on the lactating goat; to assess the response of the milk secretory process to this environmental stress and to examine possible mechanisms of the response. Previous work (Thomson, 1978) suggested that lactating goats are sensitive to low temperatures, showing significant reductions in milk yield without any obvious local effects on mammary metabolism.

The approach used in this study was to take lactating British Saanen goats from an established research herd, and expose them to cold environments in a precision climatic chamber, for periods up to three days in length. The response in terms of milk yield, milk composition and levels of certain plasma metabolites was recorded. In subsequent experiments goats were also subjected to various levels of short term dietary restriction in combination with cold exposure. Thus the response of the mammary gland to low temperatures whilst under different states could be examined.

Goats were used as the experimental animal because of their convenient size, being easier to handle and cheaper to keep than cows, and also because much is already known of their physiology of lactation (Linzell and Peaker, 1971a). The findings of this project may be useful both in the dairy goat industry, which is rapidly expanding, and in an increased understanding of the mechanisms responsible for controlling milk secretion at the level of the mammary gland.

Review of the early work

Most of the published data from the beginning of the century, in

this field, refers to bovine lactation. A detailed review of the bovine literature is beyond the scope of this project; the reader is referred to Hancock (1954) and Johnson (1965) who have published reviews of early work. One of the earliest detailed records is that of J.S. Spiers (1909) which was commissioned by The Science Committee of the Highland and Agriculture Society. In an experiment with one hundred dairy cows, increased ventilation was found to give a significant increase in health and production of the animals. This experiment also dispelled the previously held belief that cooler environmental temperatures would lead to a decreased fat content of the milk.

Also in the earlier part of the century, agriculturalists working in America, where European breeds of dairy cow had been introduced into the semitropical southern states, began to study the effects of climate on milk production. Ragsdale (1949) studied the responses of different breeds of cattle, using controlled climatic chambers at the Missouri Agricultural Experimental Station. He found that cattle were more severely affected by increasing temperature above 10°C than by decreasing it, and also that sudden reductions in air temperature were most detrimental in terms of decreasing milk yield. Over long periods animals were able to acclimatize to cold environments.

Workers in the Soviet Union (Sementovskaya and Garkavi, 1950) found that milk yield was progressively reduced by exposing cows to lower air temperatures, and that animals lost weight whilst the levels of fat in their milk increased. However, when the cows were maintained at -4.9°C and given a 25% increase in roughage intake, the cows gained weight, milk yield only fell slightly and milk fat yield rose. Therefore with extra roughage intake the animals were able to adjust to their new conditions, and maintain milk yield.

Much work has been carried out on the effects of high temperatures on dairy cattle in an attempt to improve the agricultural production of underdeveloped tropical countries. Differences between breeds in their ability to thermoregulate were discovered (Kibler and Brody, 1950) and optimal environmental conditions could be recommended.

At high environmental temperatures cattle will voluntarily reduce their food intake and thus reduce their heat production. At a temperature of 31°C, Johnson et al. (1966) found that by feeding refused food via a rumen cannula the expected decline in milk yield could almost be negated. On exposure to cold environments animals normally increase their food intake as a means of increasing heat production to maintain their body temperature. In 1940, Dice, working in North Dakota, discovered that cows reared at -2°C maintained their level of milk production, and their food conversion rate was more efficient than cows given protection from the weather. However, further work with heifers (1942) showed that growing animals were not able to compensate for the low temperature and also maintain their rate of liveweight gain.

Lactating cows are markedly resistant to cold environments (Findlay, 1958; Blaxter, 1958) and provided that extra food is available to meet increased maintenance requirements, and some protection from wind and rain is available, milk yield can be maintained. Hahn (1981) has calculated that for every 10°C decrease in temperature below +5°C results in a loss of 0.25 kg/day in Canadian dairy cows. Also an extra kilogram of hay each day will be required by each animal for every 10°C decrease. Therefore, protection from the winter weather and provision of extra fodder at low temperatures is economic. Fujita et al. (1982) have studied the performance of lactating cows in precision climatic chambers and found that a reduction in temperature (gradual or

sudden) from +10°C to -20°C caused an increased roughage intake. Although milk yield fell the concentration of fat and protein rose so that no actual decrease in milk fat or protein yield resulted.

The lactating goat, however, appears to be much more sensitive to cold stress. The response of non-productive goats of different breeds to various environmental temperatures has been studied. Appleman and Delouche (1958) found that Nubian goats subjected to +5°C for 12 days were unable to maintain homeothermy since their rectal temperature began to decline. Bianca and Kunz (1978) subjected non-productive goats of three breeds (Gemsfarbige-Gebirgsziege, Saanen and Toggenburger-sic) to -5°C for 4 hour periods and found that they were all able to maintain homeothermy; heart rate significantly increased indicating increased metabolic heat production. From these experiments the Toggenburger goats were found to be the most cold tolerant of the three breeds. The lower criticial temperature (the environmental temperature at which heat production will increase in order to maintain body temperature) will depend on many factors including the level of food intake and the level of milk production. Thomson (1978) working with the same British Saanen goats as used in the present study, found that exposure to 0°C with a windspeed of 4 m/s for two days increased the metabolic rate by 46% and decreased the rate of milk secretion to 45% of the control.

Thermoregulation in the Goat

For each species and for members of that species in defined conditions (growing, pregnant, lactating, etc.) physiologists define a thermoneutral zone within which body temperature can be maintained without a change in heat production or loss. When subjected to a thermal environment outside that zone a homeothermic animal such as the

goat must respond in order to maintain its body temperature by minimizing heat loss and increasing heat production.

At temperatures below the thermoneutral zone (below the lower criticial temperature) the rate of heat lost from the animal to the environment will rise. These losses occur by evaporation, conduction, convection and radiation. The goat may be able to minimize evaporative heat loss by reducing breathing rate, decreasing losses by evaporation from the respiratory surfaces. Losses by conduction can be minimized by avoiding contact with cold surfaces such as concrete flooring, standing instead of lying, and if possible, huddling with other animals. Convection, or loss of heat to the atmosphere, which will be increased by high air speeds as well as low temperatures, can be reduced by piloerection to trap a layer of air around the skin, and also by vasoconstriction to reduce blood flow to the body surface. Heat lost by radiation depends on the shape and surface qualities of the body and cannot be readily minimized in the short term. Selection for conformation and coat type will allow certain breeds to be adapted to particular environments.

Heat production by the animal can also be increased in order to maintain homeothermy. This can be achieved in the goat by shivering and thus producing energy in the skeletal muscles, by increasing food consumption and therefore energy resulting from metabolism and or by mobilizing adipose reserves so that more substrate is available for heat production. These processes are under central nervous and hormonal control.

The extent of these mechanisms of thermoregulation in cattle have been well studied and reviewed (Bianca, 1965; Thompson, 1973) but little in goats. Receptors in the skin trigger responses in the spinal cord and hypothalamus which then stimulate heat production (Jessen, 1977).

It appears from the studies previously mentioned that goats respond to cold environments by shivering, piloerection and an increased heart rate. Little study has been made of the relative contribution that these processes make to the maintenance of homeothermy, and little is known of the response of the lactating goat to cold stress.

The Mammary Gland, Milk Secretion and Composition

The udder of the dairy goat comprises two well developed inguinal mammary glands which share the blood and nerve supply of the inguinum. Mammary glands, being specialised skin structures, receive similar blood and nerve supplies to the skin. The arterial blood supply branches so that each lobule of the alveolar mammary tissue possesses a rich capilliary system of smaller blood vessels. The venous drainage is via the milk vein (caudal superficial epigastric) which lies cranial to the udder on the surface of the belly. In mature animals, however, valves in the pudic vein can become incompetent allowing venous blood from the abdomen to enter the gland. This can be prevented by manual clamping of the pudic vein during mammary blood flow measurements, or sampling of mammary blood. By comparing the concentration of substrates in arterial blood with the mammary venous blood, and with measurements of mammary blood flow, it has been possible to determine the rate of uptake of possible precursors for milk synthesis by the gland (Linzell, 1974).

The secretory tissue of the gland consists of specialised epithelial cells (see Linzell and Peaker, 1971a) containing a well developed Golgi apparatus with tight junctions between cells in healthy animals, so that all the components of milk must enter the lumen of the gland by passing through the cells. At the basal membrane surface metabolic precursors are taken up and then used in the synthesis of milk constituents within the cell. The precursors of milk fat, protein and lactose have been identified by means of isotopic labelling experiments (Linzell, 1978).

The precursors of milk fat are mainly acetate and β-hydroxybutyrate which are built up into fatty acids and incorporated into triglycerides before release, in the form of lipid droplets, into the lumen. The composition of milk fat varies enormously between species with most ruminants having a low fat level, made up of a higher proportion of short chain fatty acids. The goat mammary gland is also able to take up free fatty acids, triglycerides and some free glycerol directly from the plasma which contribute to the long chain fatty acid fraction of the milk fat. Lipid droplets, when exocytosed from the apical membrane of the secretory cell, are surrounded by a membrane and may carry with them small fragments of cytoplasm. This may account for the presence of intracellular metabolites in milk.

The major milk proteins - casein, α-lactalbumin and β-lactoglobulin are synthesized in the secretory cell from essential and non-essential amino acids derived from the plasma. Protein synthesis takes place on the endoplasmic reticulum and the final protein product is contained in vesicles formed by budding off of fragments of membrane at the Golgi apparatus. These vesicles move to the apical surface of the cell where their contents are released into the milk. As for lipid droplet release the exact method of exocytosis is not fully understood. Limitation of the rate of milk protein synthesis was thought to be the rate of supply of certain amino acids to the gland (Mepham, 1976) but recent work by Henderson and Peaker (1983) has shown that at peak lactation when milk secretion increases in a compensatory response to colchicine inhibition in the opposite gland, there is no accompanying increase in availability of essential amino acids.

Lactose in milk is derived purely from glucose and is synthesized in the Golgi apparatus (Kuhn and White, 1975). The glucose utilized may be derived directly from plasma glucose or can be synthesized from

amino acids or glycerol. As the ruminant derives very little glucose from its diet, conservation of glucose is very important. In the fed state gluconeogenesis is highest as precursors are available but when the requirement for metabolic glucose is raised as in starvation, pregnancy or lactation, a higher proportion of lipid must be used as an energy source. Lactose also appears to be transported to the apical membrane in membrane bound vesicles derived from the Golgi apparatus and is released with milk protein into the lumen. Lactose appears to be the major osmole of milk drawing water across the apical membrane into the milk.

The aqueous phase of goats milk contains a solution of lactose, citrate, salts and ions in water, and is isoosmotic to plasma. It has been possible to measure the potential differences between milk, extracellular and intracellular fluid and therefore determine the electrochemical gradients that exist. Peaker (1977 and 1978) has proposed a scheme to explain the movement of ions across the mammary epithelium and their resultant concentration in milk. The scheme depends on the presence of a sodium potassium pump on the basal membrane which maintains a high intracellular concentration of potassium relative to the extracellular fluid, and a low concentration of sodium. Milk is electrically positive relative to the inside of the cell and sodium and potassium ions are able to move freely across the apical membrane accordingly to the electrical gradient. Some ions will also be released into milk with lactose and protein during the exocytosis process. In goats it has been found that when lactose concentration changes there are inverse changes in sodium and potassium concentration; the ratio of sodium to potassium is maintained at 1:3. The apical membrane is permeable to chloride ions which would tend to migrate into the milk, but as levels are not high in milk, Linzell and Peaker (1971a, b)

have proposed the existance of an active chloride pump on the apical membrane to drive chloride back into the cell possibly coupled to the movement of bicarbonate ions.

The secretion of citrate into milk has recently been reviewed by Faulkner and Peaker (1982). The concentration of citrate is important in maintaining the mineral equilibrium of milk which is vital in milk processing such as cheese making (Holt, Muir, Ormrod, Zammit and Peaker, 1980). It is thought that milk citrate is derived from citrate formed in the mitochondria of the secretory cells, mainly from acetate (as acetyl CoA) and glucose. Citrate is probably included in the vesicles with lactose and casein at the Golgi apparatus and released by exocytosis at the apical membrane.

All the components of milk are thus secreted into the lumen of the alveoli and accumulate in the duct system of the gland. The composition of the stored milk is unchanged from the time of secretion until removal by milking or suckling. Initial milk removal will be from the cistern and ducts of the gland, but at the end of the milking process the peptide hormone oxytocin is released into the bloodstream causing contraction of the myoepithelial cells which expels milk from the alveoli themselves - the residual milk (Cross and Findlay, 1969). Glands which have been denervated and transplanted will receive the stimulus of oxytocin only after milking of the gland remaining in situ (Linzell, 1963).

Control of Milk Yield and Composition

Milk yielded by an individual animal during each lactation follows a defined pattern or lactation curve which is under genetic and hormonal control. Development of the mammary tissue occurs during pregnancy and lactogenesis and the initiation of milk secretion begins around the time of parturition, dependant on the species (Fleet, Goode, Haman, Laurie,

Linzell and Peaker, 1975). In early lactation as the secretory tissue is activated yield rapidly increases to a peak, at a time when the energy requirement of the offspring is highest. From peak, milk yield gradually declines but can be maintained at a relatively high level by regular milking in the dairy animal. During lactation there will be a corresponding cycle of energy balance depending on the stored fat available at parturition. In early lactation the energy required for milk production exceeds energy intake, the animal is in a state of negative energy balance and fat reserves will be mobilized. After peak milk production when yield is declining food intake may remain high and adipose stores can be replenished.

At any time during lactation various factors may influence milk yield and or composition. The rate of synthesis of each component of milk may be affected by supply of the necessary precursors: either by changes in the rate of their supply via the mammary blood flow or by alteration in their plasma concentration. Mammary blood flow may be affected by various stresses mediated by local vasoconstricting and vasodilating agents (Linzell, 1974) but is not always correlated with the rate of milk secretion (Thompson and Thomson, 1977; Henderson and Peaker, 1980). The plasma level of substrates available for milk synthesis may be influenced by the level of feeding or dietary changes (Devendra, 1982; Morand-Kehr and Sauvant, 1980) or by the hormonal state of the animal (Bines and Hart, 1978).

Low environmental temperatures, as employed in the present study may lead to an increase in metabolic rate which the animal can achieve by a higher level of food intake or by the mobilization of fat reserves. Thus changes in the metabolic state of the animal may be expected to be reflected in changes in milk yield and composition.

Non-specific stress

Various disturbances and stresses have been known to reduce or inhibit milk secretion in many species including man. A temporary inhibition in milk let down may be overcome by oxytocin treatment but more severe inhibition may lead to premature cessation of lactation. The mechanisms controlling this process are not fully understood but one hormone that has been found to rise during stress is cortisol (Friend, 1980). Methods for assessing stress factors in farm animals that may affect production, and in experimental animals which may influence results are constantly being sought but no accepted measure is recognised (Stott, 1981). Cortisol has been found to rise in the plasma of sheep exposed to cold environments (Panaretto and Vickery, 1970) and in the plasma of pregnant goats during fasting (Chaiyabutr, Faulkner and Peaker, 1982). Synthetic glucocorticoids have been shown to decrease milk production (Braun, Bergman and Albert, 1970) in the cow, possibly by their action on plasma glucose levels. Plasma cortisol levels were recorded during this study in an attempt to monitor non-specific stress and also to follow the relationship of cortisol and the rate of milk secretion.

The goats used in these experiments were all trained to handling by research workers and also previously surgically prepared as described in Chapter 2, for blood sampling. Care was taken to duplicate, as far as possible, the animals' normal routine whilst in the precision chamber, and no other procedures were carried out, other than blood sampling once daily, during exposure to the test conditions.

CHAPTER 2

METHODS

Animals

The experimental animals used in this project were exclusively female British-Saanen goats, aged between 3 and 7 years, in their second to sixth lactation either non-pregnant or in the early stages of pregnancy. They were housed in a newly refurbished goat-house in individual pens (1.8 m x 1.5 m), each pen containing an automatically refilling water bowl and a hay rick. The goats were bedded on sawdust, and the air temperature in the goat house ranged from 8°C to 24°C during the year.

After parturition the kids were removed and reared by hand, the adult goats then being milked manually, twice daily, at approximately 8.00h and 16.00h. The goats were trained to walk onto a milking stand and received their ration of concentrates whilst being milked. Each goat received concentrates (BOCM 'Red Label'; manufacturer's analysis, 4.5% oil, 16% protein and 9% fibre) at a rate of 1.5 kg/day split into two equal portions given at milking time, and 1.2 kg/day hay.

The milk yielded by each gland was recorded by weight together with the corresponding time of milking, and from this data the volume of milk secreted by each gland per hour was computed.

Experiments were carried out between the second and thirty-fourth week of lactation.

Animal preparation

Goats used for these experiments had previously been surgically modified to facilitate arterial and venous blood sampling with minimal restraint, and to reduce any distress caused to the animal.

Surgery was carried out under general anaesthesia induced by pentabarbitone sodium (SAGATAL, May & Baker Ltd., Dagenham, England) 0.22 mg/kg, and maintained on a semi-open system with halothane (FLUOTHANE, I.C.I. Ltd., Macclesfield, England) and nitrous oxide. In each animal one carotid artery was exteriorised in a skin loop on the neck, and one 'milk vein' (caudal superficial epigastric vein) was exteriorised anterior to the udder. All blood vessels crossing the udder between the two mammary glands were ligated to ensure a separate blood supply to each gland. Strict aseptic procedures were used; surgical technique being as described by the late Dr. J.L. Linzell (Linzell, 1960, 1963). Five of the goats used had previously been modified by the autotransplantation of one mammary gland to the neck, the pudic artery being anastomosed to the carotid artery (end to end), and the milk vein being sewn end to side to the jugular vein, as described by Peaker and Fleet (1979). A section of the carotid artery supplying the mammary gland with arterial blood was exteriorised, as was a section of the jugular vein below the anastomosis point, allowing samples to be taken of arterial blood entering the gland and venous blood leaving the gland (after occluding the normal jugular flow above the anastomosis point). This technique results in a complete denervation of the transplanted gland and therefore loss of the normal milk ejection reflex. When milking these goats the milker must firstly milk out the transplanted gland to empty the cistern and large ducts, then milk out the gland remaining in situ. The oxytocin released during the milking of this gland will cause the release of milk from the alveoli of both glands and this milk must now be removed by milking out the transplanted gland.

It is possible to take several blood samples in succession from the blood vessels exteriorised in skin loops, without causing stress to

the animals, but if any particular goat showed signs of discomfort (bleating, struggling or foot-stamping) she was given a subcutaneous injection of local anaesthetic (XYLOCAINE 2% with adrenaline, Astra Chemicals Ltd., Watford, England) before puncturing. However, if repeated blood samples were required over a long period (more than one week) a polyethylene catheter (DURAL PLASTICS LTD., Dural, N.S.W., Australia) was inserted into the blood vessel using an Argyle Medicut Cannula (SHERWOOD MEDICAL INDUSTRIES, St. Louis, Mo., U.S.A.). The catheters were flushed and filled with a sterile solution of trisodium citrate (7.6 g l^{-1}) in sodium chloride (7.2 g l^{-1}) (B.D.H. Poole, England), and secured with adhesive tape..

Experimental environment

Animals were exposed to the experimental conditions by transferring them to a precision climatic chamber as described by Findlay, McLean and Bennet (1959). Whilst housed in this chamber the goats were milked by their normal milker, and fed and cared for in the normal manner. There were always two goats used at a time in the experimental chamber as goats become distressed when isolated. Milk yield was recorded in the normal way, together with the time of milking.

Goats were normally moved into the climatic chamber in the afternoon, before milking time, and maintained for approximately 18h at ambient temperature to become accustomed to their new surroundings. Then the temperature was reduced to $0^{\circ}C \pm 1^{\circ}C$ during a period of approximately 2 hours, and maintained until the end of the experimental period. At the end of the prescribed period of cold exposure the temperature was returned to ambient, over a period of 2-3 hours, before the goats were returned to the goat house. If the experiment required the animals to be exposed to a simulated high wind, a high speed fan

(Smith Industries Precision Fan Co., Witney, Oxon, England) was placed approximately one metre behind the animals. (This was the lowest air temperature which could be maintained by the refrigeration apparatus).

In the initial experiments the goats were kept in standard metabolism crates whilst in the chamber, but as it was felt that this accommodation constituted a stress in itself, later experiments were conducted using pens within the chamber. These pens (1.5 m x 1.2 m) were free-standing with sheet-metal sides and a wooden slatted floor raised 30 cm from the chamber floor. The front of the pen was a mesh door to allow adequate circulation of the cold air around the goat.

A continuous record of the air temperature in the chamber during the experiment was made automatically on a chart recorder (Foxboro - Yoxall, London, England) and air movement in the chamber was monitored by timing the cooling rate of a 'kata-thermometer (Monteith, 1972) heated to 100°C.

Blood and Milk Sampling

Arterial and venous blood samples were taken at intervals as described for each experiment. Blood was withdrawn either through a needle (20g - veins, 22g - arteries) or a catheter into a plastic syringe (Brunswick-Sherwood Medical Industries Ltd., Ballymoney, N. Ireland) containing a drop of heparin (1000 u/ml, Boots Drug Co. Ltd., Nottingham, England). To separate plasma, the blood was spun in poly-prolyene centrifuge tubes in a refrigerated centrifuge (MSE-MISTRAL 2L, MSE, Crawley, England) at 4°C, with a force of 2500g for 20 mins. Plasma was stored at -20°C until required for analysis.

Milk samples were stored at 4°C with the addition of 0.05 ml of formalin before analysis of sodium, potassium, chloride, lactose, citrate and fat concentration. For the determination of the concentrations in

milk of glucose, galactose, nucleotide diphosphate, glucose-6-phosphate, phosphoenol pyruvate, iso-citrate and 2-oxo-glutarate the milk was stored at -20°C without preservative until required.

ANALYSIS OF MILK SAMPLES

Determination of the ionic composition of milk

The concentrations of sodium, potassium, chloride and lactose in milk preserved with formaldehyde were determined simultaneously using a Technicon Auto Analyser II (Technicon Industrial Systems, Tarrytown, N.Y., U.S.A.).

The determination of sodium and potassium concentration is carried out by the Technicon AAII using flame photometry. The flame photometer measures the intensity of light energies emitted by sodium and potassium as these materials are excited in the flame. Each metal ion has a peculiar wavelength at which it shows maximum emission (sodium 589 nm, potassium 768 nm) and the intensity of the emission at that wavelength allows calculation of the concentration of that ion. As the milk sample enters the autoanalyser it is mixed with a stream of air and acidic lithium sulphate; lithium is used as the internal standard. The sample stream is then dialysed against distilled water, and the ions passing into the water or analytical stream will be carried to the detector.

The concentration of chloride ions in milk is determined colorimetrically. The sample is introduced to the system with a diluent containing nitric acid, which prevents the formation of a ferric chloride precipitate, and provides the required acidity for the reaction. The stream is then dialysed to remove protein and other interfering pigments. A colour reagent is then added to the dialysed sample allowing the following reactions to take place:

(1) $Hg(SCN)_2 + 2Cl^- \rightarrow HgCl_2 + (SCN)^-$

(2) $3(SCN)^- + FE^{3+} \rightarrow Fe(SCN)_3$

Red complex.

The formation of the ferric thiocyanate complex varies directly with the level of chloride in the sample, and is measured by determining its optical density at 480 nm.

The concentration of lactose in fresh milk was also determined by a colorimetric method. The sample is diluted with saline and dialysed as it enters the system, after which it is mixed with alkaline ferricyanide and heated to 90°C. The colour change which occurs when ferricyanide is reduced to ferrocyanide is proportional to the level of lactose present in the sample and can be measured by the change in optical density of the solution at 420 nm.

Milk fat

A rapid method for estimating the level of fat in milk (Fleet and Linzell, 1964) was used. Milk was incubated at 37°C for 30 minutes, mixed by vortexing on a Whirlimixer (Griffen and George Ltd., London, England), a small volume of the milk then being drawn into a capillary tube and sealed with wax. The tubes were then centrifuged in a Micro-haematocrit centrifuge (Gelman - Hawksley Ltd., London, England) at 12,000 g for 15 minutes and within 15 minutes the percentage of fat in the whole milk was read on a Gelman - Hawksley Micro Haematocrit reader.

Milk citrate

The concentration of citrate in milk was determined by the method of Marnier and Boulet as described and modified by White and Davies (1963). A TCA (trichloracetic acid) filtrate was prepared from 0.5 ml of each sample by mixing in a 10 ml volumetric flask 0.5 ml milk and

4.5 ml water, and then making the volume up to 10 ml with 24% w/v TCA solution. The flask was shaken vigorously for a few seconds, and left to stand for 30 minutes before being filtered through a Whatman No.4 filter paper; 1.0 ml of this filtrate was then mixed in a pyrex test tube with 1.3 ml pyridine. Then to each tube at timed intervals, 5-7 ml acetic anhydride was added and the tube placed immediately into a thermostatically controlled water bath at 32°C for exactly 30 minutes. Within the following 30 minutes the optical density of the solution was measured at 428 nm using a light path of 1 cm.

Standard citrate solutions were prepared by dissolving 0.1913g sodium citrate ($Na_3C_6H_5O_7.2H_2O$) in water and making the volume up to 200 ml with water. Volumes of this solution (0, 2.0, 3.0, 4.0 and 5.0 ml) were pipetted into 25 ml volumetric flasks, and in each case the volume was made up to 12.5 ml with water, and then made up to volume with 24% (w/v) TCA. A volume of 1.0 ml of each of these solutions contains the equivalent of 0 (blank), 0.050, 0.075, 0.100, and 0.125 mg citric acid respectively, and were used to construct a calibration curve, all solutions being measured relative to the blank.

Analysis of cellular metabolites in milk

The cellular metabolites in milk were assayed by enzymatic methods as described by Bergmeyer (1974). All chemicals were obtained from Sigma London Chemical Co. Ltd. (Poole, England) including the enzyme β-galactose dehydrogenase. All the other enzymes used were supplied by The Boehringer Corporation (London) Ltd. (Lewes, England). All solutions were made up in distilled water unless otherwise specified. The Tris buffer used, contained 100 mM Tris, 10 mM EDTA, 10 mM $MgCl_2$ and 50 mM KCl and was adjusted to pH 8.0. Optical densities were measured using a Pye Unicam (Cambridge, England) SP 1805 UV Spectrophotometer, test

solutions being thermostatically maintained at 37°C.

Before analysis, milk samples were deproteinised by the following procedure. To 10 ml of fresh milk, 1.0 ml of 60% (w/v) perchloric acid ($HClO_4$) was added; after thorough mixing and centrifugation at 1500g and 0°C for 15 minutes, the fat layer was removed and 8.0 ml of supernatent were taken. The supernatent was neutralized by the addition of 1.5 ml of 5N potassium carbonate (K_2CO_3), and after mixing, the precipitate was allowed to settle out by standing at 0°C. This supernatant was then used as the sample for the analysis of cellular metabolites.

Analysis of glucose, glucose-6-phosphate and isocitrate in milk

Glucose, glucose-6-phosphate and isocitrate were measured using an assay involving glucose-6-phosphate dehydrogenase (E.C.1.1.1.49), hexokinase (E.C.2.7.1.1) and isocitrate dehydrogenase (E.C.1.1.1.42).

Into a cuvette were pipetted 50 μl ATP (5 mg/ml, pH 7-8), 50 μl NADP (2 mg/ml), 0.5 ml Tris buffer, 5 μl $MgCl_2$ (1M), a volume of sample and made up to 1.0 ml with distilled water. After mixing, the optical density was recorded at a wavelength of 340 nm until a steady level was reached, and this value designated E_0. Next 5 μl glucose-6-phosphate dehydrogenase was added and the new optical density was recorded (E_1). On the addition of 5 μl hexokinase a third value for optical density at 340 nm was recorded (E_2). Finally 5 μl isocitrate dehydrogenase was added to the contents of the cuvette, and a fourth optical density (E_3) was recorded. From these values the concentrations of the substrates could be calculated as shown below:

$$\frac{|E_0-E_1|}{6.22} \times \frac{\text{dilution of milk}}{\text{sample volume}} = \mu\text{moles glucose 6-phosphate/ml milk}$$

$$\frac{|E_1-E_2|}{6.22} \times \frac{\text{dilution of milk}}{\text{sample volume}} = \mu\text{moles glucose/ml milk}$$

$$\frac{|E_2-E_3|}{6.22} \times \frac{\text{dilution of milk}}{\text{sample volume}} = \mu\text{moles isocitrate/ml milk}$$

Analysis of phosphoenol pyruvate in milk

The enzymes lactate dehydrogenase (E.C.1.1.1.27) and pyruvate kinase (E.C.2.7.1.40) were used to determine the level of phosphoenol pyruvate in milk.

Into a cuvette were pipetted 0.5 ml Tris buffer, 0.05 ml NADH (2 mg/ml in Tris buffer), 0.1 ml ADP (4 mg/ml), a volume of sample, and distilled water to make up the volume to 1.0 ml. The contents of the cuvette were mixed thoroughly and the optical density at 340 nm recorded (E_0). Next 5 μl lactate dehydrogenase were added and when a constant optical density was reached, this was recorded (E_1). A volume (5 μl) of pyruvate kinase was then added, and the second change in optical density (E_2) was recorded when constant. Therefore:

$$\frac{|E_2-E_1|}{6.22} \times \frac{\text{dilution of milk}}{\text{sample volume}} = \mu\text{moles PEP/ml milk}$$

Analysis of β-galactose in milk

The enzyme β-galactose dehydrogenase (E.C.1.1.1.48) was used to determine the level of β-galactose in milk. This was accomplished by pipetting into a cuvette 0.6 ml Tris buffer, 0.1 ml NAD (5 mg/ml) and a volume of sample, the volume was then made up to 1.0 ml by the addition of distilled water. The optical density of the solution at 340 nm was read (E_0) and 5 μl β-galactose dehydrogenase was added. The contents of the cuvette were mixed and the new optical density at 340 nm recorded (E_1) when it reached a steady level (about 60 minutes). The concentration

of β-galactose was calculated from the following formula:

$$\frac{|E_1-E_o|}{6.22} \times \frac{\text{dilution of milk}}{\text{sample volume}} = \mu\text{moles } \beta\text{-galactose/ml milk}$$

Analysis of nucleotide diphosphate and 2-oxo-glutarate in milk

The determination of the concentrations of nucleotide diphosphate and 2-oxo-glutarate in milk utilized the enzymes lactate dehydrogenase (E.C.1.1.1.27), pyruvate kinase (E.C.2.7.1.40), and glutamate dehydrogenase (E.C.1.4.1.3). Firstly to determine the level of nucleotide diphosphate, into a cuvette were pipetted 0.5 ml Tris buffer, 0.05 ml NADH (2 mg/ml in Tris buffer), 0.05 ml phosphoenol pyruvate (5 mg/ml) and a volume of sample, and the volume was then made up to 1.0 ml using distilled water. Then, 5 µl lactate dehydrogenase was added to the cuvette and when a constant optical density at 340 nm was reached this was recorded (E_o). When 5 µl pyruvate kinase was added and the optical density had become steady the new reading was recorded (E_1). Thus:

$$\frac{|E_1-E_o|}{6.22} \times \frac{\text{dilution of sample}}{\text{sample volume}} = \mu\text{moles nucleotide diphosphate/ml milk}$$

To determine the level of 2-oxo-glutarate in the sample, 5 µl glutamate dehydrogenase was added to the cuvette, causing a decrease in optical density. When the optical density had become constant it was recorded (E_2) and the decrease used in the following calculation:

$$\frac{|E_2-E_1|}{6.22} \times \frac{\text{dilution of sample}}{\text{sample volume}} = \mu\text{moles 2-oxo-glutarate/ml milk}$$

ANALYSIS OF BLOOD SAMPLES

Packed cell volume

A small volume of each blood sample (whole fresh blood) was drawn into a heparinised glass capillary tube and one end sealed with wax. The tubes were then centrifuged in a Gelman - Hawksley Microhaematocrit centrifuge at 12,000g for 15 minutes. The percentage of cells in the whole blood was then determined using a Hawksley Microhaematocrit reader.

Plasma glucose

Glucose was measured in deproteinised plasma by an enzymatic method, using hexokinase (E.C.2.7.1.1) and glucose-6-phosphate dehydrogenase (E.C.1.1.1.49) obtained from the Boehringer Corporation (London) Ltd. The plasma was deproteinised by mixing 2.0 ml 0.33N perchloric acid (Boehringer) with 0.2 ml plasma, mixing and centrifuging in an MSE Super Minor centrifuge at 2000g for 5 minutes. The supernatant was stored at -20°C until assayed. Into a cuvette was pipetted 2.5 ml buffer (0.3 M triethanolamine, pH 7.5, containing 4 mM $MgSO_4$), 0.1 ml ATP (9 mg/ml), 0.1 ml NADP (10 mg/ml) and 0.2 ml sample. The contents of the cuvette were mixed, and the initial optical density at 340 nm was recorded (E_o) on a Pye Unicam (Cambridge, England) SP 1750 Spectrophotometer. Next 0.02 ml of the enzyme mixture was added and the optical density was allowed to reach a steady level over 10 minutes, before being recorded (E_1). The concentration of glucose in the plasma could then be calculated:

$$|E_o - E_1| \times 25.5 = \text{mmoles glucose/l.}$$

Plasma glycerol

The level of free glycerol in plasma was determined enzymatically using enzymes and chemicals obtained from the Boehringer (London) Co. Ltd. Into a cuvette was pipetted, 0.6 ml buffer (0.25 M triethanolammonium chloride, pH 7.5), 0.1 ml ATP (5 mg/ml), 0.1 ml phosphoenol pyruvate (5 mg/ml), 0.05 mg NADH (3 mg/ml in triethanolammonium chloride buffer), 0.1 ml $MgCl_2$ (1 M), 0.01 ml lactate dehydrogenase (E.C.1.1.1.27) 0.01 ml pyruvate kinase (E.C.2.7.1.40) and 0.2 ml plasma. The reagents were mixed in the cuvette and the initial optical density at 340 nm was recorded (E_0) on a Pye Unicam SP 1750 Spectrophotometer. Next 0.01 ml glycerol kinase was added and the new level of optical density recorded (E_1). The solutions were read against a reagent blank containing Medium 199 (Flow Laboratories Ltd., Irvine, Scotland) in place of plasma. The concentration of glycerol in plasma could then be calculated:

$$|E_0 - E_1| \times 876 = \mu \text{ moles glycerol/l.}$$

Plasma triglycerides and free fatty acids

Free fatty acids (FFA) and triglyceride fatty acids (TFA) were extracted from plasma, and separated before being analysed by gas-liquid chromatography.

Plasma (5-6g) was weighed into a 250 ml flask, with 0.5 ml of mixed FFA and TG standard (0.3 mg/ml hectadecanoic acid and 0.3 mg/ml trihectadecanoin). To the flask was added 30 ml methanol, and 30 ml chloroform, and the contents shaken carefully, then incubated in a water bath at 60°C for 15-20 minutes. After cooling, 30 ml chloroform was added and the liquid was filtred through a Whatman No.1 paper into a measuring cylinder. The flask was rinsed with a 2:1 mixture of chloroform and methanol, and this rinsing also filtred into the measuring cylinder. Next one fifth of the total volume, of 0.88% w/v

KCl was added, and the aqueous layer allowed to separate by standing for 4 hours, or overnight. The top (aqueous) layer was discarded and the lower layer was evaporated to dryness using a Rotavapor R (Buchi, Switzerland) rotary evaporator. When dry the contents of the flask were dissolved in approximately 5 ml of 2:1 chloroform:methanol, and evaporated to dryness again. A further wash with chloroform, and a drying, ensured the removal of the aqueous fraction. The lipid material was taken up in 1 ml of 9:1 chloroform:methanol, and 1 ml of this sample was spotted onto a 10 cm x 20 cm thin layer chromatography (TLC) plate of silica gel (Kieslgel G, Merck, Darmstadt, Germany). The sample was separated by running (vertically) for 25 minutes in a mixture of ether, hexane and formic acid (20:80:2, v/v). The plates were allowed to dry and then sprayed with dichlorofluoroscein which allows the free fatty acids and triglycerides to be identified under an ultra-violet lamp. The areas of free fatty acids and triglycerides were scraped into two test tubes.

Free fatty acids only, were quantified after separation. They were methylated by adding 1 ml of 14% (v/v) boron trifluoride methanol (B.D.H. Ltd., Poole, England) and heating to 50°C for 15-20 minutes. The methylated sample was allowed to cool and diluted with 5 ml water, and to this was added 10 ml ether and 1 ml hexane. The sample was shaken in a stoppered test tube and allowed to settle. The methylated fatty acids, which were then in hexane, separated as the upper layer and this fraction was stored at -20°C with the addition of a small quantity (approximately 5g) of drying agent (Na_2SO_4:$NaHCO_3$, 4:1 w/v), before being analysed by gas-liquid chromatography (GLC).

Gas-liquid chromatography was performed on a Pye Unicam (Cambridge, England) GCD chromatograph, using a 1.5 m column packed with 10% EGSS-X (Chromatography Services Ltd., Merseyside, England). Before analysis,

50 µl of hexane (double distilled) was added to each sample and 5-10 µl was injected onto the column. Separation of the fatty acids was carried out at 180°C, using the following mixture of carrier gas; nitrogen 50 ml/min, hydrogen 50 ml/min and air 750 ml/min. The concentration of each free fatty acid in the sample could then be calculated by peak height, and compared with the internal standards.

Plasma cortisol

Cortisol in plasma was determined using a radio-immunoassay. To 100 µl plasma or standard (2-100 ng/ml cortisol, Sigma Chemical Co. Ltd., Poole, England), was added 1 ml petroleum ether, the contents of the tube were vortexed for 30 seconds and placed in the freezer for 20 minutes. The petroleum ether layer containing plasma lipids remained liquid and could be decanted and discarded. The frozen portion was then allowed to thaw, and 1 ml dichloromethane was added to extract the cortisol. The tubes were again vortexed and placed in the freezer for 20 minutes as close as possible to the horizontal, as the aqueous layer freezes above the dichloromethane layer which then contains the cortisol. The dichloromethane fraction was then placed in a scintillation vial and the solvent was evaporated with a stream of nitrogen. To the residue in the vial was then added 1 ml phosphate buffer (0.1 M, pH 7.0 containing 0.1g/ml of bovine serum albumin, Sigma Chemical Co. Ltd., Poole, England). The sample was then left at room temperature with occasional shaking for 2 hours. Next, to each sample 50 µl cortisol antiserum (Steranti Research Ltd., St. Albans, England) and 50 µl ^{3}H-cortisol (The Radiochemical Centre, Amersham, England) was added, thereby enabling competitive binding to take place between the labelled cortisol, and cortisol in the sample or standard, for the fixed number of binding sites on the antiserum. One tube was also prepared without antiserum

and without bovine serum albumin to give a measure of the total count. To all tubes was added 250 µl of a charcoal-dextran suspension to which the protein material was adsorbed. One tube was prepared containing labelled cortisol, without sample or antisera, and without the charcoal/dextran adsorbant to check that the unbound labelled cortisol is not being removed by the adsorbant. A measure of the total binding was determined using a tube containing antisera with labelled cortisol alone. Therefore after separating the adsorbed material by centrifuging at 2,500 rpm for 10 minutes at 4°C (Centra 7R centrifuge, Damon/IEC U.K. Ltd., Dunstable, England) 200 µl of supernatant was added to 10 ml of scintillant (Aqua Luma, LKB Wallac, Finland) for ^{3}H counting by a liquid scintillation counter (Packard Model 2425). The level of labelled cortisol remaining unbound indicates the level of cortisol in the sample competing for binding sites on the antisera.

This assay was carried out routinely in the analytical laboratory, and was found to give a sensitivity of 3.3 ng/ml, with an intra-assay coefficient of variation of 5.7% and an inter-assay coefficient of variation of 7.5%.

CHAPTER 3

SECTION 1

THE EFFECT OF SOME CLIMATIC FACTORS ON GOATS' MILK YIELD AND COMPOSITION

Introduction

The initial series of experiments involved the exposure of goats, in pairs, to various conditions in the precision climatic chamber. The aim was to determine any effects of air temperature, air movement or precipitation on the rate of milk secretion and the composition of milk secreted.

Methods

Lactating goats which had been maintained as described in Chapter 2 were moved to the precision room for a 24 hour period of exposure to ambient temperature, after which the experimental conditions were imposed for approximately 72 hours. All goats received hay ad libitum and 1.5 kg of concentrates in 2 meals fed at milking times. The artificial lighting in the precision room was controlled by an automatic time switch (Sangamo Western Ltd., Enfield, England) in order that a natural daylength was maintained. Goats were milked by their normal milkers at 8.00 h and 16.00 h and the weight of milk obtained was recorded, as in the goat-house.

The first experimental condition imposed on the goats was termed the 'control' environment and consisted of an air temperature of 22^{o}C $\pm$ 1^{o}C with no additional ventilation. Four goats were subjected to this condition for 48 hours between weeks 30 and 35 of lactation, the goats being kept in pens to minimize any non-specific stress.

The second experimental condition was termed 'mild cold' and consisted of an air temperature of 0^{o}C $\pm$ 1^{o}C without additional

ventilation. Six goats were used for this experiment, between weeks 6 and 17 of lactation, and were restrained in crates within the precision room.

The third experimental condition was termed 'moderate cold' and comprised of an air temperature of $0^{\circ}C \pm 1^{\circ}C$ with an electric fan being placed behind the animals, resulting in an air-speed of 5.5 m/s as measured by the kata-thermometer. The goats (5) were kept in crates in order that they were fully exposed to the increased air movement. These conditions were imposed between weeks 17 and 22 of lactation.

The final experimental condition consisted of the 'moderate cold' as described above, with the animals also being periodically sponged with cold water so that the hair on their backs was permanently wet. This condition was termed 'cold and wet' and as it appeared to have a severe effect on the condition of the animals it was only imposed for 36 hours. Four animals were subjected to this treatment between weeks 26 and 28 of lactation and again were kept in crates during the experimental period.

For each goat the total rate of milk secretion, and concentration of various constituents in the milk from one gland was recorded for a period of 48 or 72 hours before the imposition of the experimental period (including the 24 hour acclimatization period) and compared with the results obtained during the experimental period.

Results from all the experiments were then compared using students' t-test for paired observations.

Rates of milk secretion were also determined as a percentage of the mean rate found during the 48 or 72 hour period before the experimental conditions were imposed.

Results

Rate of milk secretion

The results indicate that there is only a slight effect on the rate of milk secretion (see table 1.1 and fig. 1.1) in even the most severe of the climatic conditions applied. The so called 'control' environment was used as this has been used previously (Thomson, 1978) as the thermoneutral environment with which the effect of cold exposure was compared. Since the average temperature inside the goats' normal accommodation during late lactation (autumn) was approximately 12°C, exposure to the 'control' environment may represent exposure to a warm environment. The results show no effect on milk secretion rate during the first 36 hours exposure to 22°C, but there was a significant decrease to 78 ml/h by the morning milking on the second day. This suggests that the animals were able to tolerate these conditions for 36 hours but after this the process of milk production began to suffer. The rate of milk secretion rapidly returned to its previous level when the goats were returned to their normal environment.

The 'mild cold' environment caused a slight decrease in the rate of milk secretion which only became statistically significant ($P < 0.01$) after 3 days of exposure. However, when goats were exposed to the same temperature but with an increased air movement, there was a highly significant decrease in the rate of milk secretion. After the first day in the moderate cold environment, the average milk yield decreased to 90 ml/h, from a previous mean value of 110.2 ml/h. It can also be seen from fig. 1.1 that the rate of secretion during the day time (i.e. rate calculated from the 16.00 h milk yield) was more seriously affected than the rate of milk secretion during the night. There are a number of possible explanations for this finding, which are discussed later.

TABLE 1.1 TOTAL MILK YIELD OF GOATS SUBJECTED TO VARIOUS CLIMATIC CONDITIONS (mean ± SE in ml/h)

ENVIRONMENT		CONTROL	MILD COLD	MODERATE COLD	COLD & WET
	n	4	6	5	4
DAY 1	PM	97 ± 4.5	126 ± 7.5	110 ± 9.4	80 ± 10.4
	AM	94 ± 2.6	115 ± 9.9	105 ± 7.7	78 ± 14.6
2	PM	99 ± 6.6	124 ± 6.2	116 ± 12.7	89 ± 12.3
	AM	91 ± 5.1	120 ± 6.3	107 ± 6.6	76 ± 13.8
3	PM	97 ± 7.2[a]	121 ± 4.2	121 ± 12.4	96 ± 18.7
	AM	88 ± 5.3	115 ± 8.8	103 ± 8.6	83 ± 12.8
4	PM	94 ± 5.0	116 ± 4.5	98 ± 14.5	89 ± 19.9
	AM	78 ± 4.4	111 ± 8.3	90 ± 9.3	76 ± 9.9
5	PM	92 ± 9.2	120 ± 5.5	100 ± 14.4	95 ± 19.0
	AM	92 ± 3.3	114 ± 8.6	94 ± 10.5	76 ± 10.2
6	PM	92 ± 5.3	111 ± 8.4	107 ± 11.6	86 ± 16.8
	AM	90 ± 4.7	105 ± 6.4	89 ± 7.0	74 ± 10.7

(a - figures within boxes represent yields obtained during exposure to test conditions)

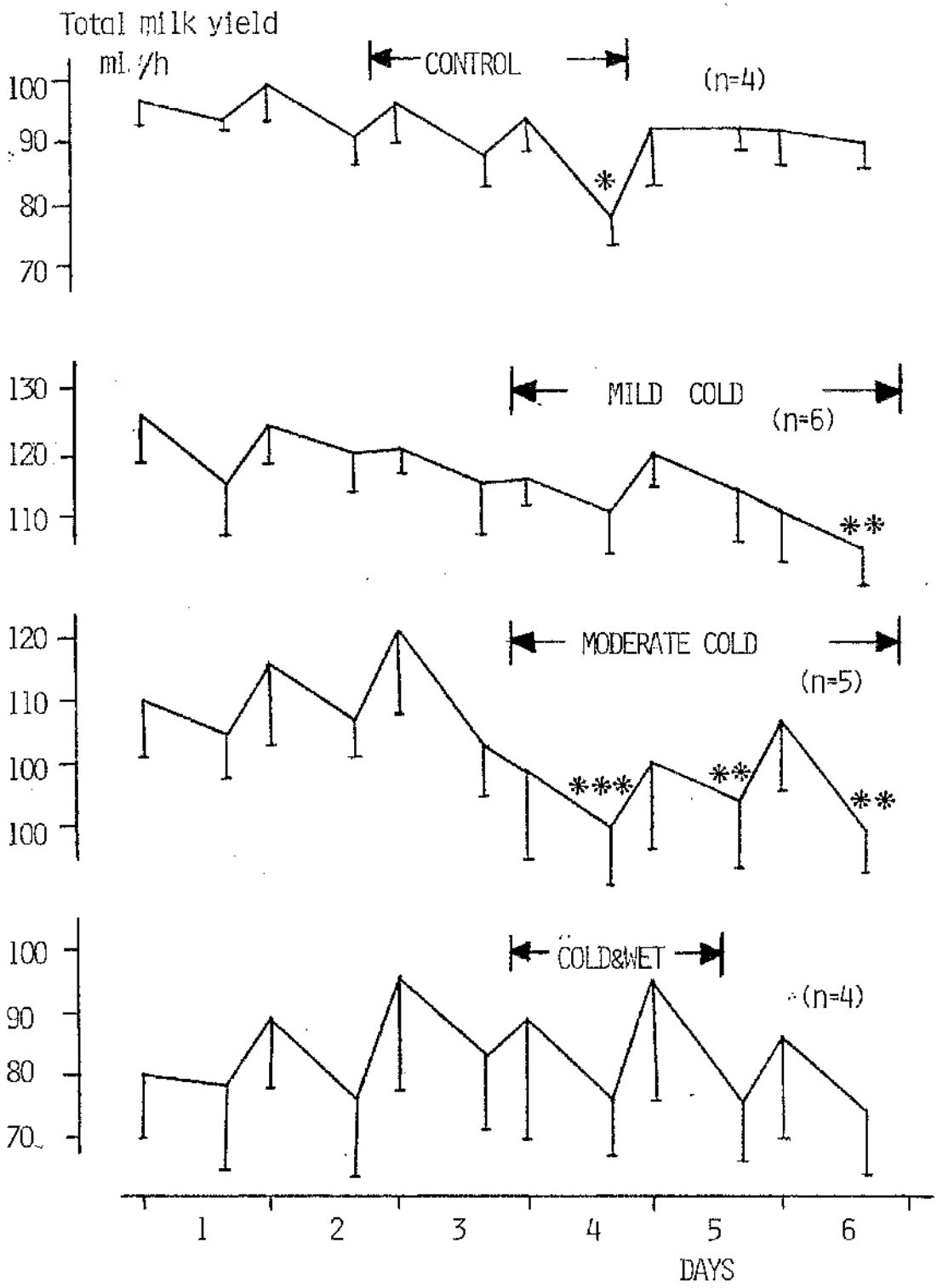

Fig 1.1 Effect of climatic conditions on milk yield in the goat (mean±S.E.)

(see text for details of climatic conditions)

* p<0.05
** p<0.01
*** p<0.001

significance of difference from previous yield

The group of goats given soakings with cold water whilst in the cold conditions ('cold and wet') showed a higher degree of variation in milk secretion rate as shown by the larger standard errors, and there was also a more marked variation between the night-time and day-time rates of secretion. There were no statistically significant changes found in the rate of milk secretion of these animals when they were subjected to the cold and wet environment or when they were subsequently returned to their normal accommodation. It is interesting to note, however, that in this group, the animals, by subjective observation, appeared to be the most severely affected, showing signs of distress; bleating, listlessness, piloerection and loss of appetite. As the animals were not consuming their rations satisfactorily they were removed from the chamber since the effect of reduced dietary intake would interfere with the effects of temperature on milk secretion. One animal that refused to eat any concentrates was returned immediately to the goat-house (where she recovered her appetite) and was not included in the results.

The effects of the various experimental conditions on the rate of milk secretion, expressed as a percentage of the previous yield for that group of animals, are illustrated in table 1.2 and figure 1.2. These show that the climatic conditions with the most severe effect on milk yield was the 'moderate cold' environment which caused the rate of milk secretion to drop to 81% of the previous rate. The 'mild cold' environment reduced the rate of milk secretion to 86% of the previous rate after 2 days exposure, but there appears to be a degree of acclimatization as on the third day the milk secretion rate had returned to 100%. The 'control' environment ($22^{O}C$) had very little effect on the rate of milk secretion, until the end of the second 24 hour period, when the rate was reduced to 81% of the previous yield.

TABLE 1.2 EFFECT OF CLIMATIC CONDITIONS ON MILK YIELD IN THE GOAT EXPRESSED AS A PERCENTAGE OF MEAN YIELD BEFORE TREATMENT

(mean ± SE in ml/h)

ENVIRONMENT		CONTROL	MILD COLD	MODERATE COLD	COLD & WET
	n	4	6	5	4
DAY 1	PM	102 ± 2.5	105 ± 4.1	101 ± 0.8	97 ± 7.1
	AM	99 ± 2.6	95 ± 3.7	96 ± 2.9	95 ± 6.5
2	PM	103 ± 5.8	103 ± 2.7	108 ± 6.5	108 ± 3.4
	AM	96 ± 5.4	100 ± 1.1	101 ± 4.8	91 ± 6.2
3	PM	102 ± 6.2[a]	102 ± 3.8	111 ± 5.4	114 ± 8.0
	AM	92 ± 5.7	95 ± 3.7	91 ± 2.5	100 ± 3.0
4	PM	99 ± 5.8	98 ± 6.2	88 ± 6.0	104 ± 7.8
	AM	81 ± 4.5	92 ± 3.6	85 ± 5.7	92 ± 3.8
5	PM	95 ± 7.1	101 ± 6.7	90 ± 8.5	112 ± 8.2
	AM	96 ± 1.6	86 ± 2.5	84 ± 2.8	92 ± 2.2
6	PM	97 ± 3.0	100 ± 8.8	99 ± 3.6	102 ± 4.1
	AM	94 ± 4.4	88 ± 2.8	81 ± 1.9	92 ± 5.1

(a - figures within boxes represent yields obtained during exposure to test conditions)

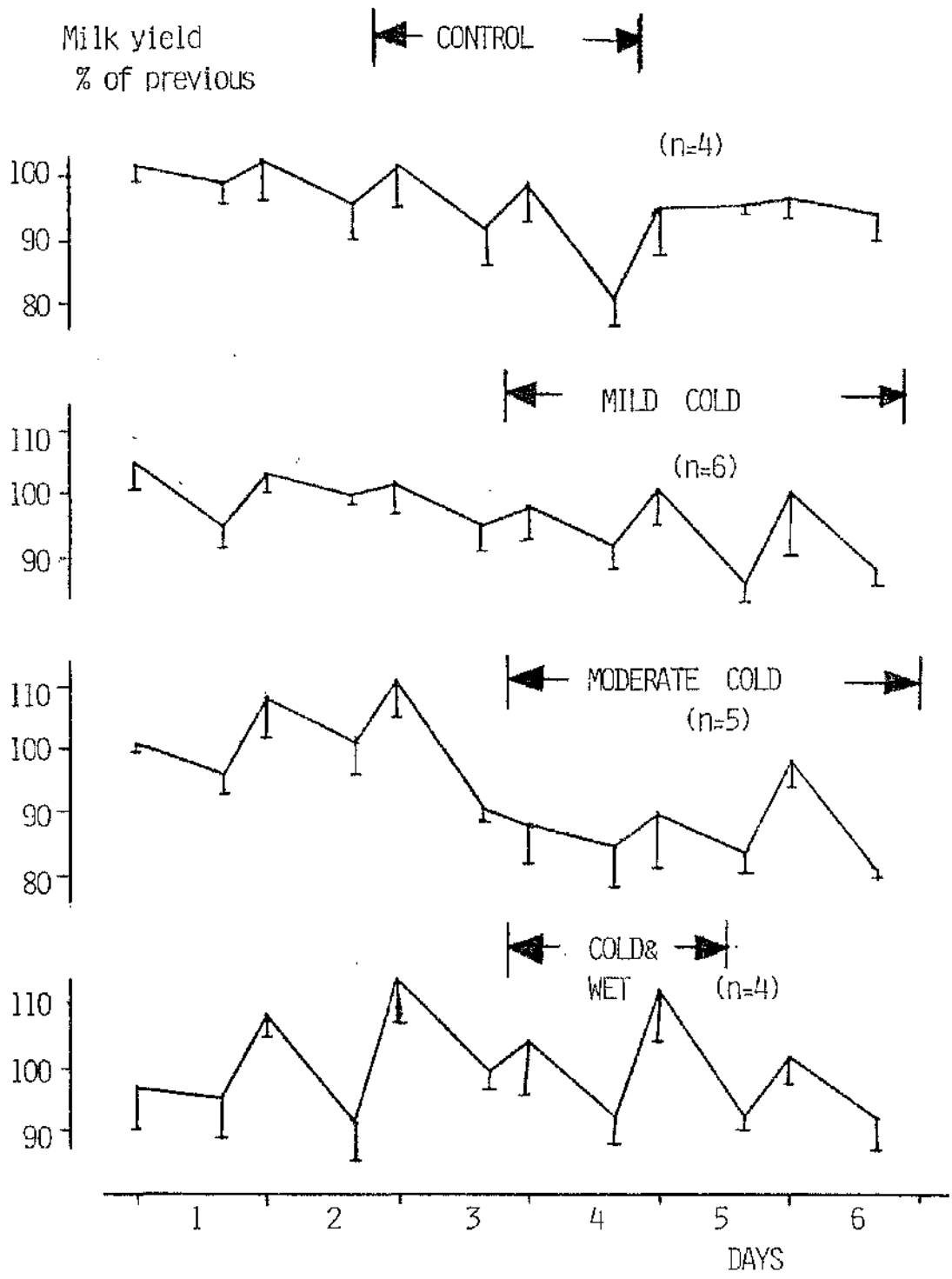

Fig 1.2 Effect of climatic conditions on goats' milk yield (total ml/h) expressed as a percentage of mean yield before treatment (mean±S.E.)

.(see text for details of climatic conditions)

The group of goats subjected to the cold and wet environment showed no significant change in milk yield during the experimental period or when they were returned to the goat-house.

Milk sodium concentration

There were only slight changes in milk sodium concentration under any of the experimental conditions (see fig. 1.3); levels varying between 14-20 mM. The control environment did show a significant decrease in sodium concentration on the second day, but in the afternoon milking only. (Note: one day is from the afternoon milking till the next afternoon). This drop precedes by one milking interval the decrease found in the rate of milk secretion (see fig. 1.1). On return to the goat-house the concentration of sodium in the milk was not significantly different from that before the experimental period. The last point on the graph is due to one spurious result of 25.2 mM in one goat, which may indicate the initial stages of an infection. The 'mild cold' environment did cause a slightly significant decrease in milk sodium concentration, but only for the first milking interval, subsequent to this there were no significant differences, the levels of sodium being maintained between 14 and 15 mM. The 'moderate cold' treatment showed a tendency to reduce the levels of sodium in the milk but this was not found to be statistically significant. The first two milkings under the experimental conditions show a slight but non-significant increase which corresponds to the decrease in rate of milk secretion at the same time. The 'cold and wet' environment showed no significant changes in milk sodium concentration, but the mean levels were noticeably higher (approximately 18-19 mM) than those found in the other 3 groups of animals.

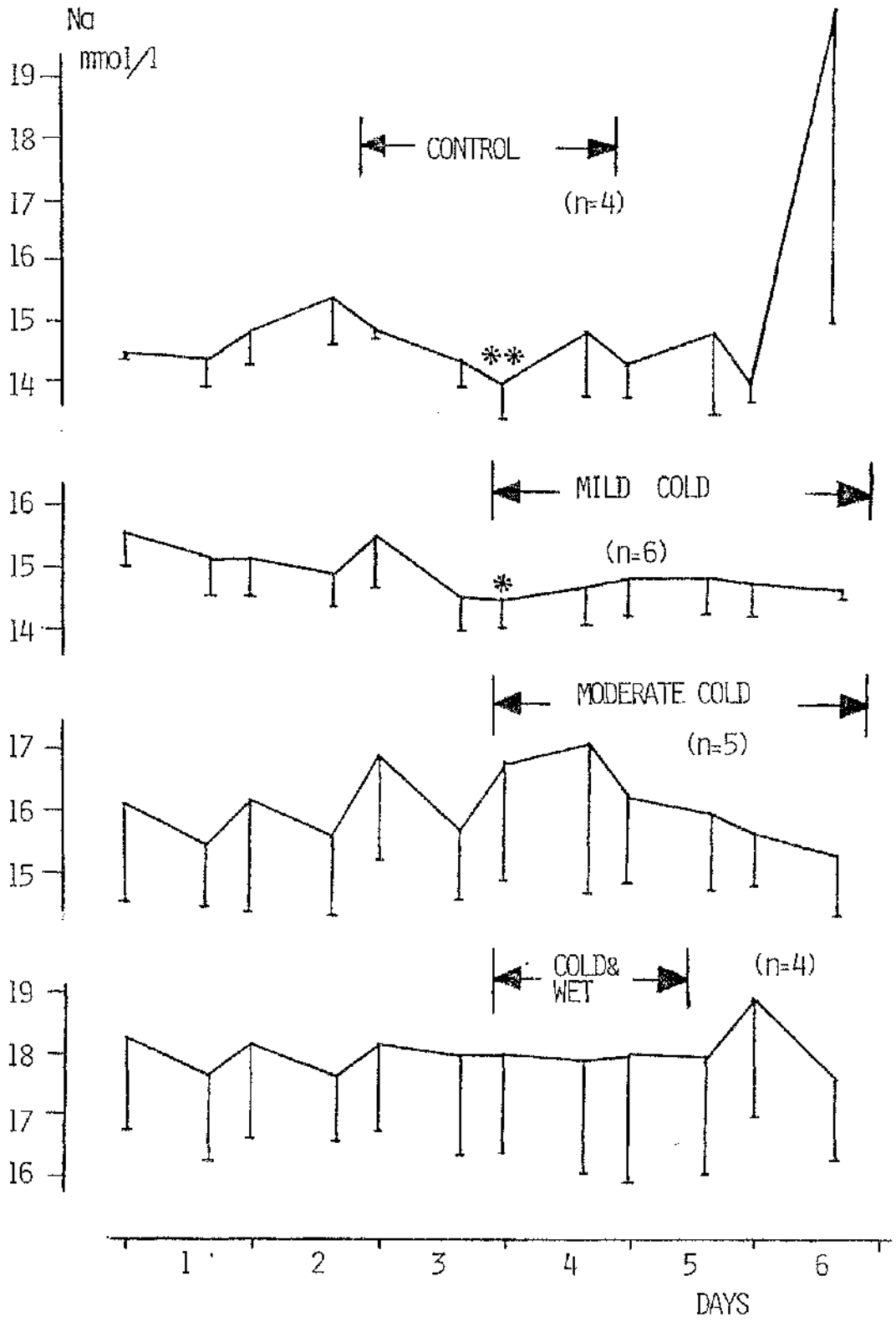

Fig 1.3 Effect of climatic conditions on sodium concentration (mmol/l) in goats' milk (mean±S.E. for one gland)

(see text for details of climatic conditions)

* $p<0.05$
** $p<0.01$ significance of difference from previous concentration

Milk potassium concentration

The concentration of potassium ions in the milk samples (see fig. 1.4) ranged from 44-58 mM. In the 'control' environment the levels dropped significantly ($P < 0.05$ after 36 hours and $P < 0.01$ after 48 hours). On the return to the goat-house there appeared to be an increase of milk potassium level - to a level (58 mM) higher than the previous level, but this was not statistically significant. The 'mild cold' environment showed a significant ($P < 0.05$) decrease in milk potassium concentration, and it was noticeable again that a diurnal variation seemed to exist, in that levels of potassium were lowest in the milk secreted during the day. The 'moderate cold' environment caused a more significant ($P < 0.01$) decrease in concentration of potassium in milk, from 50 mM before the experiment to 46 mM after 36 hours exposure. After 36 hours exposure the levels began to approach those of the pre-experimental period (although the rate of milk secretion had continued to fall). Under the 'cold and wet' environment the concentration of potassium in the milk fell significantly ($P < 0.05$) only after 48 hours exposure to the experimental conditions. On returning the goats to their normal environment there was firstly another decrease in potassium concentration, i.e. the level 45.3 mM was significantly lower ($P < 0.05$) than the level during the 'cold and wet' treatment, and secondly there was an increase in concentration so that the level of milk potassium, 48.8 mM, was significantly ($P < 0.05$) higher than the level during the treatment.

Milk chloride concentration

The concentration of chloride ions in the milk from the goats under test varied from 42-53 mM (see fig. 1.5). The 'control' environment showed no significant effect on the level of milk chloride although

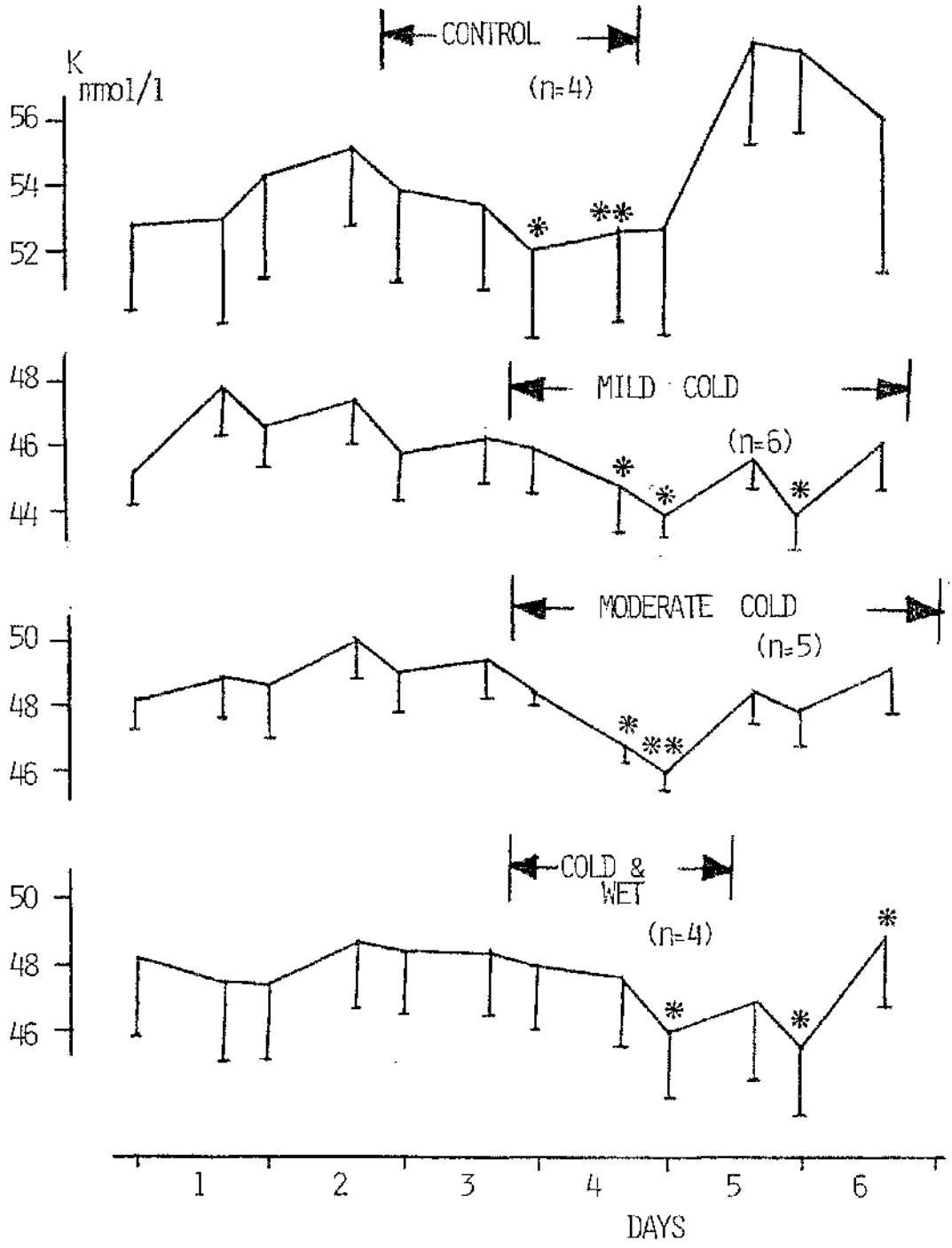

Fig 1.4 Effect of climatic conditions on potassium concentration (mmol/l) in goats' milk (mean±S.E. for one gland)

(see text for details of climatic conditions)

* $p<0.05$
** $p<0.01$ significance of difference from previous concentration

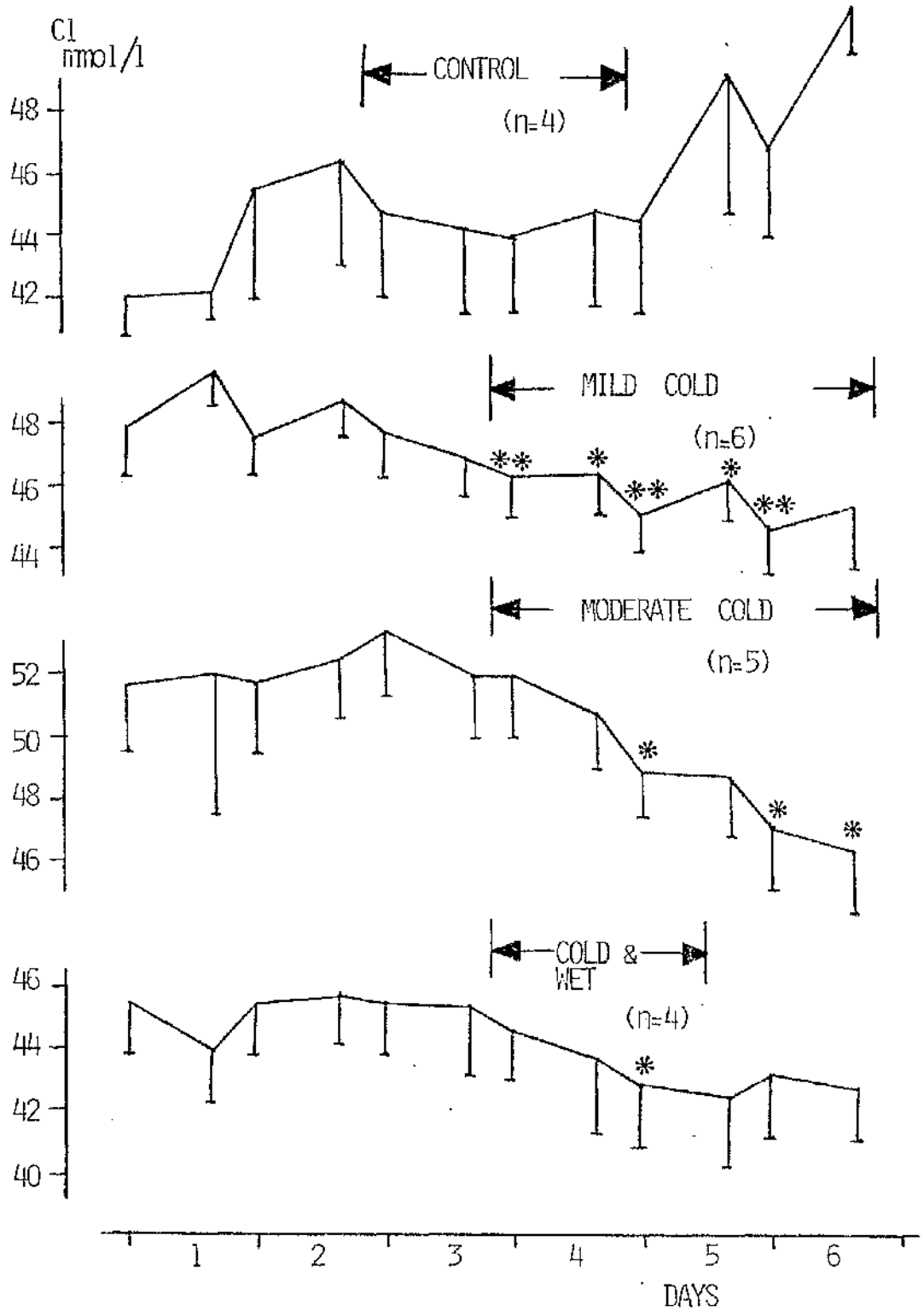

Fig 1.5 Effect of climatic conditions on chloride concentration (mmol/l) in goats' milk (mean±S.E. for one gland)

(see text for details of climatic conditions)

* $p<0.05$ significance of difference from
** $p<0.01$ previous concentration

there appeared to be a slight increase after the climatic conditions were returned to normal, but the variation within the group was large. The 'mild cold' environment caused a steady decrease in the concentration of chloride in milk which gradually fell from 46.2 mM to 44.6 mM ($P < 0.01$). The milk secreted during the day (pm milkings) showed a more significant decrease in chloride content on all 3 days of the treatment. The 'moderate cold' environment also caused a steady drop in milk chloride concentration from 51.8 mM to 46.2 mM. Owing to larger variation and smaller size of this group the decrease was only significant to the 95% level ($P < 0.05$) although the actual mean decrease in concentration was greater. The 'cold and wet' conditions only caused a slightly significant ($P < 0.05$) decrease in milk chloride concentration after 36 hours, to 42.7 mM from a mean level of 45.3 mM before treatment.

Milk lactose concentration

Milk lactose concentration in the experimental animals varied from 120-143 mM (see fig. 1.6). All the experimental climatic conditions caused a significant increase in milk lactose concentration. In the 'control' environment there was an immediate increase in milk lactose to 131.9 mM which was significantly higher ($P < 0.05$) than the mean levels (129.5 mM) before the treatment. This raised lactose level was maintained during the period in which the animals were in the climatic chamber, but on returning to the goat-house their levels of milk lactose dropped to below the level before the experiment. The 'mild cold' environment also caused an immediate significant increase ($P < 0.01$) in milk lactose concentration from 136.9 mM to 142.6 mM, which steadily increased further to 144.2 mM and represents a highly significant ($P < 0.001$) rise. In the 'moderate cold' environment there was again an immediate, significant ($P < 0.01$) increase in the concentration of

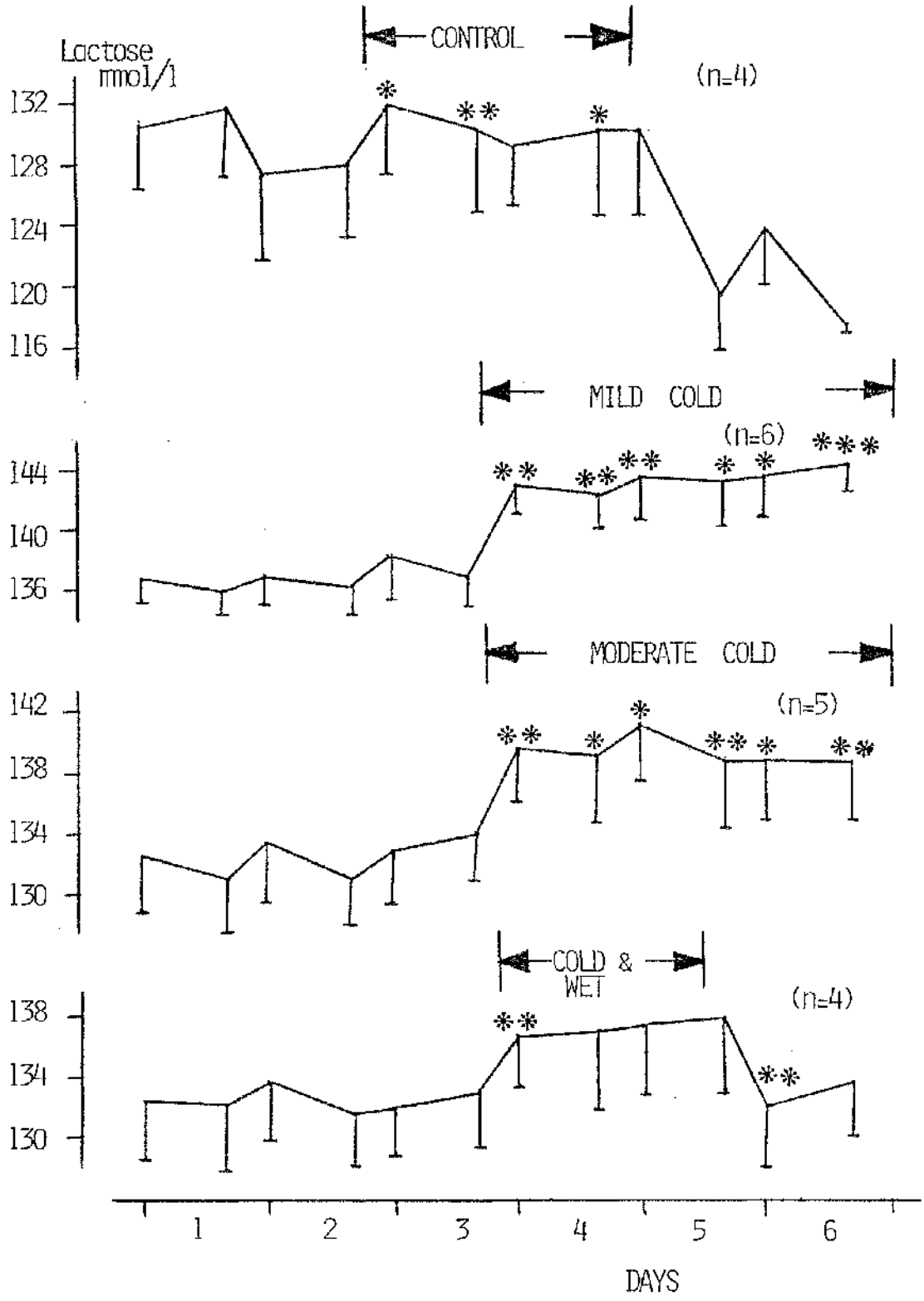

Fig 1.6 Effect of climatic conditions on lactose concentration (mmol/l) in goats' milk (mean±S.E. for one gland)

(see text for details of climatic conditions)

* $p<0.05$
** $p<0.01$
*** $p<0.001$

significance of difference from previous concentration

lactose in the milk. Before treatment the mean level of lactose concentration was 132.5 mM and it rose to a maximum of 141.2 mM during the experiment. During exposure to the 'cold and wet' environmental conditions there was also an immediate significant ($P < 0.01$) increase in milk lactose concentration from a previous level of 132.3 mM to 136.3 mM. This increase was maintained for the 36 hour period of the exposure, after which the level dropped significantly ($P < 0.01$) to 131.9 mM, when ambient conditions were restored.

Milk citrate concentration

The values for milk citrate concentration during this series of experiments ranged from 48.4 to 133.1 mg/100 ml (see fig. 1.7).

During exposure of the goats to the 'control' environment there was a small significant ($P < 0.05$) increase in milk citrate concentration from a mean level of 57.5 mg/100 ml to 74.3 mg/100 ml and although this higher level was maintained during exposure to the experimental conditions it was not found to be significant. On returning the goats to their normal environment, milk citrate concentration fell to its previous level. There was no significant change in milk citrate concentration during exposure to the 'mild cold' environment, although a slight decrease was observed. In the 'moderate cold' environment a slight increase in milk citrate concentration was observed but this was also found to be non-significant. The 'cold and wet' environment had no significant effect on the milk citrate concentration; however, on returning the goats to ambient conditions there was a significant ($P < 0.05$) increase.

Milk fat concentration

The fat content of the goats milk varied between 3.5 and 6.3 g/100 ml, as determined by the method of Fleet and Linzell (1964), (see

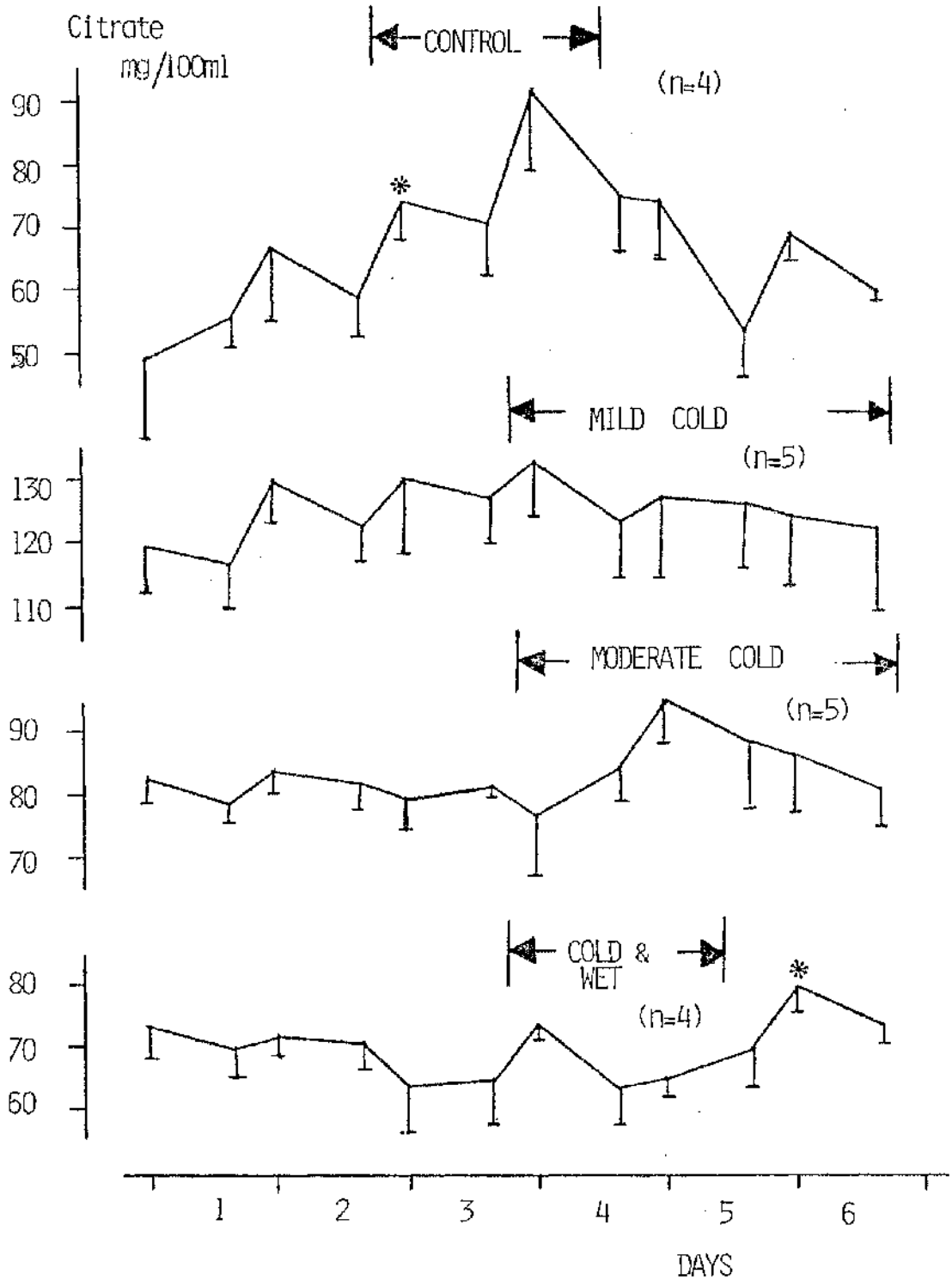

Fig 1.7 Effect of climatic conditions on citrate concentration (mg/100ml) in goats' milk (mean±S.E. for one gland)

(see text for details of climatic conditions)

* $p<0.05$ significance of difference from previous concentration

fig. 1.8). Under the conditions of the 'control' environment there were no significant changes in fat concentration. During exposure to the 'mild cold' environment there was a significant increase ($P < 0.05$) in milk fat concentration immediately, which continued to increase to a final value of 5.1 g/100 ml ($P < 0.01$) after 3 days exposure. In the 'moderate cold' environment there was a slight increase in milk fat concentration compared with the previous level, but this rise was not significant. When goats were placed in the 'cold and wet' environment there was no significant change in milk fat concentration but on returning the goats to their normal environment there was a highly significant ($P < 0.001$) increase to 6.3 g/100 ml.

Discussion

The rate of milk secretion for the British Saanen goats used in this series of experiments is higher (80-120 ml/h) than that found by Thomson (1978) and Thomson et al. (1979) working with the same breed of goats at this Institute (50-60 ml/h). Similar rates have been found in the goat by other workers; 75 ml/h by Fleet and Peaker (1978) and 80-90 ml/h by Patton (1978). This difference in the basal level of milk production is probably due to differences in breed, diet, and husbandry techniques.

The effects of the different climatic conditions on the rate of milk secretion found in this study are not as marked as those observed by Thomson (1978). The 'mild cold' environment led to a maximum reduction to 88% of the previous yield whereas Thomson et al. (1979) found a decrease to 85% of the previous yield. In the 'moderate cold' environment, with an air movement of 5.5 m/s, there was a decrease to 81% of the previous yield whereas Thomson (1978) found a decrease to 75% using an air-movement of 3.6 m/s. These figures are in approximate agreement

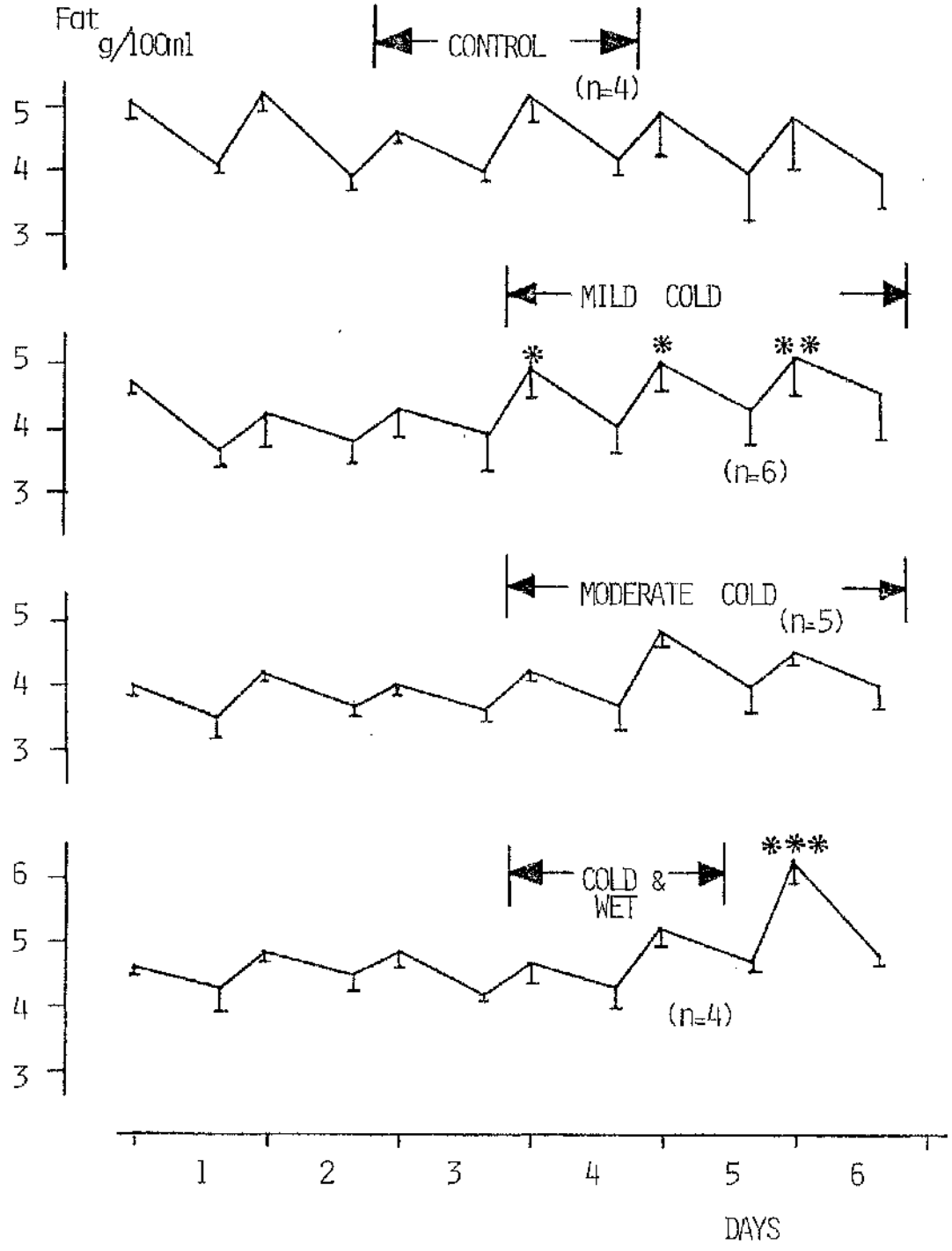

Fig 1.8 Effect of climatic conditions on fat concentration (g/100ml) in goats' milk (mean±S.E. for one gland)

(see text for details of climatic conditions)

* $p<0.05$
** $p<0.01$
*** $p<0.001$

significance of difference from previous concentration

but it is possible that the larger effects found by Thomson were due to the condition of his animals. The goats used in his study were the same breed and of similar body weights and ages, but were fed at a lower rate with a different diet.

The levels of inorganic ions in the milk found in this study are in the same range as those found by other workers (Linzell and Peaker, 1972; Peaker, 1977), and since there were only minor changes in concentration during the experimental periods this indicates that there was no major effect on ion transport in the mammary tissue, although the overall rate of milk secretion was affected. The fact that during exposure to the moderate cold environment the concentration of sodium decreased whilst milk yield decreased indicates an overall decrease in production of sodium by the gland. This may be due to an alteration in the energy available to maintain the active pump mechanism believed to be responsible for controlling the balance of inorganic sodium and potassium between blood and the secretory cell (Linzell and Peaker, 1971b). Other workers have found that the ratio of Na:K in goats' milk is 1:3 (Linzell and Peaker, 1972), and that ratio was also found in the present study.

The changes in the concentration of potassium in goats' milk during exposure to the experimental conditions were more marked than the changes in sodium concentration but also showed a tendency to decrease during the experiment and rise on the return of the animal to ambient conditions. This is to be expected if the levels of sodium and potassium are controlled by a single active pump process. Thomson (1978), however, found that during cold exposure, goats showed a small increase in milk sodium concentration and a small decrease in milk potassium concentration; these small differences ($\pm$ 2 mmol/l), which were not statistically significant, may be due to experimental error. Larger inbalances or

changes as found during destruction of the integrity of the epithelial structure or the 'tight-junctions' (Linzell and Peaker, 1974) were not noted, therefore it is unlikely that the experimental conditions caused any tissue damage - only a change in the metabolic activity of the secretory tissue.

The decrease in milk chloride concentration observed during exposure of the goats to the 'mild cold' and 'moderate cold' environments was unexpected given the apparent increase in milk potassium concentration. Chloride is believed to enter milk from the secretory cell by being drawn down concentration and electrical gradients, with only an active pump on the apical surface of the cell to restrict entry and thereby keep the final milk concentration low. If this hypothesis is correct, exposure to the experimental conditions may have reduced milk chloride concentration by increasing the activity of the active pump or by limiting the level of chloride available from the blood. Alternatively the secretion of another anion may have been increased so that its concentration in the milk is raised and electrical neutrality of the milk is maintained.

The concentrations of milk lactose found in this study are in agreement with those found in goats' milk by other workers (Linzell, 1972; Thomson, 1978) and under each of the climatic conditions there was a significant increase. Since the milk yield decreased during exposure to the 'mild' and 'moderate' cold environments this implies that there was a net increase in the secretion of lactose by the secretory cell. Previous workers have not found any increase in lactose concentration during cold exposure (Thomson, 1978; Cobble and Herman, 1951) although very low temperatures ($-11^{o}C$) caused a decrease in lactose concentration in the milk of Jersey cows. Lactose is believed to be the molecule which when released by exocytosis from

the epithelial cells to the milk, causes water to be drawn into the lumen, in order to dilute the milk to near isotonicity with the plasma. This water movement develops the transepithelial potential difference which determines the flow of cations across the apical membrane. Therefore, from the present results it appears that the rate of water movement into the milk has been reduced whilst secretion of lactose has been maintained. Lactose secretion rate could be controlled at the point of transport of the lactose-containing vesicles to the cell surface and their release, or at the actual point of synthesis by affecting substrate supply or the activity of the synthetic enzymes. As the increase in milk lactose concentration was maintained during the 3 days of exposure to the cold environments this suggests that the lactose secreting system is in a steady state. From the present experiments it is not possible to determine the nature of the changes in milk lactose production. However, if the rate of water movement into the milk is the main factor responsible for the changes in milk yield and composition it may be possible to detect changes in blood constituents and packed cell volume to corroborate this theory. The fact that the concentration of milk lactose was also slightly increased during exposure to the 'control' environment ($22^{o}C$) suggests that the effects may not be specifically due to low temperatures although the effects were less marked.

The levels of citrate found in the goats' milk during these experiments showed considerable variability but fell within the range of published results (Fleet and Peaker, 1978; Peaker et al., 1981). Exposure to the 'mild cold' environment showed no effect on milk citrate concentration but in the three remaining experimental environments there was a slight increase. These changes which mirror the decreases in milk chloride concentration suggest that the secretion of citrate and chloride ions into the milk counterbalance each other to maintain electrical

neutrality of the milk. As a large proportion of citrate in milk is present as the complex anion calciocitrate, milk calcium concentration would also be expected to rise under these conditions, so this would be an interesting area for further study.

The levels of fat determined in the goats milk from this study are in agreement with those found by Linzell (1973) and Fleet and Peaker (1978) using the same technique. The 'mild cold' environment showed the greatest increase in milk fat level of the 4 climatic conditions, but it may be that the duration of the experiments was not sufficient to give the expected increases. It is well established that in dairy cows the percentage of fat increases during exposure to low environmental temperature (Hays, 1926; Regan and Richardson, 1938; Sementovskaya et al., 1950), this is believed to be a reflection of the mobilisation of fat from the adipose tissue. It has been found in previous experiments with goats (Thomson, 1978) that the changes in milk fat concentration during cold exposure were make up of an increase in long chain fatty acid and a decrease in short chain (less than 16 carbons) fatty acids. This suggests that fatty acids are being released from triglycerides and that the rate of de novo fatty acid synthesis is reduced. During exposure to the 'cold and wet' environment there was no significant change in milk fat concentration, but on returning to ambient conditions there was a highly significant increase, which may indicate a carry-over effect, suggesting that there is a 24-36 hour time-lapse between reception of the cold stimulus and fat mobilisation as seen in higher fat levels in the milk. The 'control' environment showed no effect on the level of milk fat, suggesting that 22^{o}C is not a sufficient heat stress for the goats under test to cause a decrease in milk fat - as would be expected from previous results in cows (Moody et al., 1971).

To summarize, it appears that the climatic conditions used caused small decreases in goats milk yield, and some changes in milk composition indicating a change in mammary metabolism.

SECTION 2

THE EFFECT OF A MODERATE COLD ENVIRONMENT ON MILK YIELD AND COMPOSITION IN THE GOAT AT THREE STAGES OF LACTATION

Introduction

This series of experiments was similar to the first (see Section 1) but was carried out using one level of cold stress at three stages of lactation. The aim was to determine if there were any differences in negative response of milk yield to the cold climatic conditions, at different stages of lactation, and by examining milk composition at each stage, attempt to explain the reasons for any differences in response.

Methods

The goats used for these experiments were treated as for Section 1, and were exposed to the 'moderate cold' environment (0 $\pm$ 1°C with increased ventilation) at different stages during lactation. 'Early lactation' was taken to be the period before peak milk yield was attained, and the experiments were carried out on 8 goats between 2 and 8 weeks after parturition. 'Mid lactation' was used to define the period after peak lactation but before the decline in milk yield had begun; five goats were subjected to the cold environment between 17 and 22 weeks after parturition. 'Late lactation' was defined as the final stage of lactation in which weekly milk yield shows a marked constant decline; five goats were exposed to the 'moderate cold' environment from 28 to 33 weeks after parturition.

In all experiments pairs of goats were housed in the pens within the precision chamber, given their normal rations of concentrates and hay (see Section 1) and were milked twice daily by their normal milker. Milk yield and composition was recorded for three days prior to exposure to the test environment, and on the third day the goats were transferred to the climatic chamber. All the experiments in this section involved

exposing the goats to the moderate cold environment for three days. Throughout this period milk yield and the time of milking was recorded, and composition of milk from both glands was determined.

For each group of goats the mean and standard error (S.E.) of the mean were calculated for milk yield and milk concentration of sodium, potassium, chloride, lactose, citrate and fat.

Students' t-test for paired observations was used to determine if there was any significant effect of cold exposure on milk yield and composition by comparing the values obtained during the test period with the mean values obtained in the previous 3 days.

Results

Rate of milk secretion

In early lactation there was a slightly significant ($P < 0.05$) decrease in the rate of milk secretion (ml/h) after one day's exposure to the moderate cold environment (see fig. 2.1 + Table 2.1), but during mid-lactation there was a highly significant decrease in milk yield, which was maintained whilst the animals were exposed to the cold environment. In late lactation no significant effect was observed of the 'moderate cold' environment on the rate of milk secretion.

At the 3 different stages of lactation there were noticeably different rates of milk secretion prior to exposure to the experimental conditions. In early lactation the mean milk secretion rate was 145 ml/h; the reduction in milk secretion on the first day of cold exposure to 133 ml/h represents a decrease to 92% of the previous rate. In mid lactation the mean level of milk secretion before exposure to the cold environment was 110 ml/h, which was reduced to 89 ml/h after 3 days cold exposure, representing a decrease to 81% of the previous rate. In late lactation milk yield was lower (between 69 and 88 ml/h) but was not

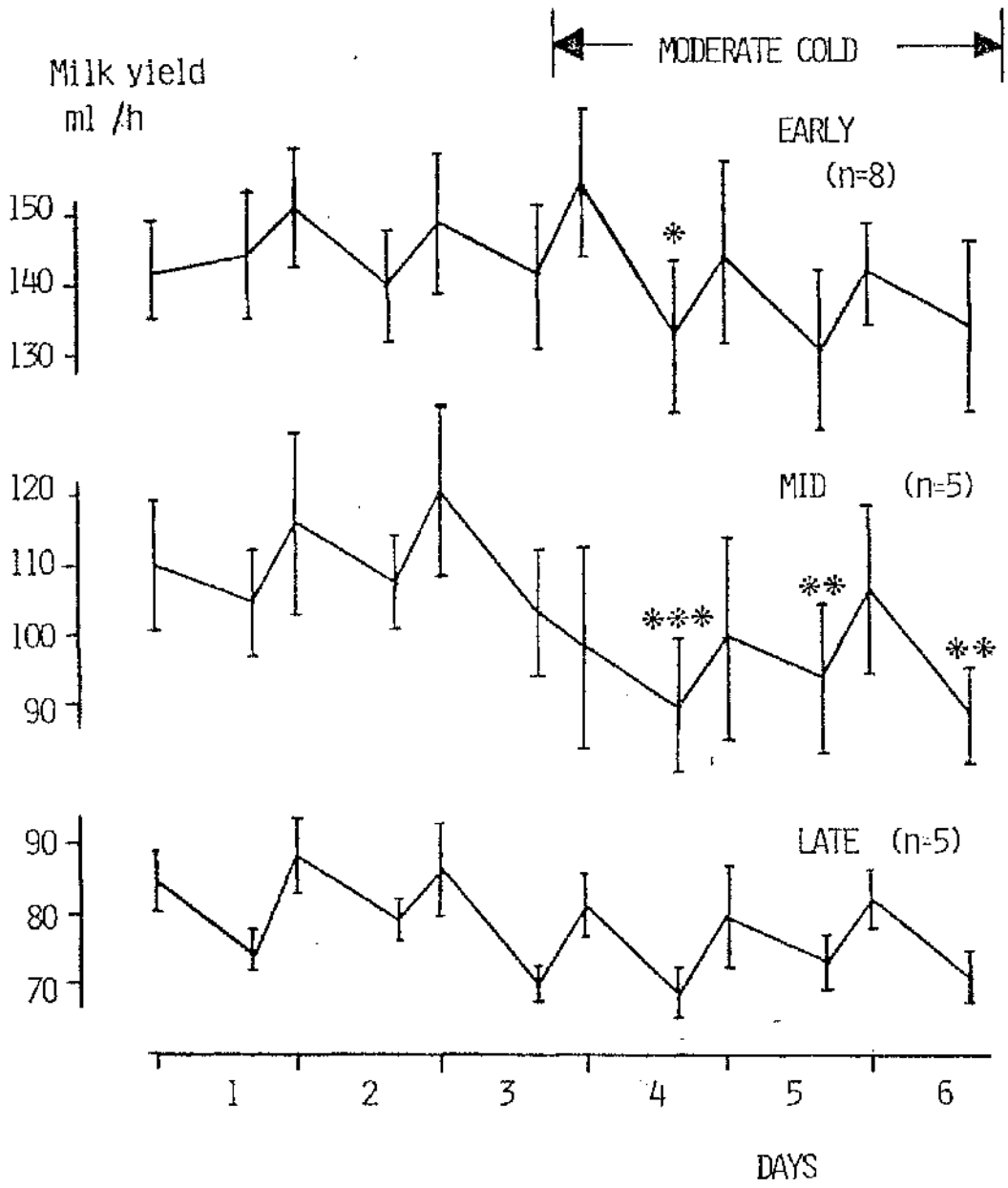

Fig.2.1 Effect of a 'moderate cold' environment on total milk yield(mls/h) in the goat, in 3 stages of lactation (mean±S.E)
(see text for details of climatic conditions)

* $p<0.05$
** $p<0.01$
*** $p<0.001$

significance of difference from previous yield

TABLE 2.1 EFFECT OF 'MODERATE' COLD EXPOSURE ON MILK YIELD IN THE GOAT DURING THREE STAGES OF LACTATION (mean ± SE in ml/h)

STAGE OF LACTATION		EARLY	MID	LATE
	n	8	5	5
DAY 1	PM	142 ± 7.2	110 ± 9.4	85 ± 4.4
	AM	144 ± 9.2	105 ± 7.7	75 ± 2.6
2	PM	151 ± 8.4	116 ± 12.7	88 ± 5.5
	AM	140 ± 7.8	107 ± 6.6	79 ± 2.9
3	PM	149 ± 10.0	121 ± 12.4	87 ± 6.6
	AM	142 ± 10.3	103 ± 8.6	70 ± 2.5
4	PM	154 ± 9.8[a]	98 ± 14.5	81 ± 3.8
	AM	133 ± 10.7	90 ± 9.3	69 ± 3.3
5	PM	144 ± 13.4	100 ± 14.4	80 ± 7.4
	AM	131 ± 11.3	94 ± 10.5	73 ± 3.8
6	PM	142 ± 6.8	107 ± 11.6	82 ± 4.0
	AM	135 ± 12.3	89 ± 7.0	71 ± 2.7

(a - figures within boxes represent milk yields obtained during exposure to cold environment)

reduced by the cold conditions. It was also noticed that at each stage of lactation the rate of milk secretion during the night (as calculated from the morning yield) was regularly lower than the day-time yield, although this difference was not significantly accentuated by the experimental conditions.

Representing the changes in milk secretion rate as a percentage of the previous rate illustrates the larger effect of the cold environment on milk yield during mid-lactation (fig. 2.2 & table 2.2)

Milk sodium concentration

The levels of sodium found in the goats' milk during these experiments are shown in fig. 2.3 (see Appendix 2, table 1 for data) and lie between 12.3 and 17.2 mM. It can be seen that for the goats in early lactation, the 'moderate cold' environment caused a gradual decrease in milk sodium concentration, which was slightly significant ($P < 0.05$) after 3 days exposure. In mid-lactation there was no significant change in milk sodium concentration, and in late lactation there appeared to be an increase after 36 hours exposure but this was found to be not significantly higher than the previous levels.

Milk potassium concentration

The levels of potassium in the milk ranged from 42.3 to 49.7 mM, as shown in fig. 2.4 (see Appendix 2, table 2 for data). The 'moderate cold' environment showed a significant effect on milk potassium concentration only in goats during mid-lactation. After 12 hours the levels were reduced to 46.3 mM ($P < 0.05$) and after 24 hours further reduced to 46.1 mM ($P < 0.01$). During early and late lactation there were no significant effects on milk potassium concentration. The average level of milk potassium during early lactation was 43.8 mM, but during mid and late lactation the average levels were higher (48.8 and 46.9 mM

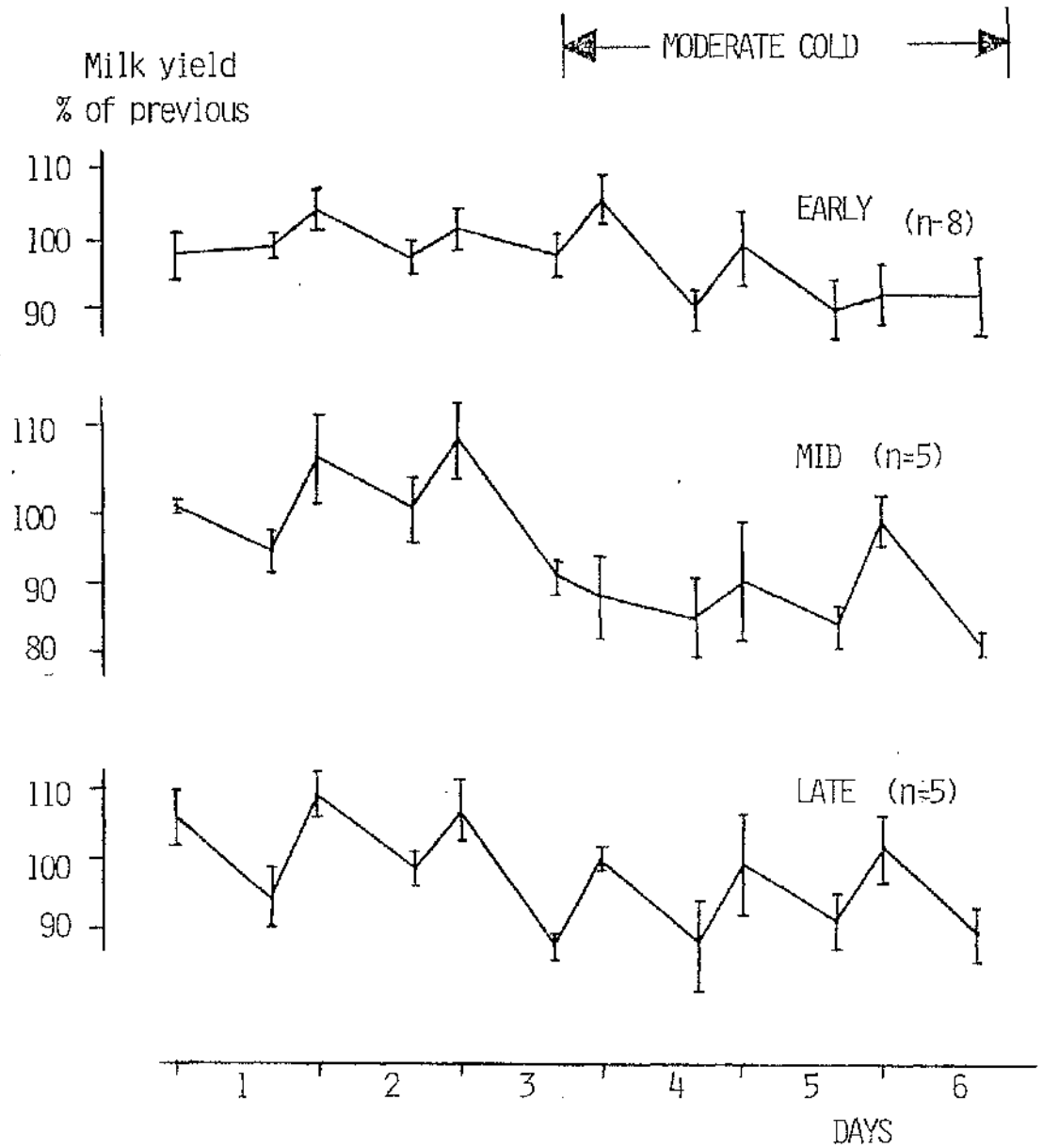

Fig.2.2 Effect of a 'moderate cold' environment on total milk yield (ml/h) in the goat, expressed as a percentage of yield for three days previous (mean±S.E) in 3 stages of lactation.

(see text for details of climatic conditions)

TABLE 2.2 EFFECT OF 'MODERATE' COLD EXPOSURE ON MILK YIELD IN THE GOAT, EXPRESSED AS A PERCENTAGE OF PREVIOUS YIELD, DURING THREE STAGES OF LACTATION (mean ± SE in ml/h)

STAGE OF LACTATION		EARLY	MID	LATE
	n	8	5	5
DAY 1	PM	98 ± 3.6	101 ± 0.8	106 ± 4.0
	AM	99 ± 2.1	95 ± 2.9	94 ± 4.2
2	PM	104 ± 2.9	108 ± 6.5	109 ± 3.2
	AM	97 ± 1.9	101 ± 4.8	98 ± 2.5
3	PM	102 ± 2.8	111 ± 5.4	107 ± 4.5
	AM	98 ± 3.4	91 ± 2.5	87 ± 2.0
4	PM	106 ± 3.4[a]	88 ± 6.0	100 ± 1.7
	AM	90 ± 3.1	85 ± 5.7	87 ± 6.6
5	PM	99 ± 5.4	90 ± 8.5	99 ± 7.3
	AM	90 ± 4.4	84 ± 2.8	91 ± 4.1
6	PM	92 ± 4.2	99 ± 3.6	102 ± 5.1
	AM	92 ± 5.1	81 ± 1.9	89 ± 3.7

(a - figures within boxes represent milk yields obtained during exposure to the cold environment)

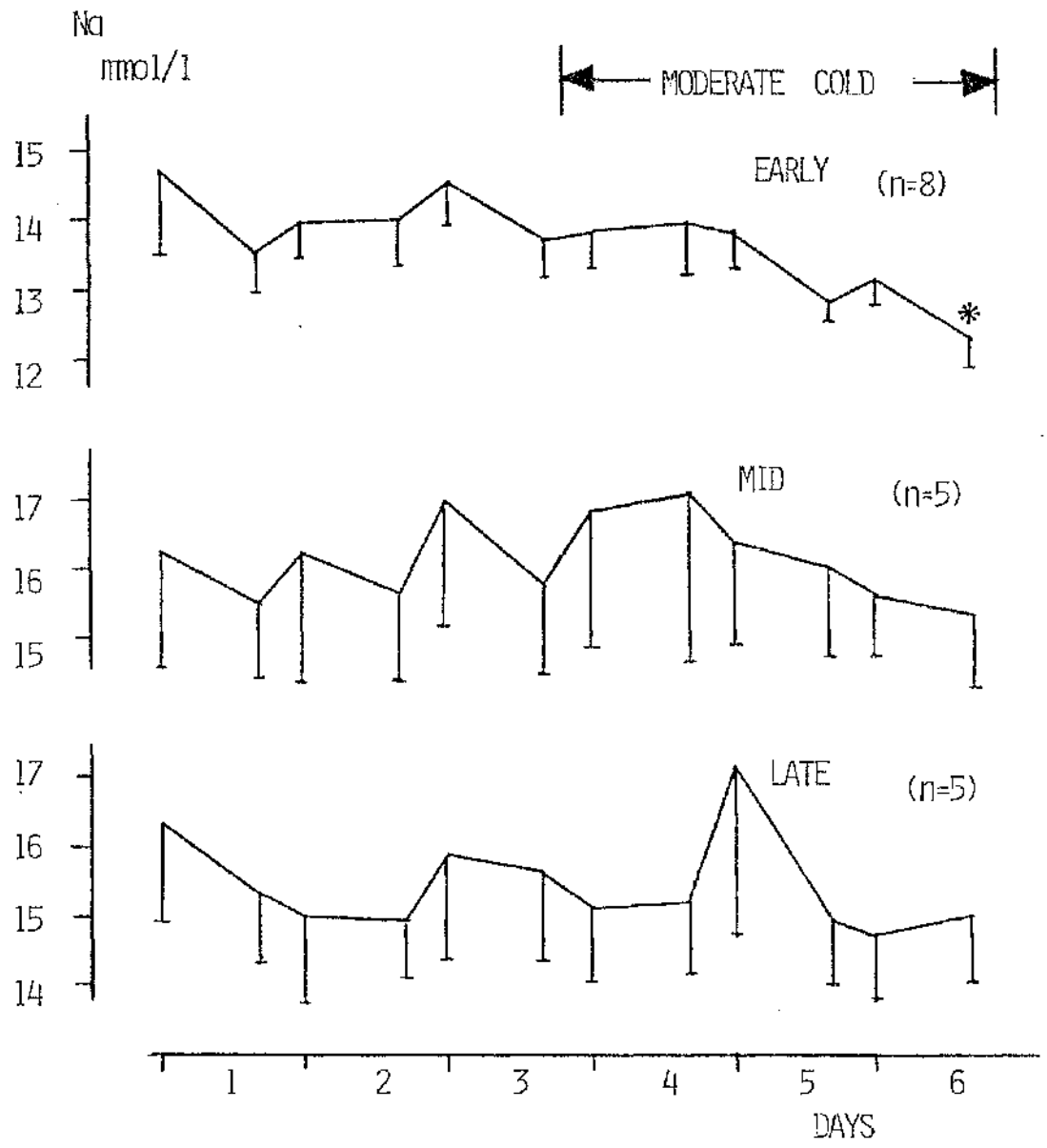

Fig.2.3 Effect of a 'moderate cold' environment on sodium concentration (mmol/l) in goats' milk (mean±S.E.) in 3 stages of lactation.

(see text for details of climatic conditions)

* $p<0.05$ significance of difference from previous concentration

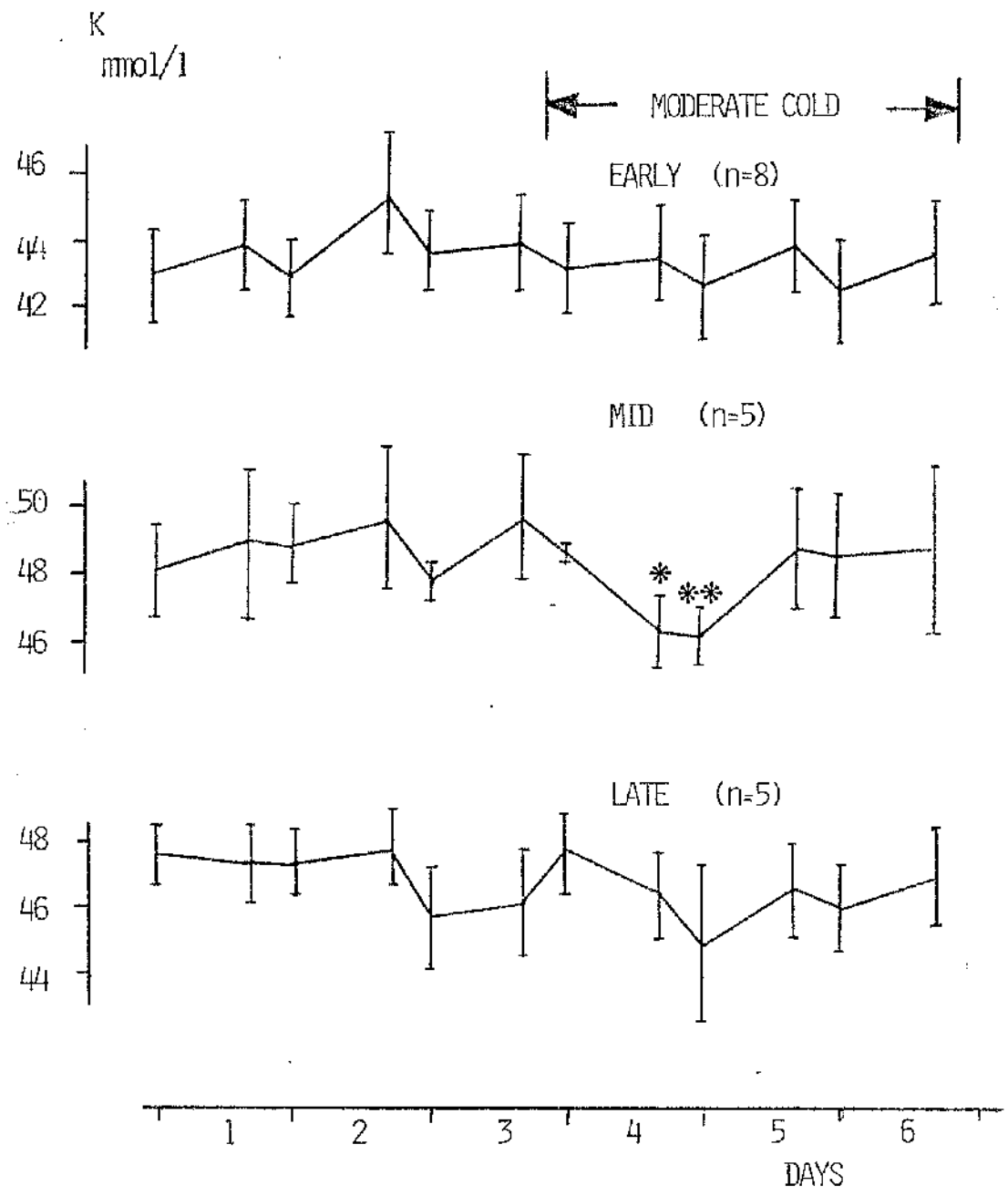

Fig.2.4 Effect of a 'moderate cold' environment on potassium concentration (mmol/l) in goats' milk (mean±S.E.) in 3 stages of lactation

(see text for details of climatic conditions)

* $p<0.05$ significance of difference from
** $p<0.01$ previous concentration

respectively).

Milk chloride concentration

Concentrations of chloride in milk are shown in fig. 2.5 (see Appendix 2, table 3 for data) and range from 36.2 mM to 55.5 mM. The effect of the 'moderate cold' environment on milk chloride concentration was most marked during early lactation when a highly significant ($P < 0.001$) decrease was observed at each afternoon milking, gradually decreasing to a level of 36.2 mM. Chloride concentration was also significantly lower ($P < 0.01$) in the mornings' milk during exposure to the cold conditions. During mid-lactation there was a slightly significant ($P < 0.05$) decrease in milk chloride concentration from a mean level of 54.6 mM before treatment to a minimum of 47.8 mM after 3 days cold exposure. In late lactation there was also a slightly significant ($P < 0.05$) decrease in milk chloride concentration, from 42.7 mM to 40.8 mM after 2 days cold exposure, which decreased further to 39.5 mM ($P < 0.01$) after 3 days.

Mean levels of milk chloride were higher in the goats tested at mid-lactation than those used at early or late lactation.

Milk lactose concentration

Milk lactose concentration ranged from 127.3 mM to 152.2 mM, as shown in fig. 2.6 (see Appendix 2, table 4 for data). At all stages of lactation there was a significant increase in milk lactose concentration during exposure to the cold environment.

In early lactation milk lactose concentration rose from a mean level of 145.1 mM to 151.6 mM after 3 days cold exposure. In mid-lactation milk lactose concentration rose significantly from a mean value of 129.1 mM to 137.1 mM ($P < 0.01$) after approximately 6 hours cold exposure, and this elevated level was maintained through exposure to

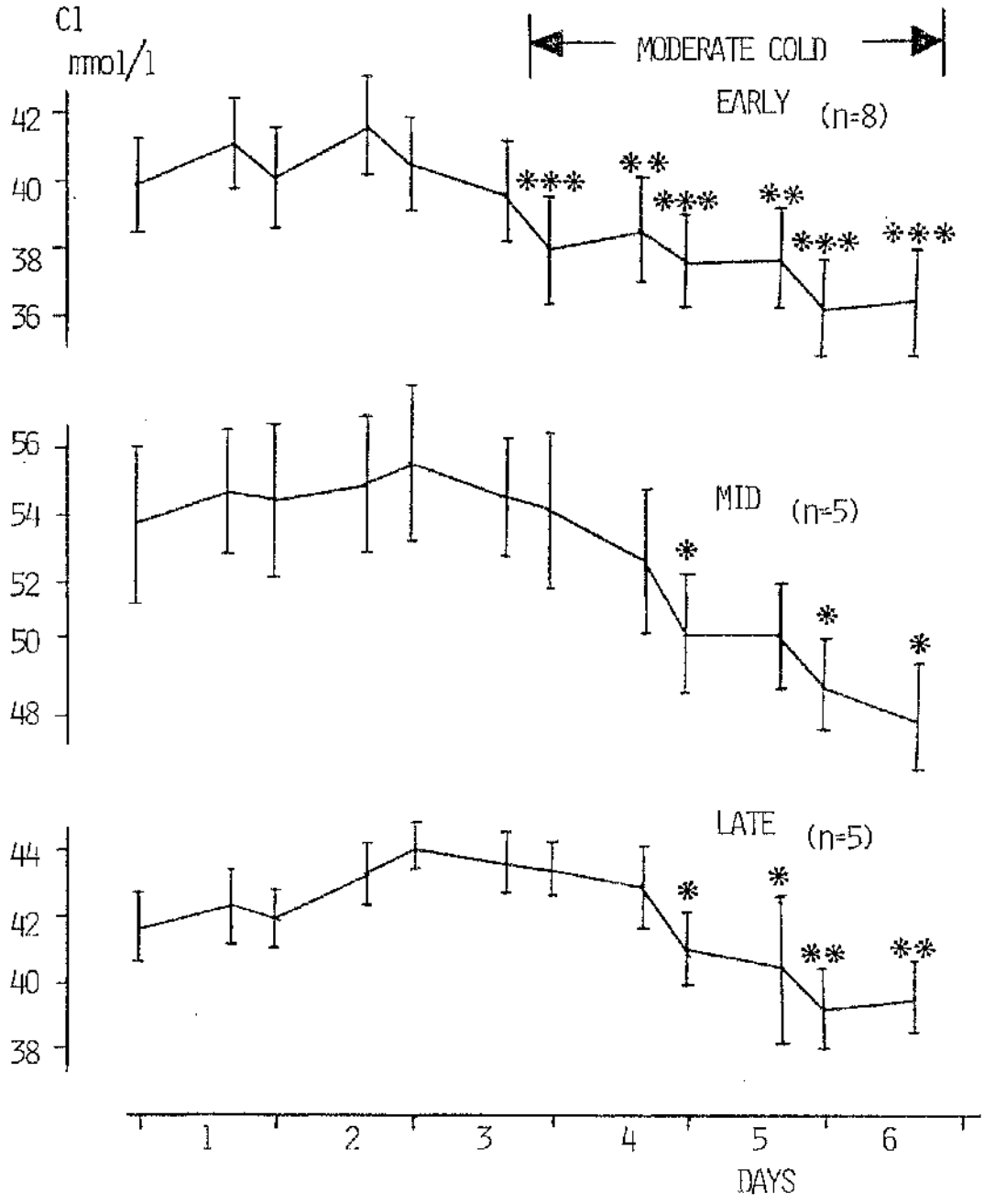

Fig.2.5 Effect of a 'moderate cold' environment on chloride concentration (mmol/l) in goat' milk (mean±S.E.) in 3 stages of lactation

(see text for details of climatic conditions)

* p<0.05
** p<0.01
*** p<0.001

significance of difference from previous concentration

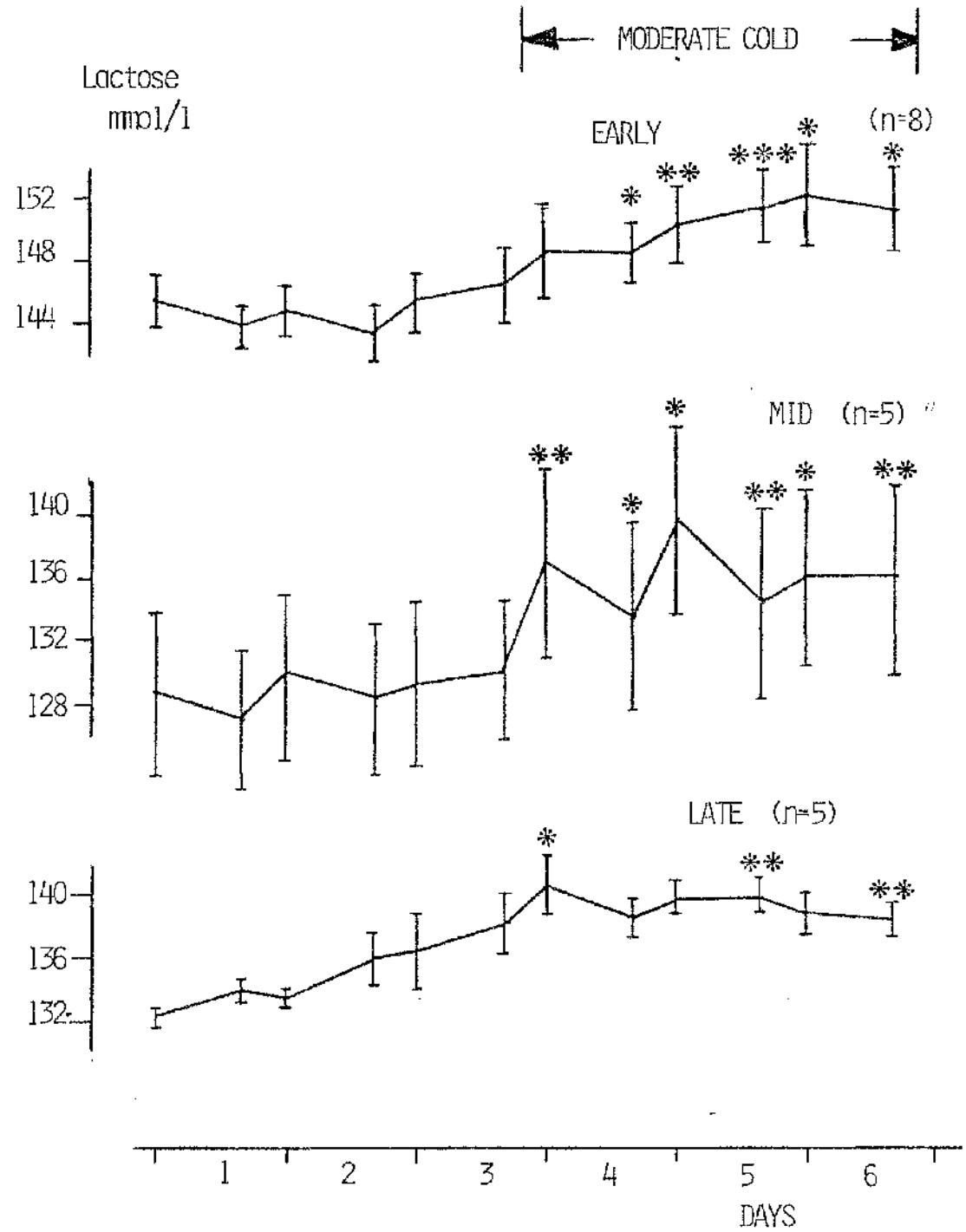

Fig 2.6 Effect of a ' moderate cold ' environment on lactose concentration (mmol/l) in goats' milk (mean±S.E.) in 3 stages of lactation.

(see text for details of climatic conditions)

* p<0.05
** p<0.01
*** p<0.001

significance of difference from previous concentration

the cold environment, reaching a peak of 139.9 mM ($P < 0.01$) after 2 days. In late lactation the milk lactose concentration rose significantly ($P < 0.05$) from a mean value of 135.3 mM to 140.8 mM in the first milking interval in the cold; a significantly higher lactose concentration was maintained throughout exposure to the experimental conditions. The highest levels of milk lactose were found in the goats used during early lactation.

Milk citrate concentration

The levels of citrate in milk were found to range from 57.1 to 178.5 mg/100 ml as shown in fig. 2.7 (see Appendix 2, table 5 for data). In each group of goats there was considerable variation in milk citrate concentration so that although noticeable differences were observed between the 3 stages of lactation, there were no significant effects during exposure to the 'moderate cold' environment. The highest milk citrate levels were found during early lactation (150.5-178.5 mg/100 ml) whilst the lowest were in late lactation (57.1-74.3 mg/100 ml).

Milk fat concentration

Milk fat concentration ranged from 3.3 to 5.5 g/100 ml in these experiments as illustrated in fig. 2.8 (see Appendix 2, table 6 for data). The most significant effect of the cold exposure on milk fat level was an increase during early lactation from a previous mean value of 3.9 g/100 ml to a maximum of 5.5 g/100 ml ($P < 0.01$) after 2.5 days. During mid-lactation the 'moderate cold' environment had no significant effect on milk fat concentration. There was a slightly significant ($P < 0.05$) increase in milk fat level during late lactation, after 2.5 days exposure to the test environment, to 5.5 g/100 ml from a previous average value of 4.55 g/100 ml.

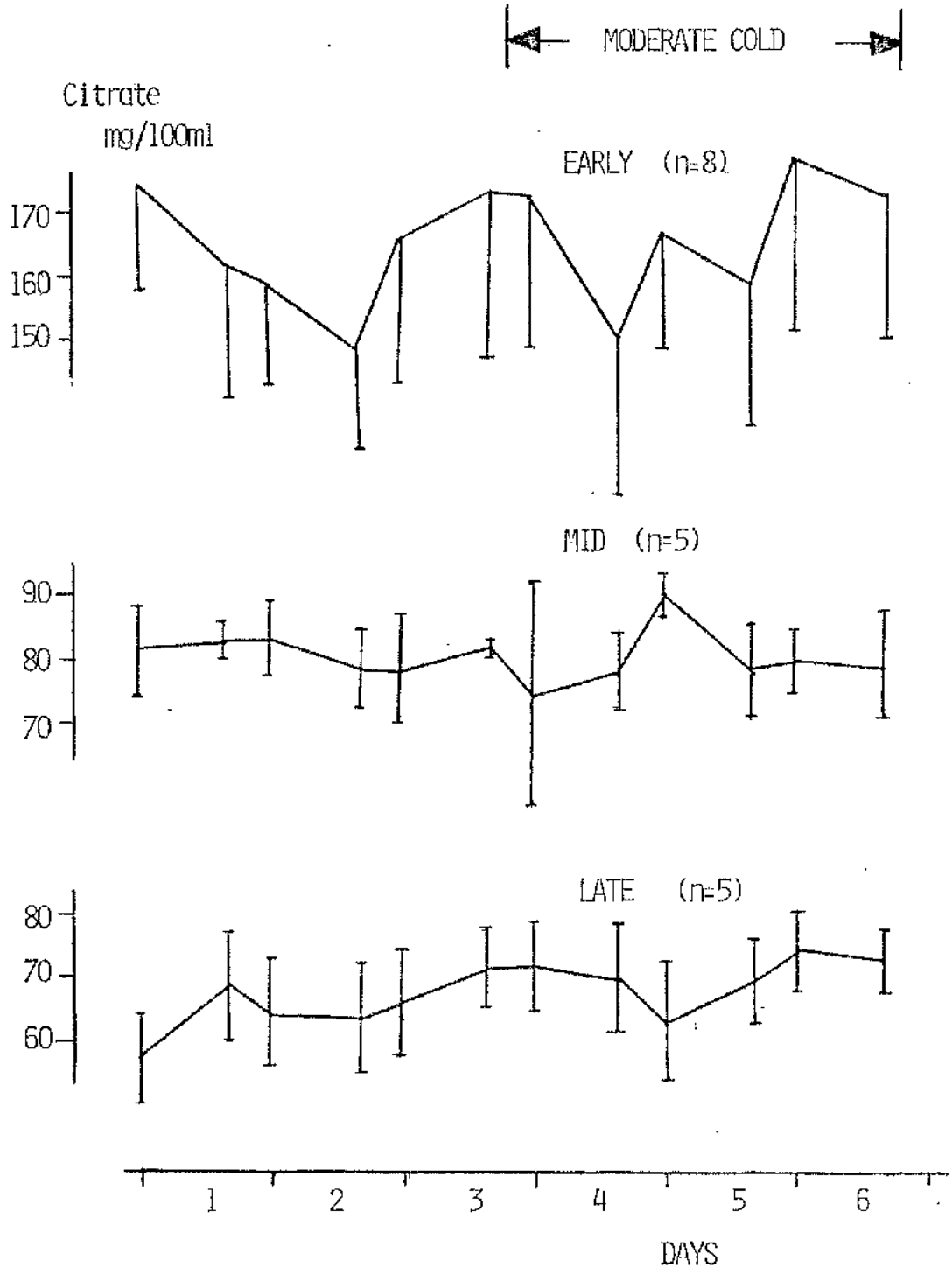

Fig 2.7 Effect of a 'moderate cold' environment on citrate concentration (mg/100ml) in goats' milk (mean±S.E.) in 3 stages of lactation

(see text for details of climatic conditions)

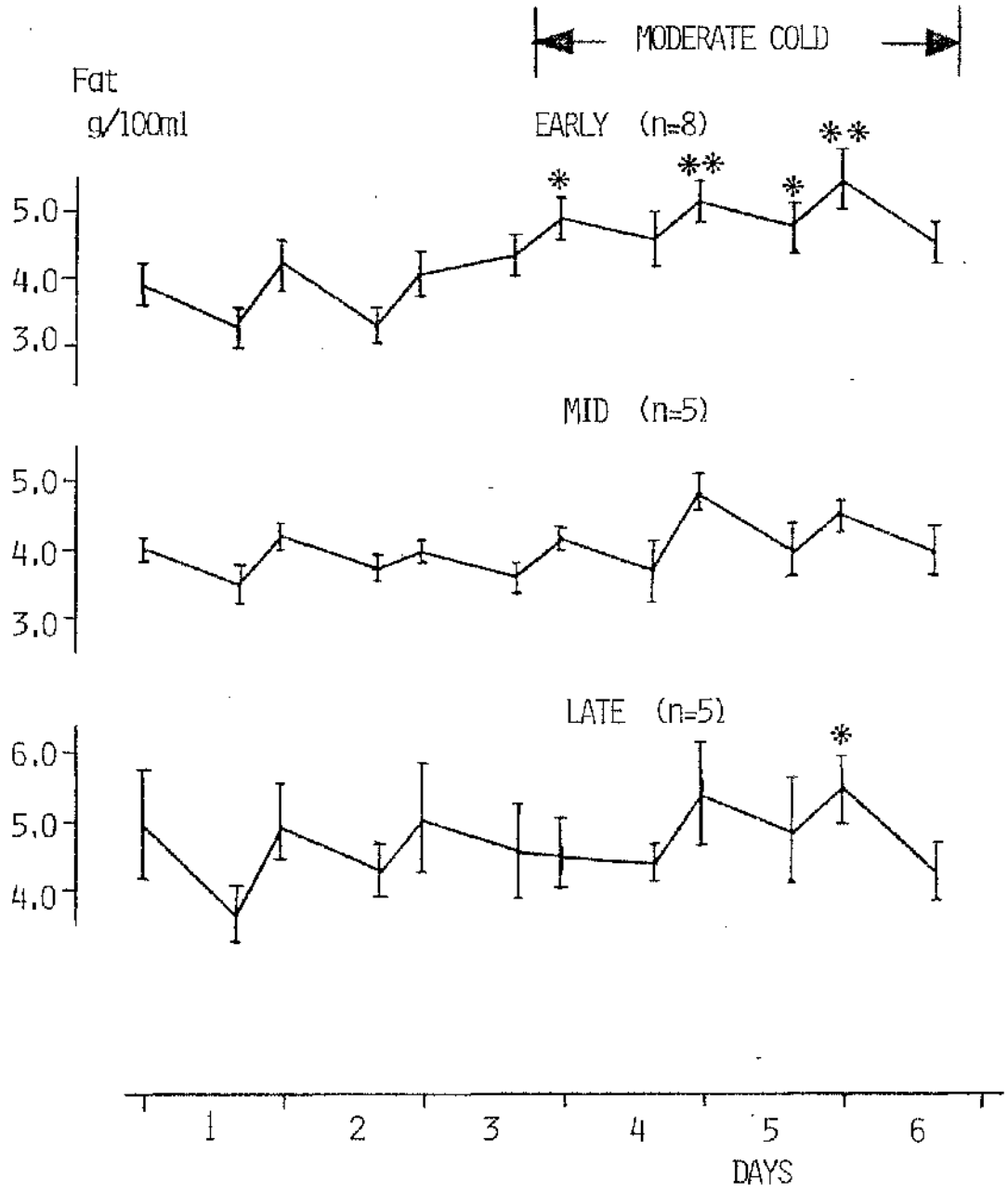

Fig 2.8 Effect of a 'moderate cold' environment on fat concentration (g/100ml) in goats' milk (mean S.E.)in 3 stages of lactation.

(see text for details of climatic conditions)

* $p<0.05$
** $p<0.01$

significance of difference from previous concentration

Discussion

The values for milk secretion rate obtained in this section agree with those found by other workers as described in Section 1. The results illustrate the changing rate of milk secretion as lactation progresses, as shown by Linzell (1973). Linzell recorded a seasonal cyclicity of goats milk secretion which could be maintained for several years without the stimulus of pregnancy, suggesting that mammary tissue can undergo growth and involution in response to another stimulus, possibly day-length. The goats in this study, which were exposed to natural daylength throughout, showed the greatest rate of milk secretion (144.6 ml/h) in early lactation; in the spring and early summer when daylength in Scotland is longest. In mid-lactation, after midsummer, when daylength is shorter, the rate of milk secretion was reduced to a mean value of 110.3 ml/h. In late lactation, which for these goats is late autumn, the milk yield had fallen to 80.6 ml/h. Dietary composition and quantity was maintained to the end of lactation; therefore there was not a direct nutritional cause for this decline.

The response of the milk secretion rate to the 'moderate cold' environment was only slightly significant in early lactation ($P < 0.05$) after 24 hours exposure, but during mid-lactation the decrease was highly significant ($P < 0.001$) after 24 hours; this lower rate of milk secretion was maintained throughout the 3 days. In late lactation there was no significant effect of cold stress on milk yield. It has been suggested (Peaker and Blatchford, 1982) that the process of goats' milk secretion passes through 'zones of responsiveness' which are related to the ratio of milk yield to weight of secretory tissue for each goat. The zone represents a time when the rate of milk secretion can be stimulated, for example by frequent milking, and implies that at that time substrate supply was not limiting. In general it may be said that

in early lactation, yield is ascending to a peak and mammary tissue is in a receptive state. At peak lactation the tissue is 'working' to its maximum capacity and even with increased substrate supply could not be stimulated. As the rate of milk secretion begins to decline the tissue becomes sensitive to frequent milking, but at the time of late lactation the tissue is no longer sensitive. The present results, therefore, could suggest that in early and late lactation, substrate supply is not rate-limiting, so thermoregulatory functions can be carried out by the body whilst exposed to a cold environment without significantly depleting supplies of nutrients and, or, energy required by the mammary gland. At mid-lactation however (which is near peak lactation) the gland is operating at full capacity and therefore when an added demand is put on the body - thermogenesis - there is an insufficiency of nutrients and or energy for milk production. This theory could be tested by restricting the dietary intake during early and late lactation and determining whether the response of milk secretion to cold stress is increased.

Some small but significant changes in milk composition were noted after exposure to the moderate cold environment and these changes varied according to the stage of lactation. The decrease in milk sodium concentration was most significant in early lactation as was the chloride decrease. Milk potassium levels decreased when sodium concentration fell, but was most significant during mid-lactation. The most significant changes in milk composition, as in Section 1, were the increases in milk lactose concentration, and the decrease in chloride concentration which were both most marked during early lactation. These effects seen in early lactation may indicate that at this stage, the lactation process is not fully established and the tissue may be more susceptible to

outside influences such as hormone levels and substrate supply. Milk fat levels rose, during cold exposure, most significantly ($P < 0.01$) in early lactation and also slightly ($P < 0.05$) in late lactation. This could be related to the energy supplies available for lactation; in early lactation the goats have a sufficient reserve of energy, probably in the form of fat, to combat the cold stress and also maintain synthesis of fat droplets in the mammary cell. Similarly in late lactation when yield is lower and energy intake is maintained at a high level, there may be surplus energy available for milk fat production, but at mid or peak lactation, the gland is unable to increase its fat production. The lack of response of the mammary gland regarding milk fat production during cold stress may be due to substrate supply or some other limiting factor. The role of substrate supply could be estimated by determining if milk yield or milk fat yield during cold exposure could be increased by frequent milking.

SECTION 3

THE RELATIONSHIP OF ENERGY METABOLISM IN THE GOAT TO THE RESPONSE TO COLD EXPOSURE OF MILK YIELD

Introduction

The following series of experiments were carried out in an attempt to determine the relationship between energy metabolism in the goat, and the effect of exposure to a 'moderate cold' environment on milk yield.

With knowledge of the energy intake in the form of metabolisable energy of food consumed per day, and the energy content of the milk produced, it is possible by subtraction to calculate a total figure, E, representing the energy used by the animal for maintenance, heat production, and energy required for any tissue gains or losses.

The metabolisable energy of the diet can be calculated assuming a value of 8.2 MJ/kg for moderate quality hay, (Technical bulletin 33 MAFF, 1975), and a value of 10 MJ/kg dry matter for concentrates substituting the results of the manufacturer's analysis into the following equation:

$$ME = 0.12\ CP + 0.31\ EE + 0.05\ CF + 0.14\ NFE$$

where, CP = % crude protein

EE = % ether extract

CF = % crude fibre

NFE = % nitrogen free extract (by subtraction)

and assuming an ash value of 8%. (Equation taken from Feeding-stuffs Evaluation Unit, Third Report 1981, DAFS).

The energy content of the milk produced can be calculated, given the concentrations of fat, lactose and protein from the following formula (after Peterson + Turner, 1939):

$$E_{MILK} = (9.11\ F + 5.86\ P + 3.95\ L) \times 4.184 \times 10 \times \frac{\text{DAILY YIELD}}{1000}$$

= MJ/DAY

where F = fat concentration in g/100 ml

L = lactose concentration in g/100 ml

P = protein concentration in g/100 ml.

Protein concentration was estimated as 4% for the purpose of these calculations as the milk protein was not determined.

The efficiency of conversion of energy intake to milk energy for goats has been estimated at 70% (Armstrong and Blaxter, 1965) which is slightly higher than the value of 62% normally found in dairy cattle (McDonald, Edwards and Greenhalgh, 1973) but may vary with diet and the proportion of fatty acids produced by the rumen fermentation. Adjusting the energy content of the milk produced by the efficiency of milk production, allows for the heat energy consumed by milk production to be included, and therefore the value of E includes only heat utilized for non-lactational purposes.

Methods

Groups of British-Saanen goats, from the herd used previously, were exposed to the 'moderate cold' environment in the precision climatic room, as for Sections 1 and 2. Experiments were carried out during early, mid, and late lactation, and for one group the levels of concentrates included in the ration were reduced. Hay was fed at a constant daily rate and water was available at all times. In each experiment milk yield was recorded for 3 days under normal conditions in the goat house, then for 3 days whilst exposed to the 'moderate cold' environment as described previously. For each day the metabolisable energy of the ration fed was calculated, and after chemical analysis of the milk, the energy content of the milk produced under both environments was determined.

Experiments were conducted as shown in table 3.1, the mean result for each group being given (see Appendix 3 for individual data). The

TABLE 3.1 MEAN ENERGY BALANCE OF LACTATING GOATS SUBJECTED TO THE 'MODERATE COLD' ENVIRONMENT

GROUP	n	STAGE OF LACTATION (weeks)	ENERGY INTAKE MJ/DAY	ENERGY OUTPUT IN MILK MJ/DAY		ENERGY REMAINING 'E' MJ/DAY		MEAN % DECREASE IN MILK YIELD
				control	cold	control	cold	
1	5	3-5	24.84	9.22	10.68	11.67	9.58	-5.38
2	4	9-19	19.84	6.60	6.60	10.41	10.41	-14.75
3	5	17-22	26.34	6.48	5.82	17.08	18.02	-14.42
4	5	28-33	27.34	5.16	5.04	20.05	19.95	-5.84

energy intake was constant for each group throughout the experiment, at the rate indicated. The energy content of milk produced as a mean daily value under ambient and cold conditions are shown as 'E_{milk}'. The value for 'E' refers to the mean energy available for maintenance, heat production and tissue gain or loss, and is corrected for a milk efficiency of 70%. The value for percentage decrease in milk yield was calculated from the difference between mean milk yield during the 3 days immediately before exposure to the cold environment, and the mean milk yield recorded during the cold exposure.

Body weights were recorded for most of the goats immediately before and after cold exposure.

Results

The results shown in table 3.1, illustrate the decreasing quantity of energy lost as milk as the lactation period progresses, and as the energy intake was maintained until late lactation, the increasing amount of energy available to the animal for maintenance. The energy intake calculated from the hay and concentrates fed to each goat, ranged from 24.84 MJ/DAY to 27.34 MJ/DAY, during the season, which was the standard level of feeding for all goats in the herd. In the experiment in which the level of feeding was reduced the energy intake was 19.84 MJ. The energy content of the milk under ambient conditions ranged from 9.22 MJ/DAY in early lactation to 5.16 MJ/DAY in late lactation. When the 'moderate cold' environment was imposed on the goats, although milk yield decreased, there was no significant change in the energy content of the milk secreted each day.

In early lactation the 'moderate cold' environment resulted in a 5.38% decrease in milk yield, but a 15.83% increase in milk energy. In mid-lactation, under the standard feeding regime, exposure to the

'moderate cold' conditions resulted in a 14.42% decrease in milk yield, and a 10.2% decrease in milk energy produced per day. When the goats were subjected to the 'moderate cold' environment during late lactation, under the standard feeding regime, there was a decrease in milk yield of 5.84% and a decrease in milk energy of 2.32%. When the energy intake of the goats was reduced to 19.84 MJ/DAY, there was a similar decrease in milk yield (-14.75%) but there was no change in milk energy.

The value for 'E', the energy available for maintenance, heat production and tissue gain, is related to the energy intake, and the energy lost in the milk for each group of animals. Therefore when the intake is maintained at the normal level, and milk energy lost is increased, there is a decreased quantity of 'E' available. In early lactation 'E' was calculated to be 11.67 MJ/DAY under ambient environmental conditions, and was reduced to 9.58 MJ/DAY in the 'moderate cold' environment. In mid-lactation, whilst under ambient conditions the value for 'E' was 17.08 and increased to 18.02 MJ/DAY after 3 days 'moderate cold' exposure. The value during late lactation was 20.05 decreasing to 19.95 MJ/DAY after exposure to the cold conditions. When energy intake was reduced during mid-lactation there was no reduction in milk energy produced, and therefore, in comparison with the group of goats used at the same stage of lactation but fed normally, there was less energy available (10.41 MJ/DAY compared with 17.08 MJ/DAY) for maintenance. Under the 'moderate cold' conditions, there was no change in milk energy produced (6.60 MJ/DAY) although there was a 14.75% decrease in milk yield.

The results for the changes in body weight of the goats are shown in table 3.2 (see Appendix 3, table 2 for detailed data) and show that the goats lost weight during exposure to the cold environment in early lactation (2.53 kg N.S.) and also in mid-lactation when the dietary

TABLE 3.2 CHANGES IN BODY WEIGHT (mean ± SE in kg) OF GOATS EXPOSED TO THE 'MODERATE COLD' ENVIRONMENT

GROUP	n	INITIAL WEIGHT	WEIGHT AFTER COLD EXPOSURE
1. EARLY LACTATION	4	61.25 ± 1.39	58.72 ± 2.35
2. MID LACTATION/ REDUCED DIET	4	58.37 ± 3.71	53.62 ± 3.88**
3. MID LACTATION	3	56.67 ± 2.73	57.50 ± 3.40
4. LATE LACTATION	1	61.0	63.0

(** - significant decrease at the $p < 0.001$ level)

intake was reduced (4.75 kg, $P < 0.01$). However, from the data available it appears that during late lactation and in mid-lactation with the standard diet, there was a slight increase in body weight (0.83 kg and 2.0 kg respectively). No account was taken of the degree of gut fill in each animal, which can vary to a great extent in ruminants, although all measurements were made at the same time of day.

Discussion

The results of this section show that the goats used in these experiments were receiving a diet containing a higher energy intake than those used by Thomson (1978). This may indicate, since the goats used in these studies were of similar body weights to his, that the animals had a greater reserve of adipose tissue. The energy produced by the animals in this study in the form of milk, was higher, especially during early lactation, and was not significantly affected by cold stress. Thomson (1978) found that the energy lost as heat by the animals increased by almost 50% after exposure to the 'moderate cold' environment. From the present results it is impossible to ascertain the effect of cold stress on heat production, but it is safe to assume from previous studies that an increase in heat production and loss will ensue. The fact that the goats in this study appeared to maintain their milk energy production under climatic stress whilst the yield per se was reduced can be explained by the increase in fat and lactose concentration during cold exposure, combined with the decreased yield.

If the goats have a higher quantity of body fat than those used by Thomson (1978), and are increasing the rate of secretion of fat into the milk under cold stress we would expect to see a rise in the level of plasma free fatty acids, and possibly a drop in body weight after prolonged exposure. Experiments were carried out to follow the changes in

plasma free fatty acids during cold exposure (see Section 6) and preliminary findings do indicate losses in body weight during exposure to 'moderate cold' conditions during early lactation.

These changes in body weight suggest that in early lactation body reserves are being depleted to supply energy or nutrients for thermogenesis and milk production. When dietary intake was reduced during mid-lactation a significant weight loss occurred, showing that the reduction in intake was sufficient to change the energy balance during cold stress at this stage of lactation. It must be remembered, however, that there is considerable variation in body weight due to variation in rumen contents and more accurate experiments are required to confirm these findings. Also, more detailed experiments are required to determine the exact changes in energy metabolism in lactation in the goat and particularly with respect to response to cold stress at different stages of lactation.

The present study, using relatively high energy intakes indicate that stage of lactation is more significant in determining the response of milk secretion to cold stress than energy intake. When the energy intake was reduced by approximately 25% during mid-lactation the percentage decrease in milk yield was relatively unaffected, although 'E' was reduced by 40%. This suggests that at mid-lactation, the reduced level of energy supply, even under climatic stress, was sufficient for the needs of body's metabolism - and milk production could be maintained. If in future experiments the energy content of the diet was reduced further, causing a percentage decrease in milk yield greater than 14%, this may indicate the level of energy required for basal metabolism.

Alternatively, it could be argued that the level of cold exposure applied in these experiments was not a sufficient stress to alter the

metabolic balance of the lactating goat. Therefore, it would appear that a temperature of $0^{o}C$ and a wind speed of 5.6 m/s will not cause a significant energetic deficit to the process of milk production, in the goats used. The group of goats exposed to the 'moderate cold' environment during late lactation were concurrently in the very early stages of pregnancy (1-4 weeks). The demands of the foetal development may have influenced the energy metabolism in these goats, but as the energy intake was maintained at a comparatively high level for the rate of energy produced as milk, the value for 'E' was highest of all the groups, and appears to adequately supply the body's needs.

It is interesting to note that the mean percentage decrease in milk yield in early and late lactation are very similar although the energy produced as milk was double in early lactation, and consequently the energy remaining available for maintenance and heat production was double. This may reflect an increased efficiency of the mammary gland during early lactation, and, or a greater cold tolerance in the spring - following a winter of acclimatization.

SECTION 4

THE EFFECT OF MODERATE COLD EXPOSURE DURING THREE STAGES OF LACTATION, ON MILK YIELD OF INTACT AND DENERVATED MAMMARY GLANDS, IN THE GOAT

Introduction

Some of the animals studied in this and earlier (Thomson, 1978) projects had one mammary gland denervated and transplanted to the neck as described in Chapter 2. It was decided therefore to compare the response of these transplanted glands with the gland remaining in situ, and also with glands of 'normal' intact animals, to moderate cold exposure. All udders were surgically divided and provided with an exteriorised mammary vein loop as previously described (see Chapter 2), so that a comparison of individual mammary glands was possible.

Methods

British Saanen goats were exposed to the moderate cold environment for 3 days, in crates within the precision chamber, as described for previous experiments.

Group 1 consisted of three goats with normal glands and five goats with one gland transplanted to the neck, and were studied between weeks 2 and 8 of lactation (early lactation). Group 2 was made up of three 'normal' goats and two with one transplanted gland; these animals were exposed to the cold environment between weeks 17 and 22 of lactation (mid lactation). The final group of animals (Group 3) comprised of five 'normal' animals and two with one gland transplanted; they were studied between weeks 28 and 33 of lactation (late-lactation).

All animals were fed and milked according to the normal schedule as described in Chapter 2, and milk yield for each gland was recorded twice daily.

For each group the response of individual glands in terms of mean

milk yield per hour was determined. Students' t test for paired observations was used to test for statistically significant differences between mean yield during cold exposure and the previous three days. Then response between groups and stages of lactation were compared.

Results

During early lactation the goats' milk yield ranged from 49.6 to 83.2 ml/h as shown in Table 4.1 and illustrated in Fig. 4.1. The effect of moderate cold exposure on 'normal' mammary glands appeared to cause an increase in the diurnal variation in milk secretion rate. Also the mean milk yield was slightly decreased ($P < 0.1$), during the experimental period. The 'in situ' control glands and the transplanted glands showed no significant response to the moderate cold exposure in terms of decreasing milk yield although a slight reduction was suggested.

In mid lactation milk yield ranged from 29.5 to 71.0 ml/hr/gland as shown in Table 4.2 and illustrated in Fig. 4.2. The effect of moderate cold exposure on 'normal' mammary glands at this time was a highly significant decrease ($P < 0.005$) in milk secretion rate to a mean value of 51.3 from 56.7 mls/hr. When animals with one gland transplanted were exposed to the moderate cold environment in mid lactation, and individual glands were considered, no significant change in milk secretion rate was observed. The sample size was small ($n = 2$) and although the results appear to show a decline in milk yield in both transplanted and in situ control glands it is not possible to determine if either type of gland has a significant response.

A similar situation occurred when the goats were exposed to the moderate cold environment during late lactation. Milk yield ranged from 32.5 to 52.5 ml/hr (see Table 4.3 and Fig. 4.3). Milk secretion rate showed a significant ($P < 0.05$) decrease in the normal mammary

TABLE 4.1 EFFECT OF MODERATE COLD EXPOSURE IN EARLY LACTATION ON MILK YIELD IN TRANSPLANTED AND NON-TRANSPLANTED MAMMARY GLANDS (mean ± SE in ml/h)

		NORMAL GLANDS	IN SITU CONTROL GLANDS	TRANSPLANTED GLANDS
	n	6	5	5
DAY 1	PM	78.8 ± 3.24	70.0 ± 4.51	62.8 ± 13.17
	AM	71.8 ± 4.28	78.2 ± 9.11	66.4 ± 11.33
2	PM	81.8 ± 1.97	77.0 ± 10.13	67.4 ± 10.63
	AM	72.5 ± 2.66	72.0 ± 11.91	66.2 ± 12.58
3	PM	78.5 ± 6.54	76.0 ± 9.88	66.2 ± 12.58
	AM	71.8 ± 3.73	79.2 ± 9.22	62.2 ± 11.21
4	PM	87.0 ± 3.42[a]	81.2 ± 10.07	61.8 ± 8.58
	AM	67.7 ± 4.93	72.0 ± 10.19	59.0 ± 10.09
5	PM	83.2 ± 3.46	72.0 ± 9.85	59.4 ± 7.05
	AM	64.4 ± 5.41	75.6 ± 8.58	57.2 ± 13.33
6	PM	77.5 ± 4.64	70.8 ± 8.86	49.6 ± 3.01
	AM	66.8 ± 3.31	75.0 ± 11.03	62.4 ± 11.38

(a - figures within boxes represent milk yields obtained during exposure to the moderate cold environment)

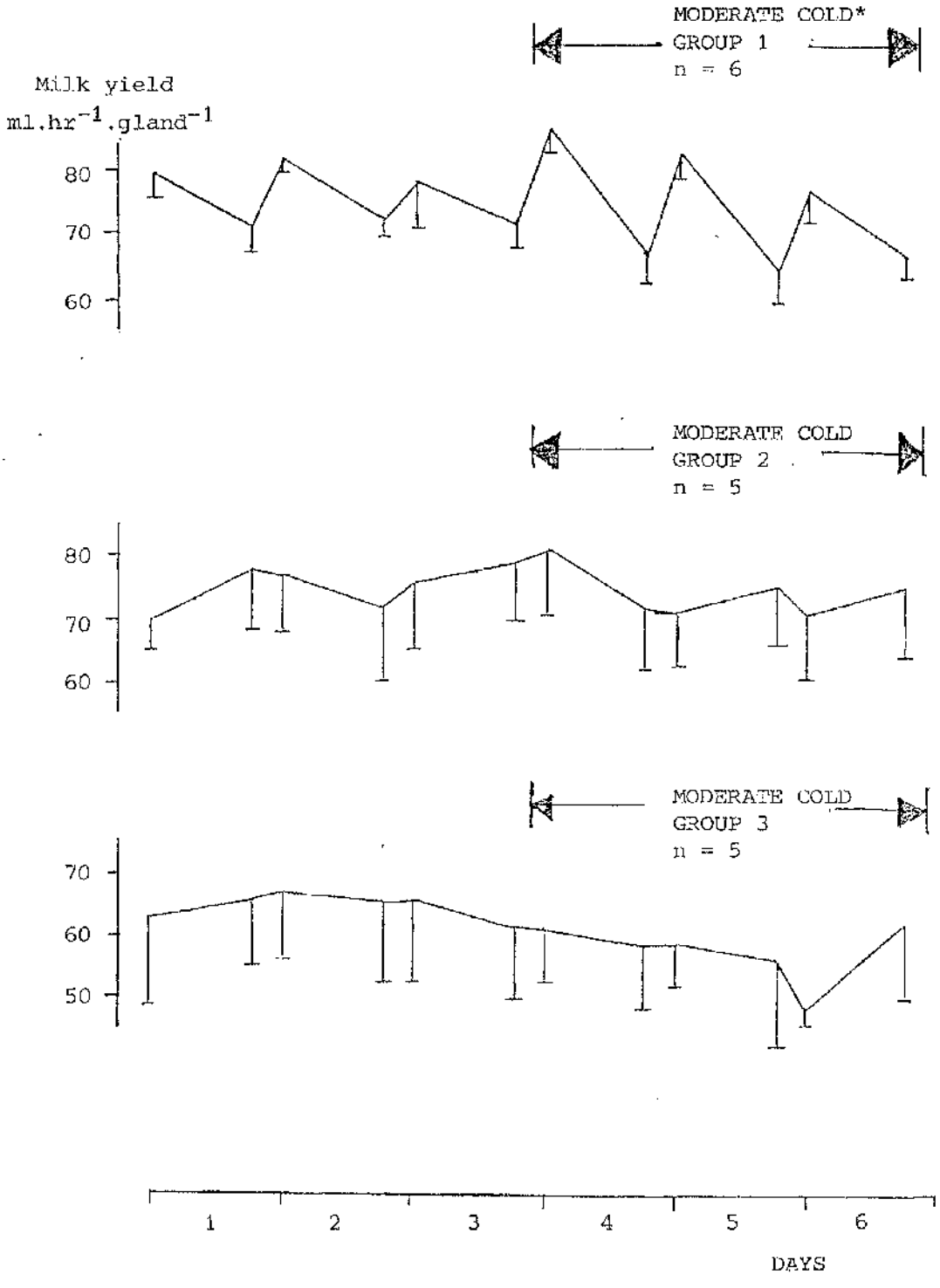

Fig 4.1. Effect of moderate cold exposure during early lactation on goats' milk yield (ml.hr^{-1}.gland^{-1}) in normal mammary glands (Group 1), 'in situ' control glands (Group 2) and transplanted glands (Group 3)

* $P < 0.10$

TABLE 4.2 EFFECT OF MODERATE COLD EXPOSURE IN MID-LACTATION ON MILK YIELD IN TRANSPLANTED AND NON-TRANSPLANTED MAMMARY GLANDS (mean ± SE in ml/h)

		NORMAL GLANDS	IN SITU CONTROL GLANDS	TRANSPLANTED GLANDS
	n	6	2	2
DAY 1	PM	57.2 ± 6.14	66.0 ± 7.00	37.5 ± 2.50
	AM	54.3 ± 5.45	64.0 ± 8.00	34.5 ± 3.50
2	PM	61.3 ± 7.39	68.5 ± 14.50	38.5 ± 1.50
	AM	53.3 ± 4.87	68.0 ± 9.00	39.5 ± 3.50
3	PM	62.3 ± 8.38	71.0 ± 10.00	44.0 ± 2.00
	AM	53.5 ± 6.21	64.5 ± 8.50	34.0 ± 3.00
4	PM	53.8 ± 8.78[a]	49.0 ± 6.00	34.0 ± 5.00
	AM	46.2 ± 5.91	56.0 ± 13.00	29.5 ± 2.50
5	PM	53.0 ± 7.10	57.0 ± 26.00	35.0 ± 6.00
	AM	50.7 ± 6.40	52.0 ± 9.00	30.5 ± 0.50
6	PM	57.7 ± 6.92	60.0 ± 14.00	41.5 ± 7.50
	AM	46.8 ± 5.82	52.5 ± 6.50	29.5 ± 4.50

(a - figures in boxes represent milk yields obtained during exposure to the moderate cold environment)

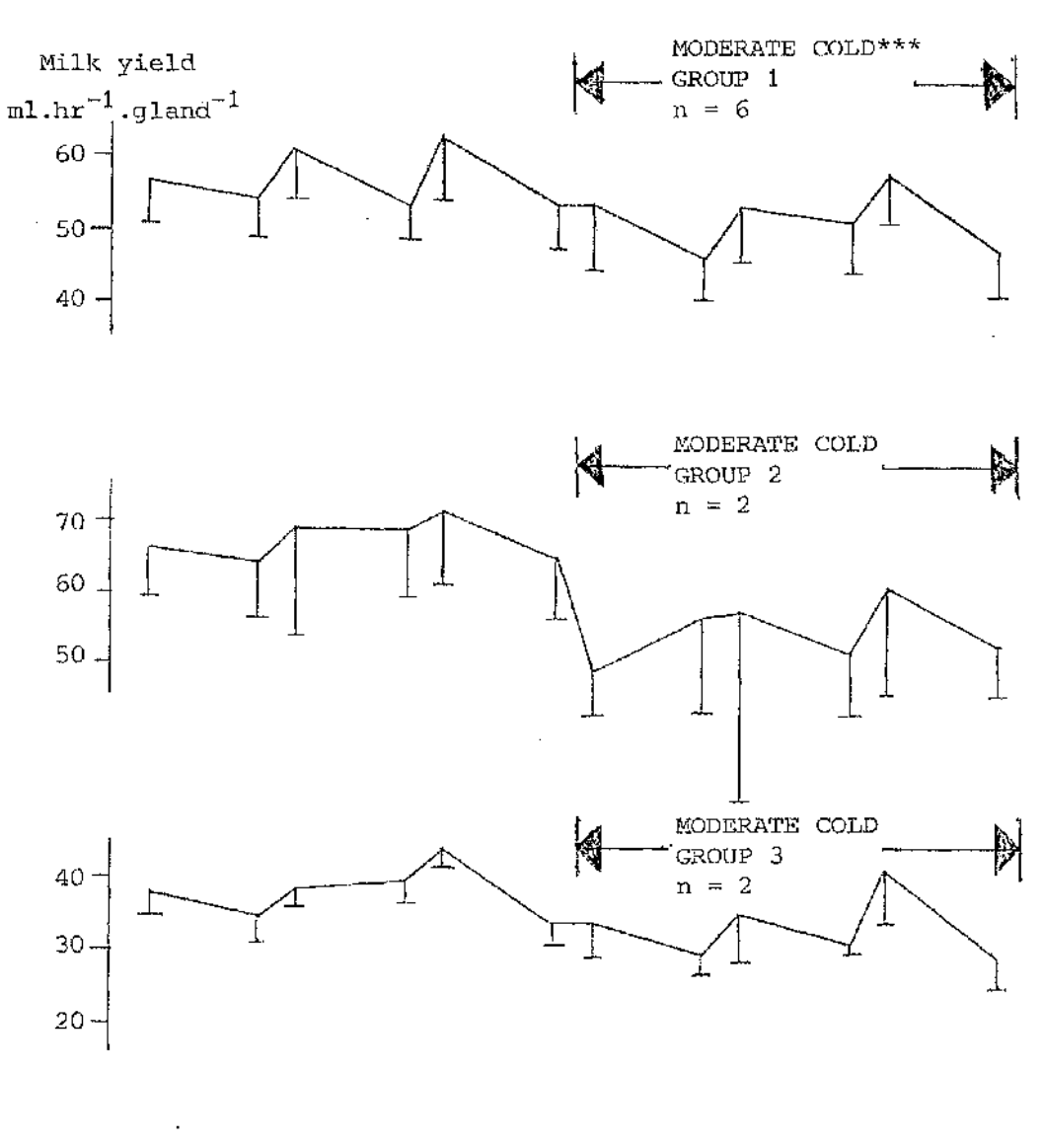

Fig 4.2. Effect of moderate cold exposure during mid-lactation on goats' milk yield (ml.hr^{-1}.gland^{-1}) in normal mammary glands (Group 1), in situ control glands (Group 2) and transplanted glands (Group 3) (mean ± SEM)

*** $P < 0.005$

TABLE 4.3 EFFECT OF MODERATE COLD EXPOSURE DURING LATE LACTATION ON MILK YIELD IN TRANSPLANTED AND NON-TRANSPLANTED MAMMARY GLANDS (mean ± SE ml/h)

		NORMAL GLANDS	IN SITU CONTROL GLANDS	TRANSPLANTED GLANDS
	n	10	2	2
DAY 1	PM	42.5 ± 2.65	47.0 ± 8.00	36.5 ± 5.50
	AM	37.7 ± 1.87	48.0 ± 7.00	36.0 ± 6.00
2	PM	43.9 ± 2.50	50.0 ± 8.00	35.0 ± 4.00
	AM	39.4 ± 2.34	44.0 ± 7.00	35.0 ± 8.00
3	PM	43.5 ± 3.01	44.0 ± 7.00	35.0 ± 6.00
	AM	35.0 ± 2.05	44.0 ± 5.00	32.5 ± 3.50
4	PM	40.3 ± 1.79[a]	46.5 ± 8.50	37.5 ± 6.50
	AM	34.6 ± 2.47	51.5 ± 10.50	36.5 ± 6.50
5	PM	39.8 ± 2.87	43.5 ± 6.50	35.0 ± 6.00
	AM	36.4 ± 2.17	49.0 ± 10.00	36.5 ± 7.50
6	PM	41.1 ± 1.80	44.0 ± 6.00	36.5 ± 6.50
	AM	35.7 ± 1.94	52.5 ± 11.50	35.5 ± 8.50

(a - figures in boxes represent yields obtained during exposure to the moderate cold environment)

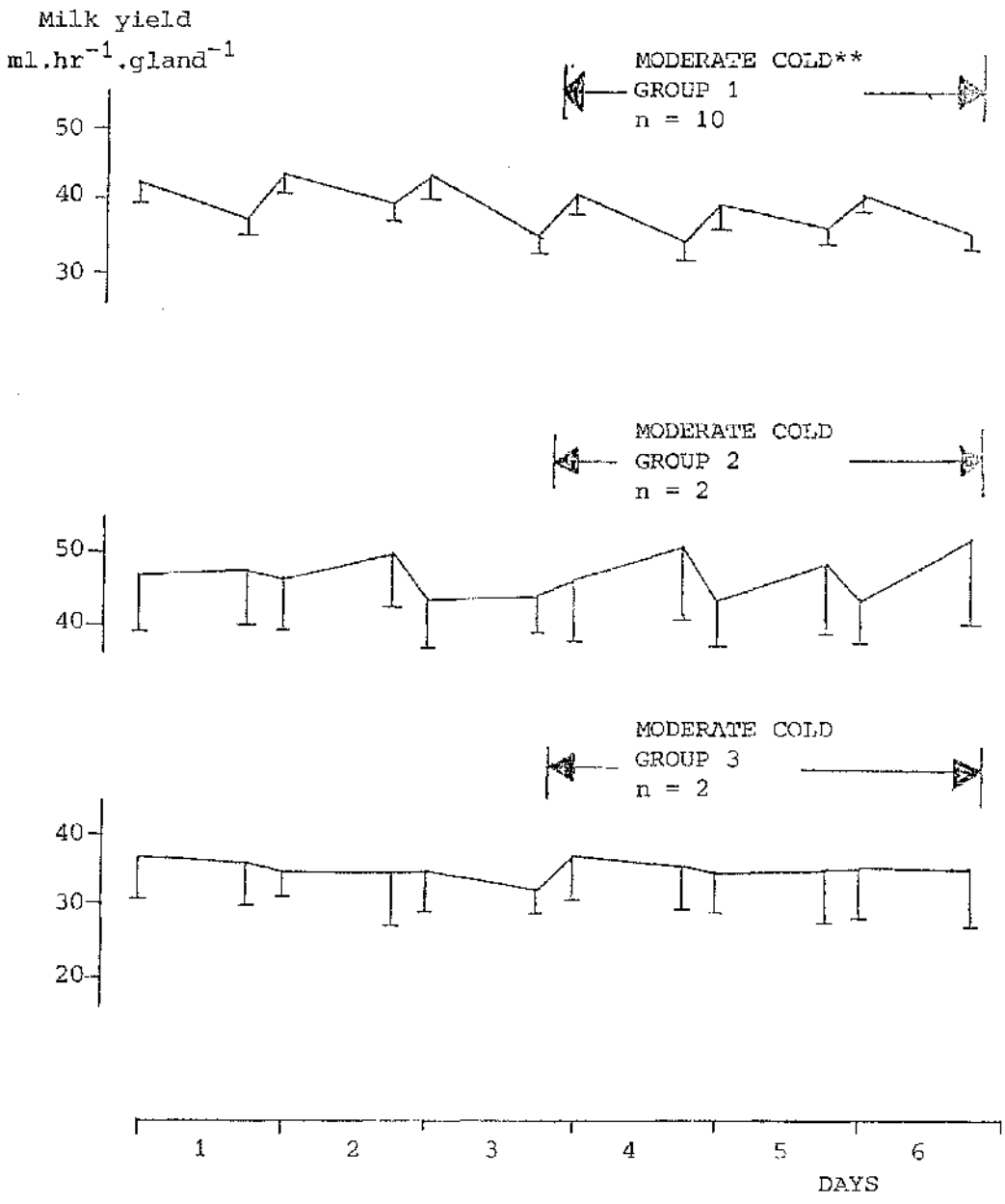

Fig 4.3. Effect of moderate cold exposure during late lactation on goats' milk yield (ml.hr^{-1}.gland^{-1}) in normal mammary glands (Group 1), in situ control glands (Group 2) and transplanted glands (Group 3) (mean ± SEM)

** $P < 0.05$

glands from 40.3 to 39.9 ml/h (n = 10) but the transplanted and in situ control glands (n = 2) were not significantly affected.

Discussion

The results of this section again illustrate the differences in rate of milk secretion in early, mid and late lactation (see this chapter, section 2). Also it can be seen that the transplanted glands generally have a slightly lower milk yield than the control glands, although similar yields were reported in both types of gland by Linzell (1963) and Peaker and Fleet (1979). Denervation eliminates the milk ejection reflex from the mammary gland, as explained in chapter 1, but oxytocin released during milking of the in situ control gland will allow contraction of the alveolar myoepithelium of the transplanted gland and milk ejection will follow. Therefore milk removal is not affected by transplantation, and any changes in yield must be due to changes in synthetic processes.

From the data shown in this section it can again be seen that the moderate cold environment causes the greatest decrease in milk yield during mid-lactation and the least in late lactation. In Group 2 and 3 it is unfortunate that it was only possible to study the response to cold in 2 animals with transplanted glands so that statistically significant effects were not detected. It is apparent, however, that even with these small samples there were no differences in response between the transplanted and in situ control glands.

We may have expected some difference because of local physical effects; the goats were positioned with hind quarters and hence the in situ gland towards the fan so that the transplanted gland may have been protected. However, the in situ gland may receive some insulation from the hind legs, therefore the cooling effects of the environment

may have been equalised. Mammary gland surface temperature was not recorded in this study but may have been useful as Ewebank (1968) found that direct cooling of one mammary gland in the cow increased the rate of heat loss eleven times when compared with the contralateral gland. Thomson (1978) working with the same Saanen goats as those of the present study, found that when environmental cooling lowered mammary surface temperature by 18°C, the temperature of blood leaving the gland was unchanged. He does not mention any differences between transplanted and in situ glands. As the transplantation operation leaves the blood supply to the gland intact we would not expect any differences in the response of the gland to the environment to be related to blood supply.

SECTION 5

THE EFFECT OF COLD EXPOSURE ON THE LEVELS OF CELLULAR METABOLITES IN GOATS MILK

Introduction

The levels of certain cellular metabolites in milk have been shown to reflect their cytosolic and Golgi-vesicular concentration (Faulkner, 1980). Therefore, the concentration and yield of milk glucose, galactose, phosphoenol pyruvate, glucose 6-phosphate, nucleotide di-phosphate, iso-citrate and 2-oxo-glutarate were determined during exposure of goats to four levels of cold stress.

Changes in the concentration of these metabolites in milk secreted during cold stress may be interpreted in terms of changes in mammary metabolism or the secretory process.

Methods

Groups of female British Saanen goats drawn from the herd previously used (see Section 1) were subjected to various environmental conditions, and milk yield and composition were determined. In each case the milk was sampled for three of four days prior to exposure to the test conditions, and during the three days of cold exposure. In certain cases samples were monitored on the return of the goats to their normal environment. In all cases milk from a single gland only was studied (the left gland or the gland in situ). Assays to measure milk concentration of phosphoenol pyruvate, glucose-6-phosphate, glucose, iso-citrate, 2-oxo-glutarate, nucleotide di-phosphate and galactose were carried out as described in Chapter 2.

All goats were fed and maintained as described in Chapter 2 unless otherwise specified. Milking was carried out twice daily throughout the experimental period, at approximately 8.00h and 16.00h, by the

normal milkers. The goats were transferred from the goathouse to the precision chamber in pairs on day 3, and on the fourth day the experimental conditions were imposed. Natural daylength was maintained throughout, and water was available at all times.

The first three goats (Group 1) were exposed to the moderate cold environment (0°C ± 1°C, with wind speed of 5.6 m/s) during mid-lactation and were maintained on their normal diet. The second group of three goats (Group 2) were treated as Group 1 (also in mid-lactation) but the level of concentrates fed was reduced to 1.0 kg/day three days before sampling began, and maintained on the lower level of feeding until the end of the experimental period, when normal feeding was resumed. The third group of goats, Group 3, comprised of 4 goats which were subjected to the 'cold and wet' environment (as described in Section 1) during mid-lactation for two days. The last group, Group 4, contained two goats which had the concentrate part of this diet removed for 36 hours prior to exposure to the moderate cold environment, during late lactation. Feeding was restored to the normal regime on installation of the animals in the precision chamber where they were subjected to the moderate cold environment for three days.

Levels of the various intracellular metabolites in milk could, therefore, be monitored during exposure of the lactating gland to increasing physiological strain.

Results were expressed in terms of concentrations of metabolites measured and also in yield secreted per hour during the experimental period, and were tested for statistical significance by use of Students t-test for paired observations.

Results

Phosphoenol pyruvate in milk

Milk yield, and concentration of phosphoenol pyruvate (PEP) in milk was measured prior and during exposure of goats to the four experimental conditions as previously described. The levels and amounts of PEP found are shown in Table 5.1 and illustrated in Fig 5.1.

Goats milk contained between 14.6 and 84.7 nM/ml PEP and the secretion rate varied from 0.92 to 5.11 nM/h.

The moderate cold environment caused a significant ($P < 0.01$) decrease in concentration of PEP from 68.7 to 23.8 nM/ml (Group 1), but the decrease in yield was less significant ($P < 0.02$). In Group 2, reduction of the food intake appeared to result in a decreased concentration of PEP in milk before exposure to the moderate cold environment (40.4 nM/ml), but subsequent exposure to the test conditions had no significant effect on PEP concentration or yield. Exposure of the goats to the cold and wet environment (Group 3) caused no significant changes in the concentration or yield of PEP in milk. Removal of the concentrate part of the diet from the goats in Group 4 caused a decrease in milk PEP concentration which recovered slightly on refeeding even through refeeding was concurrent with cold exposure. The concentration of PEP in milk did not return to the previous level during this period suggesting that cold exposure did have an effect, but the sample was too small to test for statistical significance.

The results also suggest that milk PEP concentration may be higher in goats in late lactation than early lactation.

Glucose-6-phosphate in milk

The concentration of glucose-6-phosphate (G6P) in goats milk during this study ranged from 66.5 to 160 nM/ml and the yield varied

TABLE 5.1 EFFECT OF VARIOUS CLIMATIC CONDITIONS ON THE MEAN CONCENTRATION AND YIELD OF PHOSPHOENOL PYRUVATE IN GOATS' MILK

		GROUP 1		GROUP 2		GROUP 3		GROUP 4	
	n	3		3		4		2	
		nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h
DAY 1	PM	66.6	4.47	-	-	59.7	2.67	82.0	4.92
	AM	73.6	5.11	41.0	3.10	60.0	3.45	81.5	4.83
2	PM	67.3	4.91	40.6	2.07	77.2	3.60	70.0	4.10
	AM	72.6	5.06	43.3	3.05	79.2	3.18	50.5	2.11
3	PM	65.0	5.07	42.6	2.39	80.0	4.32	47.5	2.04
	AM	67.0	4.54	34.3	2.34	84.7	3.92	36.5	1.25
4	PM	55.6[a]	3.05	32.3	1.88	66.0	3.74	47.5	1.90
	AM	21.0	1.22	32.6	1.69	79.2	3.28	56.5	2.49
5	PM	16.0	0.93	26.3	1.59	68.0	3.61	56.5	1.97
	AM	17.3	1.08	17.0	0.97	78.5	3.13	50.5	2.32
6	PM	14.6	0.96	20.6	2.33	71.0	3.34	34.0	1.37
	AM	18.0	1.04	25.0	1.19	91.2	3.79	43.5	1.94

(a - figures in boxes represent results obtained during exposure to the test environments; see text for details)

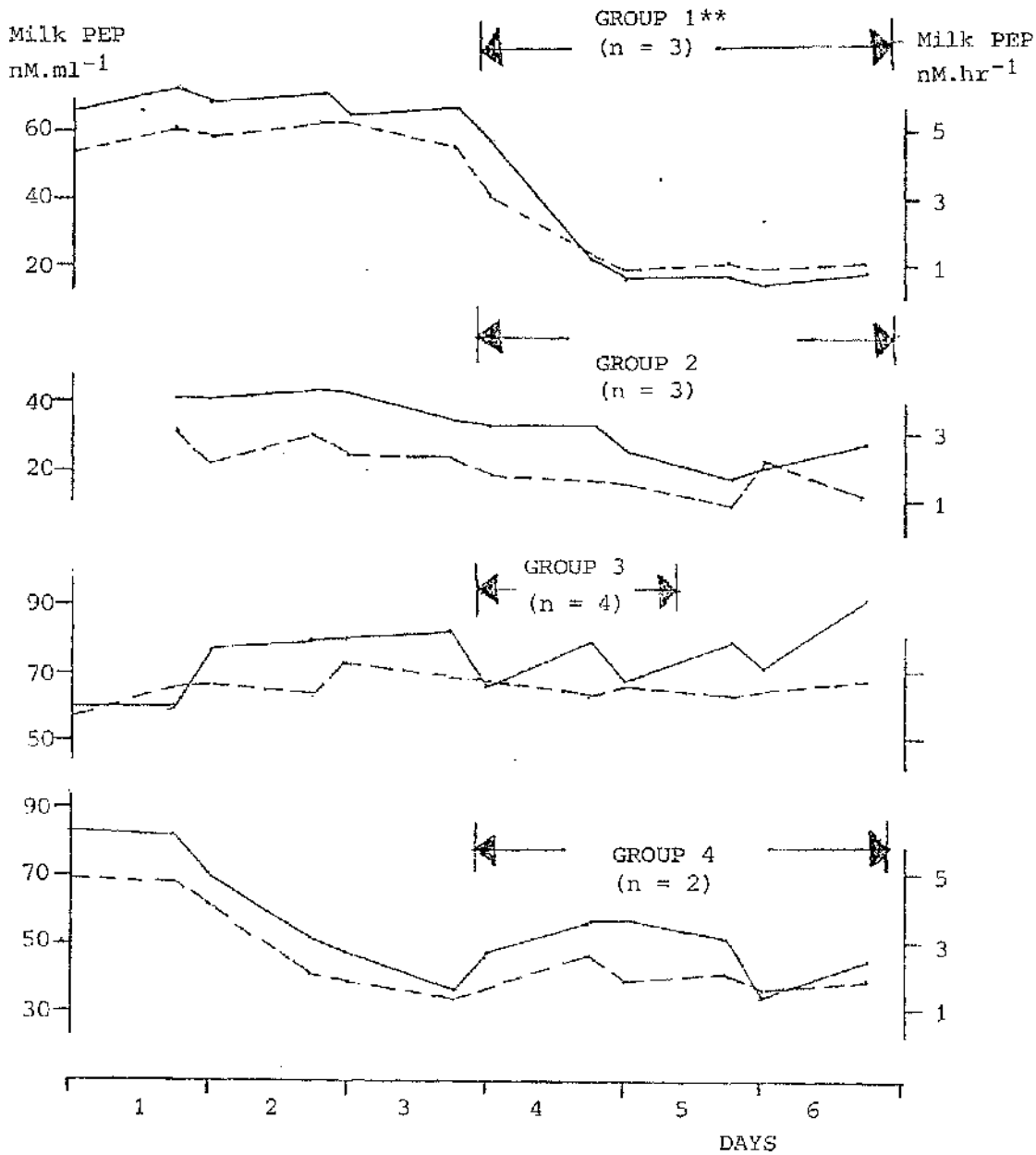

Fig 5.1. Effect of different cold environments on mean concentration (— $nM.ml^{-1}$) and yield (--- $nM.hr^{-1}$) of phosphoenol pyruvate in goats' milk.

See text for details of climatic conditions.

** $P < 0.01$ significance of difference from previous concentration

from 2.41 to 7.43 nM/h as shown in Table 5.2 and illustrated in Fig 5.2.

The goats exposed to the moderate cold environment after normal and reduced feeding (Groups 1 and 2 respectively) showed no significant change in concentration or yield of G6P in milk. In Group 3 the concentration and yield of G6P were more variable between milkings, but no significant effect was found during cold exposure. The level of G6P in milk in this group was higher than in the other 3 groups. In group 4 removal of the concentrate ration for 3 feeds had no significant effect on G6P level in the milk, but refeeding and cold exposure lead to a slight, non-significant, increase. Concentration of G6P returned to the previous level during the period of cold exposure and G6P yield was unaffected.

Glucose in milk

The concentration of glucose in goats milk found in this study ranged from 54.0 to 124 nM/ml, and the yield varied from 2.72 to 9.88 nM/h as shown in Table 5.3 and illustrated in Fig 5.3.

Exposure of the goats to none of the test regimes caused any statistically significant change in the concentration or yield of milk glucose, although there was considerable variation between milkings. As for G6P, the levels of milk glucose appeared higher in milk from animals in group 3. There was no obvious difference in milk glucose between animals sampled in mid or late lactation, and reduction in food intake lead to an apparent decrease.

Nucleotide di-phosphate in milk

The concentration of nucleotide-di-phosphate (NDP) found in goats' milk during these experiments varied from 67.5 to 248 nM/ml and the yield ranged from 3.32 to 15.39 nM/h, as shown in Table 5.4 and illustrated in Fig 5.4. Levels of NDP in milk found during exposure

TABLE 5.2 EFFECT OF VARIOUS CLIMATIC CONDITIONS ON THE MEAN CONCENTRATION AND YIELD OF GLUCOSE-6-PHOSPHATE IN GOATS' MILK

		GROUP 1		GROUP 2		GROUP 3		GROUP 4	
	n	3		3		4		2	
		nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h
DAY 1	PM	78.3	5.61	-	-	125.2	6.21	78.5	4.76
	AM	60.6	4.20	68.0	4.93	121.0	5.44	74.0	4.32
2	PM	76.3	5.57	70.0	3.60	125.0	6.84	83.5	4.55
	AM	78.3	5.87	71.3	4.90	113.0	5.04	76.0	3.01
3	PM	92.3	7.26	74.3	4.11	128.7	6.18	67.5	2.91
	AM	77.3	5.24	83.3	5.99	126.5	5.71	68.0	2.41
4	PM	80.3[a]	5.08	80.3	4.69	131.5	7.43	123.5	4.79
	AM	88.3	5.07	80.6	4.35	121.5	5.45	79.5	3.26
5	PM	73.0	4.19	83.6	5.12	146.0	8.49	100.5	3.68
	AM	96.0	5.47	81.6	4.45	141.7	6.53	66.5	2.70
6	PM	92.3	5.78	79.0	4.80	160.5	5.75	88.5	3.52
	AM	92.0	4.94	87.0	3.81	131.7	5.90	93.5	4.10

(a - figures in boxes represent results obtained during exposure to the test environments; see text for details)

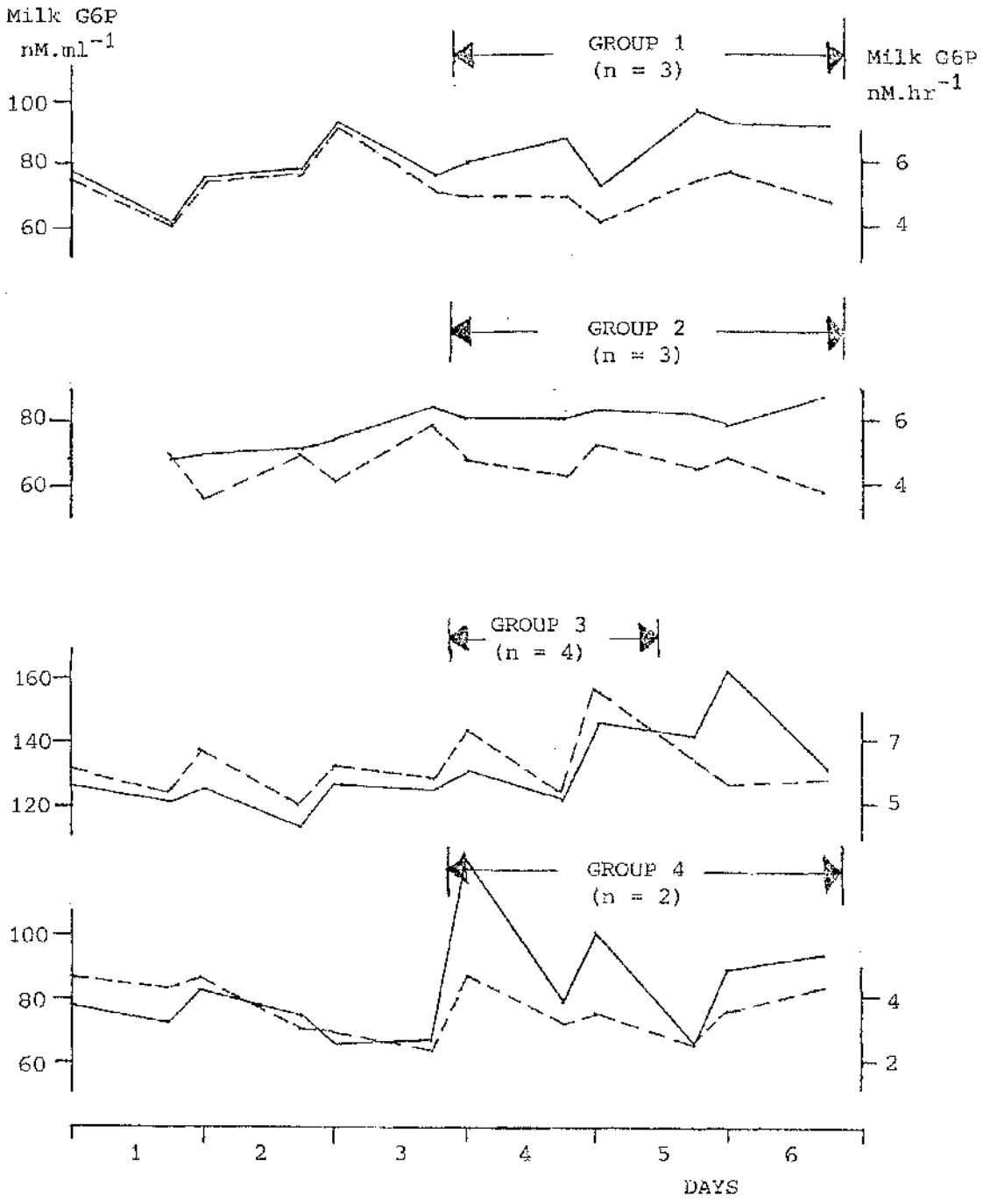

Fig 5.2. Effect of different cold environments on mean concentration (—— nM.ml^{-1}) and yield (--- nM.hr^{-1}) of glucose-6-phosphate in goats' milk.
See text for details of climatic conditions.

TABLE 5.3 EFFECT OF VARIOUS CLIMATIC CONDITIONS ON THE MEAN CONCENTRATION AND YIELD OF GLUCOSE IN GOATS' MILK

		GROUP 1		GROUP 2		GROUP 3		GROUP 4	
	n	3		3		4		2	
		nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h
DAY 1	PM	92.0	6.61	-	-	107.0	5.60	95.5	5.90
	AM	65.0	4.52	56.3	4.13	110.2	5.04	96.0	5.67
2	PM	88.0	6.61	58.3	2.98	113.2	6.69	97.0	5.63
	AM	86.6	6.09	55.0	3.81	110.0	5.06	74.0	3.19
3	PM	123.3	9.88	75.0	4.50	115.0	7.56	67.5	3.00
	AM	104.0	7.10	70.6	4.81	124.2	6.40	106.0	3.54
4	PM	104.3[a]	8.91	79.0	4.52	121.2	7.45	77.0	2.97
	AM	101.0	8.27	109.3	5.89	109.7	5.22	84.5	3.59
5	PM	96.3	6.15	76.3	4.72	112.7	6.91	103.5	3.89
	AM	94.6	5.78	66.6	3.88	109.7	5.07	83.0	3.65
6	PM	90.0	6.16	87.6	5.81	113.5	6.75	98.5	3.95
	AM	74.6	5.80	54.0	2.72	108.5	4.82	102.5	4.56

(a - figures in boxes represent results obtained during exposure to test environments; see text for details)

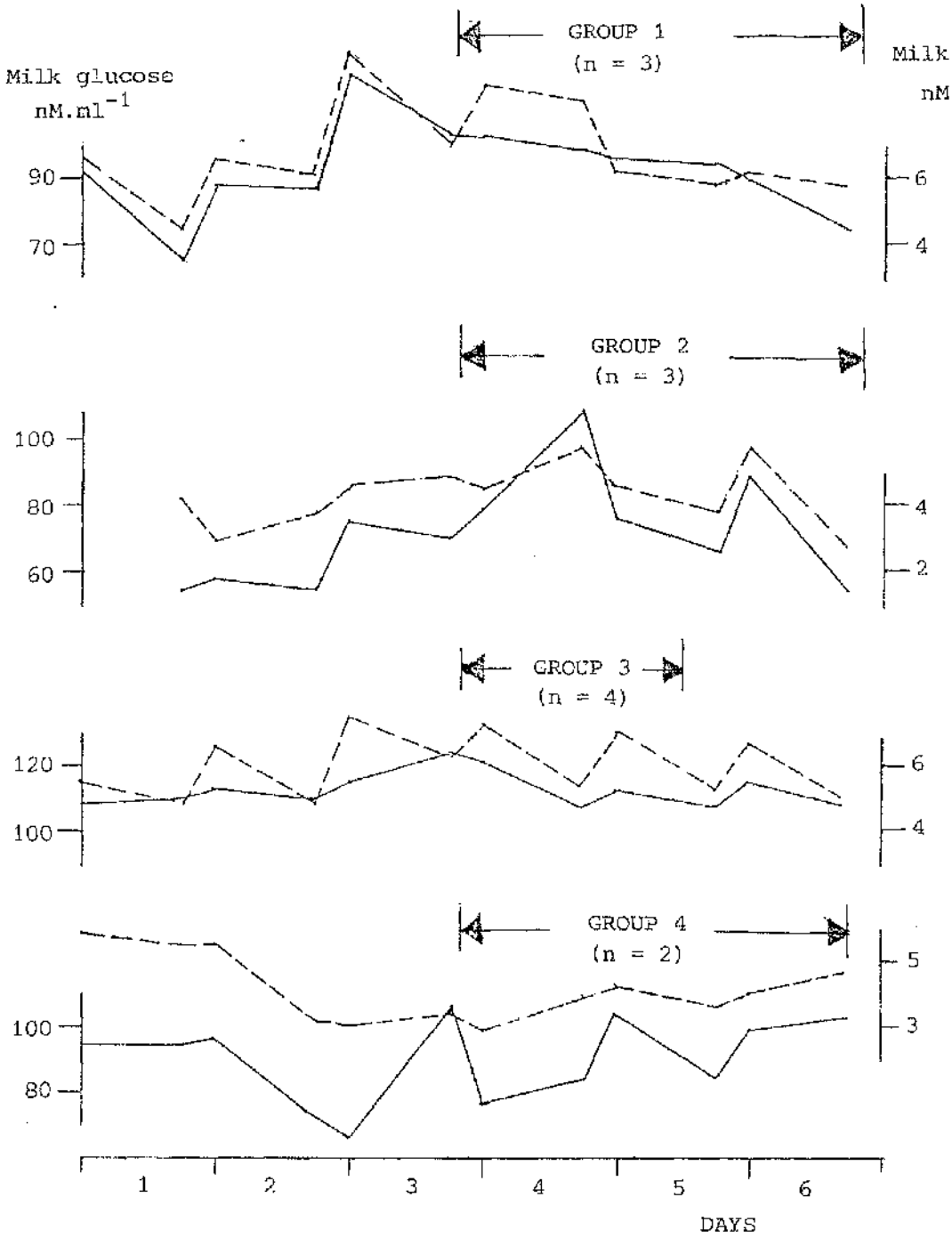

Fig 5.3. Effect of different cold environments on mean concentration (——— nM.ml^{-1}) and yield (--- nM.hr^{-1}) of glucose in goats' milk.

See text for details of climatic conditions.

TABLE 5.4 EFFECT OF VARIOUS CLIMATIC CONDITIONS ON THE MEAN CONCENTRATION AND YIELD OF NUCLEOTIDE-DI-PHOSPHATE IN GOATS' MILK

		GROUP 1		GROUP 2		GROUP 3		GROUP 4	
	n	3		3		2		2	
		nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h
DAY 1	PM	150.3	10.83	-	-	137.5	8.48	68.5	4.15
	AM	165.3	11.70	195.6	15.24	131.5	6.90	67.5	3.96
2	PM	185.6	14.02	225.3	12.90	95.5	6.71	112.0	6.16
	AM	191.3	13.57	246.6	17.40	146.5	8.25	109.5	4.31
3	PM	166.3	13.27	248.0	13.28	135.0	10.98	231.0	9.11
	AM	151.0	10.52	226.3	15.39	117.5	7.07	134.0	4.45
4	PM	179.3[a]	11.47	235.6	13.85	114.5	9.00	116.5	4.50
	AM	172.3	10.68	246.6	13.53	116.0	6.56	123.5	5.00
5	PM	172.3	11.11	223.3	13.57	110.0	8.63	156.0	5.48
	AM	143.6	9.17	194.0	10.28	97.5	5.60	166.5	6.93
6	PM	171.0	10.65	234.3	14.52	92.5	6.30	158.5	6.31
	AM	151.3	8.47	224.3	10.76	120.5	3.32	137.0	5.60

(a - figures in boxes represent results obtained during exposure to the test environments; see text for details)

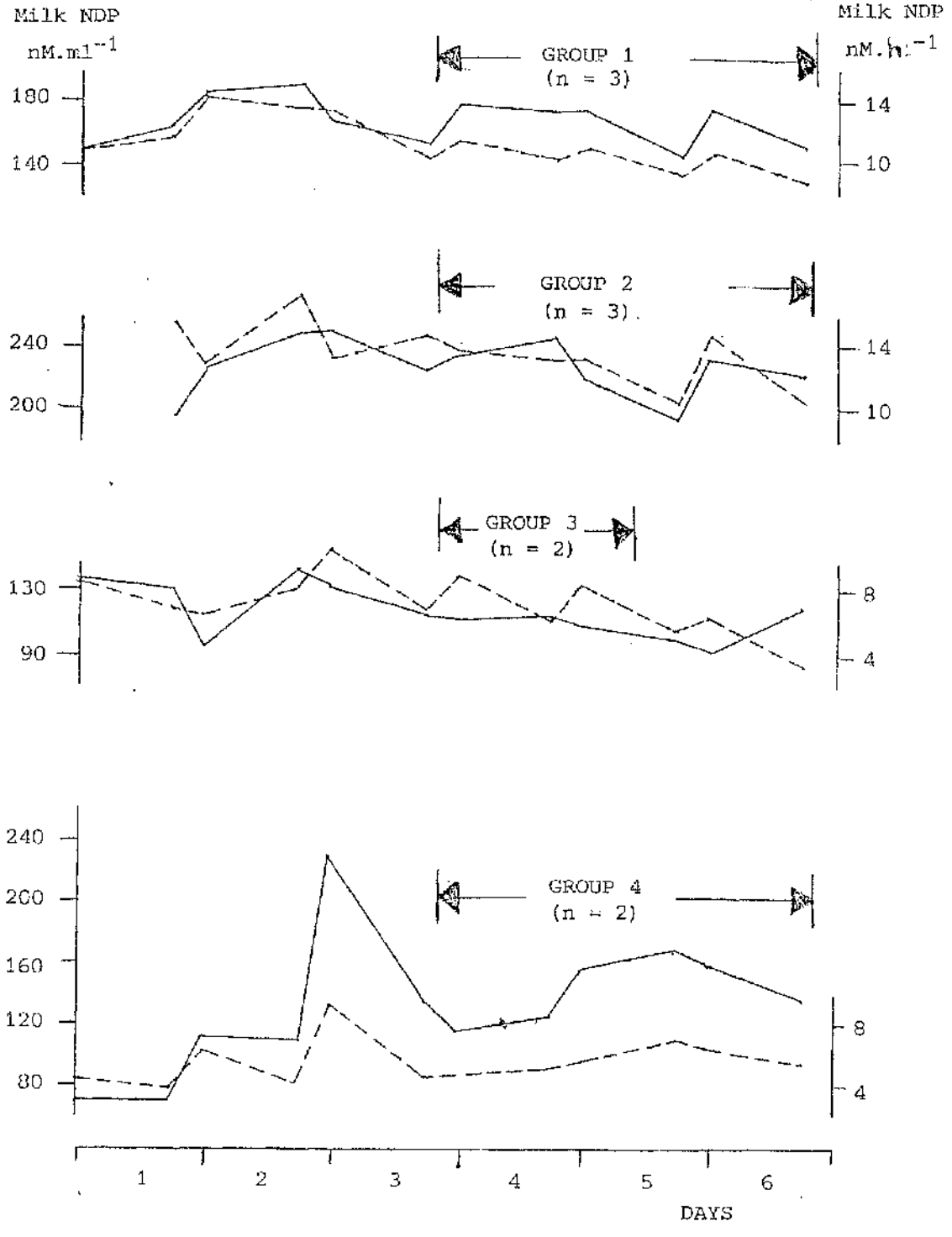

Fig 5.4. Effect of different cold environments on mean concentration (——$nM.ml^{-1}$) and yield (---$nM.hr^{-1}$) of nuclectide diphosphate in goats' milk.

See text for details of climatic conditions.

to the experimental conditions were not statistically different from levels found under control conditions. Animals sampled during late lactation (Group 4) appeared to have a lower milk NDP level compared with mid-lactation. The effect of reducing or removing the concentrate ration from the diet was to increase the NDP content of the milk, to between 200 and 240 nM/ml.

Iso-citrate in milk

The concentration of iso-citrate found in goats' milk during this study ranged from 29.3 to 75.0 nM/ml and the yield varied from 1.18 to 4.08 nM/h as shown in Table 5.5 and illustrated in Fig 5.5.

Exposure of the animals to the experimental procedures produced no significant changes in yield or concentration of iso-citrate in milk. There were differences in level of iso-citrate in milk between groups before exposure to the cold environments but these differences could not be explained in terms of food intake or stage of lactation.

2-oxo-glutarate in milk

The concentration of 2-oxo-glutarate found in goats' milk during these experiments ranged from 25.0 to 136 nM/ml and the yield ranged from 1.15 to 8.00 nM/h as shown in Table 5.6 and illustrated in Fig 5.6.

Exposure to the four different experimental regimes gave no significant changes in concentration or yield of milk 2-oxo-glutarate. The results suggest that a reduced food intake leads to a decreased concentration of milk 2-oxo-glutarate, but the large differences in level between the groups was not related to food intake or stage of lactation.

TABLE 5.5 EFFECT OF VARIOUS CLIMATIC CONDITIONS ON THE MEAN CONCENTRATION AND YIELD OF ISO-CITRATE IN GOATS' MILK

		GROUP 1		GROUP 2		GROUP 3		GROUP 4	
	n	3		3		4		2	
		nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h
DAY 1	PM	39.0	2.75	-	-	61.2	2.82	33.0	2.00
	AM	29.3	2.00	52.3	3.74	53.5	2.25	34.5	2.03
2	PM	40.6	2.95	46.6	2.40	66.0	3.33	46.5	2.69
	AM	41.3	2.85	51.3	3.41	61.2	2.54	40.0	1.64
3	PM	41.0	3.20	51.3	2.81	75.0	4.08	34.0	1.45
	AM	35.3	2.41	53.6	3.52	59.0	2.78	35.0	1.18
4	PM	35.6[a]	2.31	59.6	3.49	64.5	3.42	44.5	1.73
	AM	43.0	2.53	58.0	3.07	59.0	2.54	36.5	1.48
5	PM	37.6	2.20	52.3	3.19	65.5	3.59	41.5	1.51
	AM	49.6	2.81	56.6	3.03	66.7	2.89	39.0	1.62
6	PM	48.0	2.94	53.3	3.25	74.2	3.72	33.5	1.34
	AM	47.6	2.53	58.6	2.60	64.7	2.70	43.5	1.88

(a - figures in boxes represent results obtained during exposure to the test environments; see text for details)

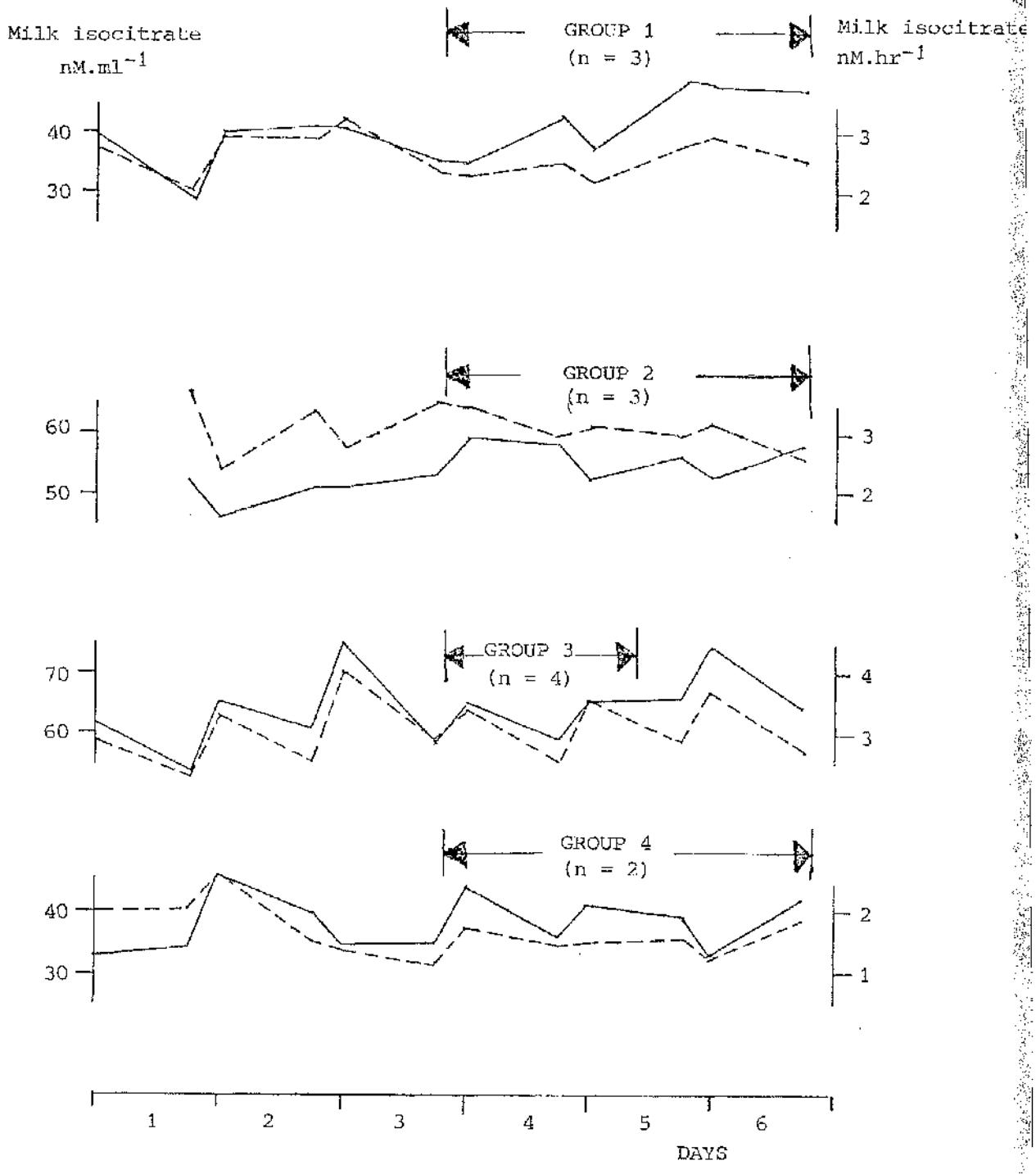

Fig 5.5. Effect of different cold environments on mean concentration (—— $nM.ml^{-1}$) and yield (--- $nM.hr^{-1}$) of iso-citrate in goats' milk.

See text for details of climatic conditions.

TABLE 5.6 EFFECT OF VARIOUS CLIMATIC CONDITIONS ON THE MEAN CONCENTRATION AND YIELD OF 2-OXO-GLUTARATE IN GOATS' MILK

		GROUP 1		GROUP 2		GROUP 3		GROUP 4	
	n	3		3		4		2	
		nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h
DAY 1	PM	53.3	3.68	-	-	130.5	6.40	129.0	7.85
	AM	56.0	3.57	40.3	3.13	114.0	5.01	103.0	6.10
2	PM	62.3	4.23	40.6	2.08	132.0	7.19	109.0	6.43
	AM	37.0	2.43	36.3	2.57	123.0	5.31	69.5	2.97
3	PM	47.6	3.64	32.3	1.73	136.2	8.00	62.0	2.73
	AM	47.3	2.95	29.0	2.21	120.7	5.92	104.5	3.48
4	PM	34.3[a]	1.91	30.0	1.77	123.7	7.10	106.0	4.27
	AM	36.3	1.83	30.6	1.69	98.7	4.59	115.0	5.12
5	PM	36.3	1.77	31.6	1.92	120.7	6.95	130.0	5.03
	AM	32.6	1.74	34.0	1.98	121.0	5.53	97.5	4.41
6	PM	42.0	2.53	37.0	2.37	118.2	5.86	95.5	3.82
	AM	36.0	1.82	25.0	1.15	134.7	5.68	92.0	4.04

(a - figures in boxes represent results obtained during exposure to the test environments; see text for details)

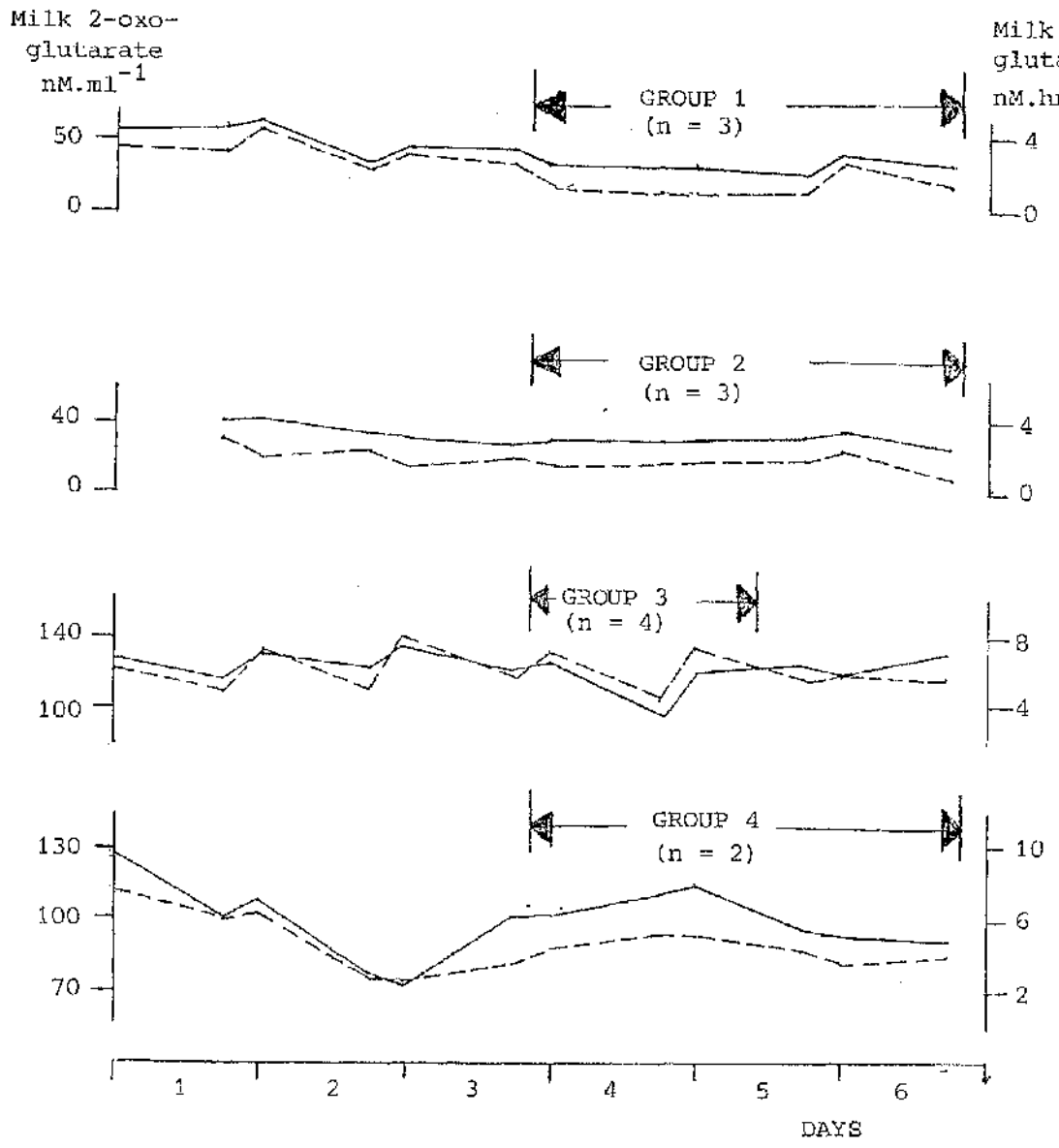

Fig 5.6. Effect of different cold environments on mean concentration (—— nM.ml^{-1}) and yield (--- nM.hr^{-1}) of 2-oxo-glutarate in goats' milk.

See text for details of climatic conditions.

Galactose in milk

The concentration of galactose found in goats' milk during this study ranged from 61.5 to 205.0 nM/ml and the yield varied between 3.75 and 9.55 nM/h as shown in Table 5.7 and illustrated in Fig 5.7.

No statistically significant effects were found when comparing milk galactose concentration during cold exposure with the corresponding level previously. Reduction in food intake increased the level of galactose in milk but subsequent cold exposure still did not have any significant effect. The results do not suggest any differences due to stage of lactation.

Discussion

Concentrations of phosphoenol pyruvate in milk, which have been found to correlate well with levels in mammary tissue (Faulkner, 1980) were in the same range as previously reported by Faulkner. A significant decrease ($P < 0.01$) in concentration resulted from exposure of the goats to the moderate cold environment during mid-lactation. The significance of a lower milk concentration of this glycolytic intermediate may indicate a decreasing flux through the pentose phosphate shunt (Chaiyabutr et al., 1981).

All other intracellular intermediates studied were in the same range as those found by Faulkner (1980) and Faulkner et al. (1982). In each of the four groups the test conditions caused little change in the rate of milk secretion. Reduction in food intake was more effective in decreasing milk secretion especially in high yielding goats than cold exposure. Any decrease in yield may be expected to lead to an apparent increase in concentration of intracellular metabolites in milk, but this was not apparent. (See Appendix 4 for milk yield data). Also any changes in milk yield would be expected to

TABLE 5.7 EFFECTS OF VARIOUS CLIMATIC CONDITIONS ON THE MEAN CONCENTRATION AND YIELD OF GALACTOSE IN GOATS' MILK

		GROUP 1		GROUP 2		GROUP 3		GROUP 4	
	n	3		3		2		2	
		nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h	nM/ml	nM/h
DAY 1	PM	86.0	6.23	-	-	77.5	4.76	61.5	3.75
	AM	95.6	6.73	121.0	8.32	71.0	4.19	65.5	3.85
2	PM	102.6	8.05	129.0	6.64	75.0	5.23	96.0	5.41
	AM	85.3	6.36	115.0	8.12	86.0	4.77	120.5	4.85
3	PM	95.6	7.65	135.0	8.08	92.0	7.44	181.5	7.48
	AM	101.3	7.19	135.6	8.95	92.5	5.74	205.0	6.99
4	PM	79.0[a]	5.39	126.0	7.28	91.5	7.20	110.5	4.18
	AM	71.0	4.33	129.0	7.38	91.5	5.14	130.0	5.49
5	PM	78.3	5.19	129.0	7.97	87.0	6.95	150.5	5.47
	AM	90.6	5.67	129.0	6.44	79.0	4.55	149.5	5.87
6	PM	97.6	5.26	159.6	9.55	76.5	5.38	130.5	5.18
	AM	95.0	5.41	144.6	6.61	79.0	4.38	108.0	4.51

(a - figures in boxes represent results obtained during exposure to test environments; see text for details)

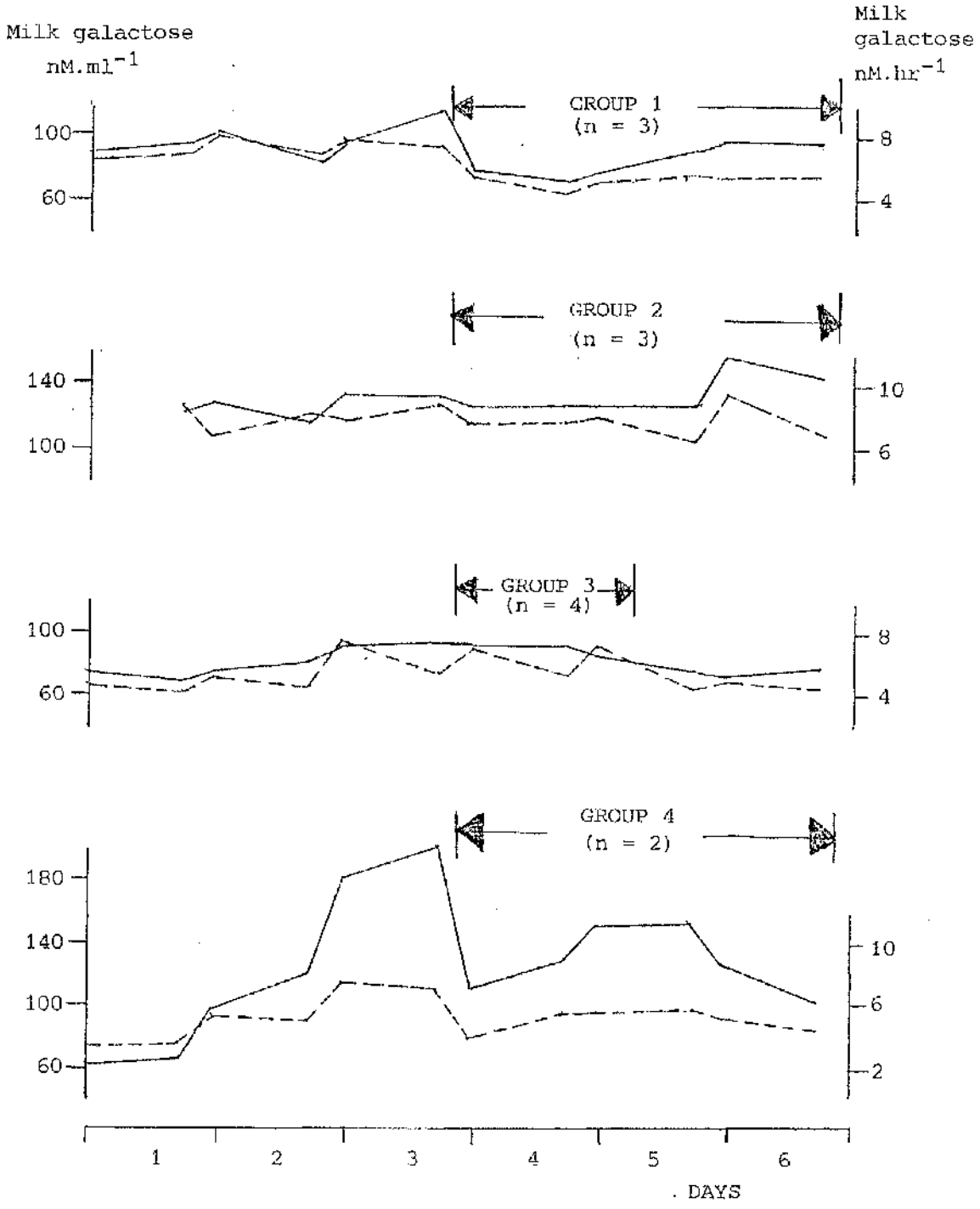

Fig 5.7. Effect of different cold environments on mean concentration (—— $nM.ml^{-1}$) and yield (--- $nM.hr^{-1}$) of galactose in goats' milk.

See text for details of climatic conditions.

be accompanied by changes in rates of lactose synthesis. Previous results (Section 1) suggested an increase in lactose concentration in milk. Results from this section did not indicate any significant differences in the substrates for lactose synthesis, galactose, glucose or nucleoside di-phosphate.

In summation it appears that if concentrations of intracellular metabolites in milk do correlate to their concentrations in mammary tissue, then the environmental stresses imposed by this series of experiments had little effect on mammary metabolism.

SECTION 6

THE EFFECT OF DIFFERENT COLD ENVIRONMENTS ON PLASMA COMPOSITION IN THE GOAT

Introduction

The aim of this section of the study was to determine any changes in plasma composition and packed cell volume in lactating goats during exposure to various climatic conditions in the precision chamber, and to correlate any observations with the change in rate of milk secretion. Plasma glucose was measured as this is the precursor of lactose synthesis and therefore its availability may influence milk secretion. Plasma cortisol has been shown to rise in sheep exposed to stresses including cold stress (Panaretto and Vickery, 1970 and Falconer, 1976) and increased plasma levels have triggered increased milk yield in cattle (Brush, 1960).

Plasma free fatty acids and free glycerol were measured in order to determine whether the cold environments used initiated lipolytic changes in the lactating goats. However, other workers (Vernon, 1980) have found increased levels of plasma free fatty acids in stressed animals, therefore results must be interpreted with care.

Packed cell volume, or haematocrit, was measured in order to determine if any gross changes in blood composition occurred in response to the environmental changes.

Methods

Lactating goats, maintained as described in Chapter 2, were subjected to the 'moderate cold' and 'cold and wet' environments as described in Section 1 of this Chapter.

Eight goats were exposed to the 'moderate cold' environment for 3 days during early lactation, between 2 and 8 weeks after parturition.

Five goats were studied during mid lactation, 17-22 weeks after parturition, whilst being exposed to the 'moderate cold' environment for 3 days. Four goats were subjected to the 'cold and wet' environment for 2 days between weeks 26 and 28 of lactation (mid-lactation).

Arterial blood samples (from the exteriorised carotid loop) were taken from each goat during the experimental period as described in Chapter 2. Samples were taken at 14.00 hours each day in order to minimise the effects of any circadian rhythms, or feeding and milking intervals. One sample was taken before the goats were moved from the goat-house to the precision climatic chamber, to give a base level; 3 samples were taken whilst the goats were living under the test conditions (except in the 'cold and wet' environment where only 2 days exposure was given) and one was taken on the day that the goats were returned to ambient conditions.

Milk yield for each goat was recorded over the experimental period (see Section 1). Blood was centrifuged and plasma stored at -20°C until assayed as described in Chapter 2. Student's t-test for paired observations was used to determine if there was any significant effect of cold exposure on blood composition.

Results

Plasma glucose concentration

Plasma glucose concentration in the three groups of goats ranged from 3-4 mmol/l during the experimental period (see Table 6.1 and Fig 6.1).

Exposure to the 'moderate cold' environment during early lactation caused a slight but non-significant increase from 3.45 to 3.78 mmol/l after 24 hours. Levels then fell but rose on the return of the goats to ambient conditions.

In mid-lactation there was a significant ($P < 0.05$) increase in

TABLE 6.1 EFFECT OF VARIOUS CLIMATIC CONDITIONS ON THE ARTERIAL PLASMA GLUCOSE LEVEL IN LACTATING GOATS (mean ± SE in mmol/l)

ENVIRONMENT		MODERATE COLD	MODERATE COLD	COLD & WET
STAGE OF LACTATION		EARLY	MID	MID
	n	7	5	4
DAY	1	3.45 ± 0.33	3.25 ± 0.13	3.30 ± 0.11
	2	3.78 ± 0.27[a]	3.88 ± 0.11	3.75 ± 0.08
	3	3.61 ± 0.07	4.04 ± 0.15	3.48 ± 0.39
	4	3.52 ± 0.17	3.81 ± 0.12	3.48 ± 0.11
	5	3.88 ± 0.26	3.27 ± 0.23	-

(a - figures in boxes represent results obtained during exposure to the test environments)

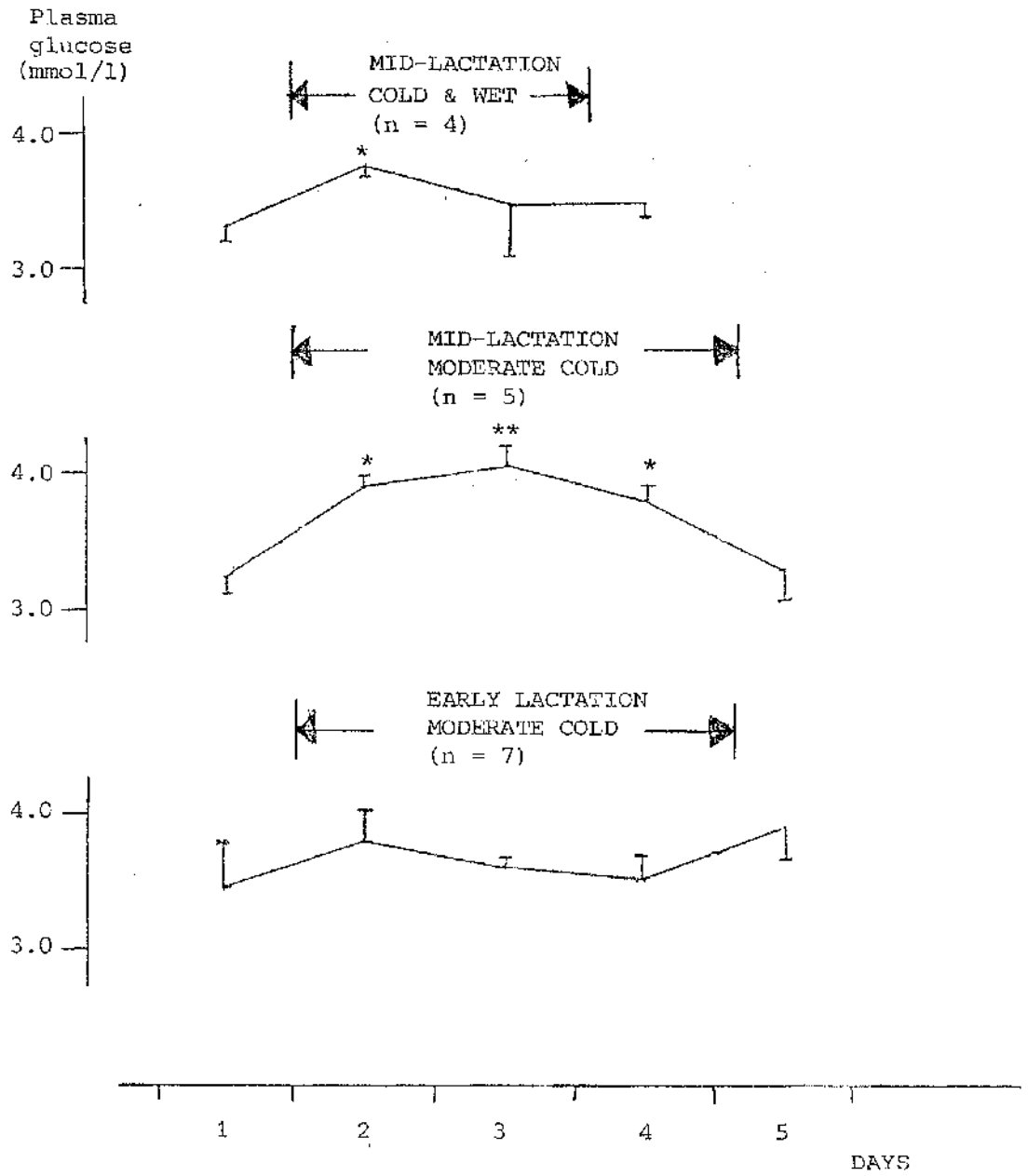

Fig 6.1. Effect of different cold environments on plasma glucose concentration in the goat.

(mean ± SE in mmol/l)

See text for details of climatic conditions.

* P < 0.05)
** P < 0.01) significance of difference from previous concentration

the arterial glucose concentration after 24 hours exposure to the moderate cold environment, from 3.25 to 3.88 mmol/l. This increase was maintained on the second day of cold exposure, but on the third day levels had fallen slightly. On the return of the goats to their normal environment their plasma glucose levels had returned to the previous level.

Exposure to the 'cold and wet' environment during mid lactation also caused a significant ($P < 0.05$) increase in arterial plasma glucose level on the first day, from 3.30 to 3.75 mmol/l, but on the second day there was no significant change.

Plasma cortisol concentration

Plasma cortisol levels found initially in the three groups of goats studied ranged from 8.37 to 18.30 ng/ml which is relatively low, indicating a non stressed condition (see Table 6.2 and Fig 6.2). During exposure to the moderate cold environment during early lactation plasma cortisol concentration remained steady, but on the return of the animals to their normal environment there was a small (non-significant) increase.

When lactating goats were subjected to the moderate cold environment during mid-lactation, however, there were increases in cortisol concentration over the first 48 hours exposure and a reversal on the third day. These changes, however, were not statistically significant. There was no change in cortisol concentration on the return of the animals to the goat-house.

Exposure to the cold and wet environment during mid-lactation showed only a small, non-significant increase in plasma cortisol level on the second day.

The highest mean level of cortisol reached in any of the

TABLE 6.2 EFFECT OF VARIOUS CLIMATIC CONDITIONS ON THE LEVEL OF CORTISOL IN THE ARTERIAL PLASMA OF LACTATING GOATS

(mean ± SE in ng/ml)

ENVIRONMENT		MODERATE COLD	MODERATE COLD	COLD & WET
STAGE OF LACTATION		EARLY	MID	MID
	n	7	5	4
DAY	1	15.13 ± 5.06	18.30 ± 6.62	8.97 ± 3.23
	2	12.64 ± 3.17[a]	21.92 ± 3.12	7.00 ± 2.44
	3	11.80 ± 4.12	26.70 ± 6.70	18.77 ± 5.53
	4	11.84 ± 2.19	19.32 ± 3.81	9.20 ± 4.23
	5	18.07 ± 4.28	18.22 ± 5.18	

(a - figures in boxes represent results obtained during exposure to the test environments)

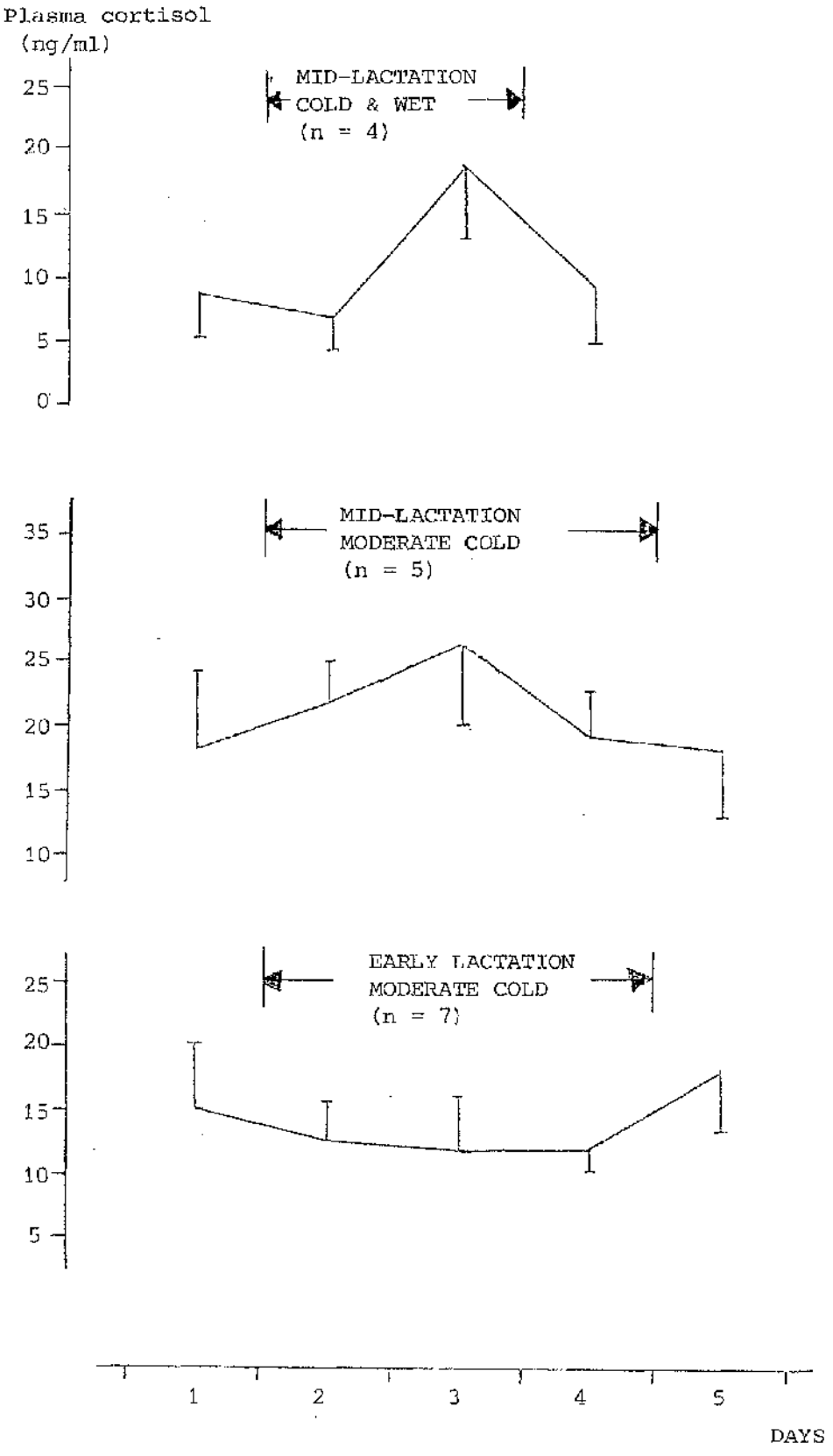

Fig 6.2. Effect of different cold environments on plasma cortisol concentration in the goat.
(mean ± SE in ng/ml)
See text for details of climatic conditions.

experimental conditions was 26.70 ng/ml on the second day of exposure to the moderate cold environment during mid-lactation.

Plasma free palmitic acid concentration

The levels of free palmitic acid found in the plasma of goats in this study ranged from 25.74 to 192.06 µmol/l (see Fig 6.3 and Appendix 5, Table 1 for data).

The most obvious differences in concentration of free palmitic acid observed was between the groups studied; those in early lactation ranging between 161.31 and 192.06 µmol/l and those in mid lactation ranging between 25.74 and 76.04 µmol/l.

During early and mid-lactation, exposure to the moderate cold environment caused no significant change in plasma free palmitic acid concentration. When the goats were exposed to the cold and wet environment in mid-lactation, there was a significant ($P < 0.01$) increase in free palmitic acid. After 48 hours the levels were raised still further, and on return of the animals to their normal environment the levels fell to a point not statistically different from the initial level.

Plasma free palmitoleic acid

The levels of free palmitoleic acid found in the plasma of the lactating goats studied ranged from 1.69 to 12.01 µmol/l (see Fig 6.4 and Appendix 5, Table 2 for data).

Levels were found to be higher in goats studied in early lactation (7.48 - 12.01 µmol/l) than those studied in mid-lactation (1.69 - 5.03 µmol/l).

Exposure of the goats to the moderate cold environment in early or mid-lactation caused no statistically significant changes in the levels of free palmitoleic acid in the plasma. However, exposure to

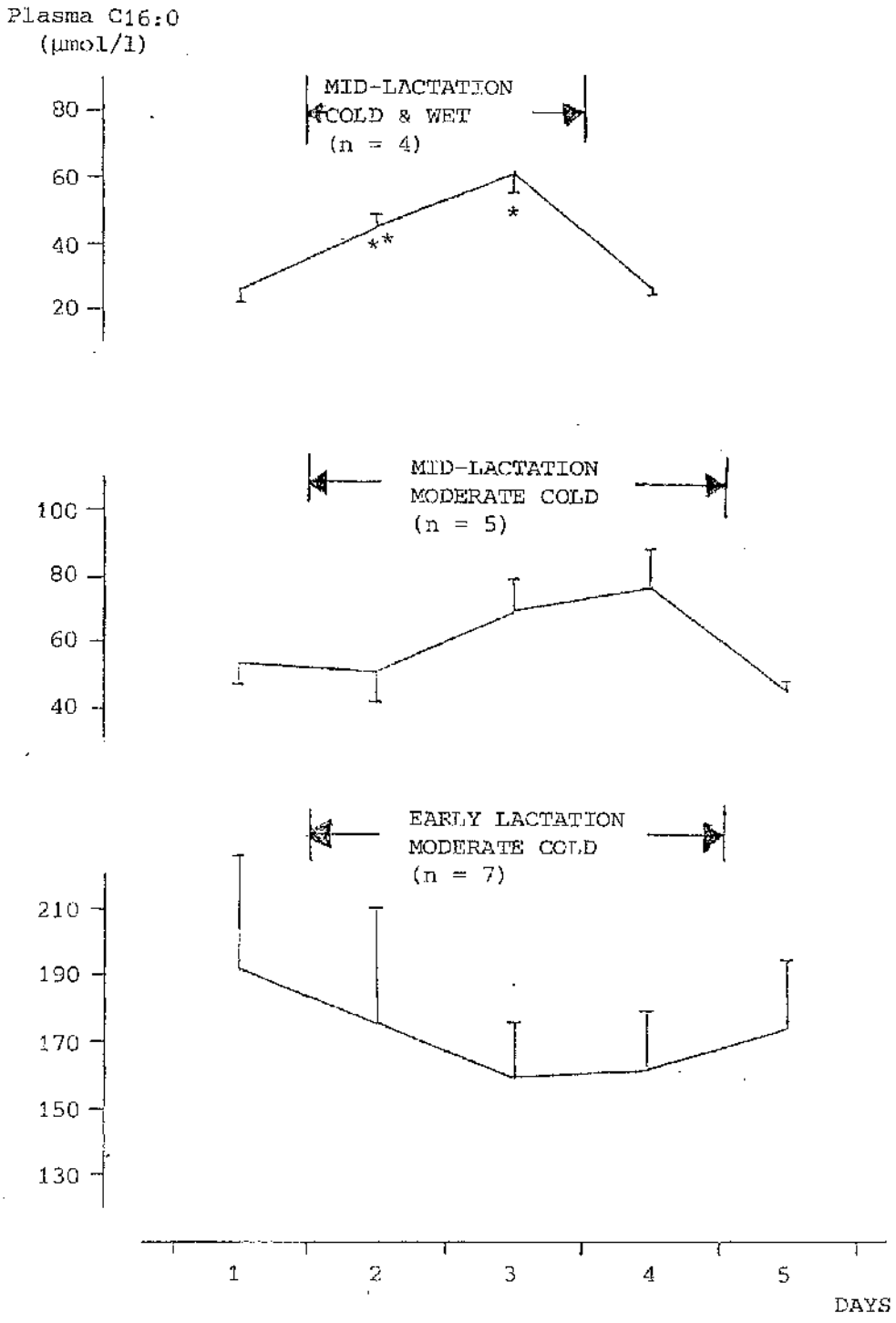

Fig 6.3. Effect of different cold environments on plasma free palmitic acid ($C_{16:0}$) in the goat.

(mean ± SE in µmol/l)

See text for details of climatic conditions

* $P < 0.05$)
** $P < 0.01$) significance of difference from previous concentration

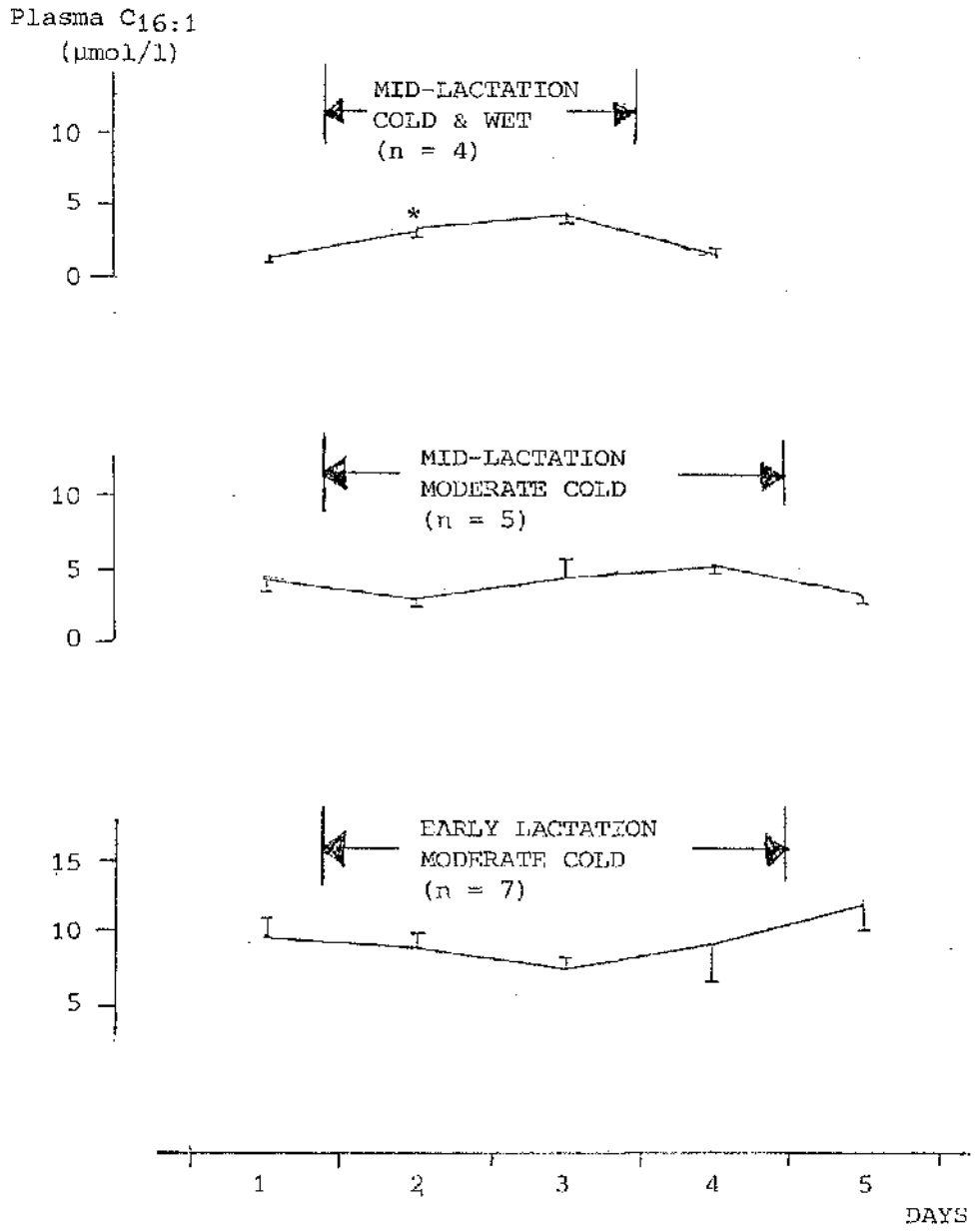

Fig 6.4. Effect of different cold environments on plasma free palmitoleic acid ($C_{16:1}$) in the goat.
(mean ± SE in μmol/l)
See text for details of the climatic conditions.
* $P < 0.05$ significance of difference from previous concentration

the cold and wet environment during mid-lactation did cause a small, but significant ($P < 0.05$), increase in the level of free palmitoleic acid. Returning the goats to their normal environment after exposure to the cold and wet environment caused a significant decrease ($P < 0.01$) to 1.69 µmol/l of palmitoleic acid in the plasma.

Plasma free stearic acid

Levels of free stearic acid found in goats' plasma during this study ranged from 43.15 to 332.46 µmol/l (see Fig 6.5 and Appendix 5, Table 3 for data).

During early lactation when the overall concentration of free stearic acid was found to be the highest, there was a gradual but non-significant decline in concentration during exposure to the moderate cold environment.

Exposure to the moderate cold environment during mid-lactation, when basal levels of free stearic acid in the plasma were lower, lead to a steady increase but this was also found to be non-significant.

However exposure, at the same stage of lactation, to the cold and wet environment caused a significant increase in plasma stearic acid after 24 hours ($P < 0.01$) and a further increase ($P < 0.01$) after 48 hours to 45.51 µmol/l. On the return of the animals to their normal environment their plasma levels of free stearic acid had fallen to their original levels.

Plasma free oleic acid concentration

During this study the plasma concentration of free oleic acid ranged from 31.89 to 334.10 µmol/l (see Fig 6.6 and Appendix 5, Table 4 for data).

The highest levels were found in goats studied during early lactation (245.34 to 334.10 µmol/l) but exposure to the moderate cold

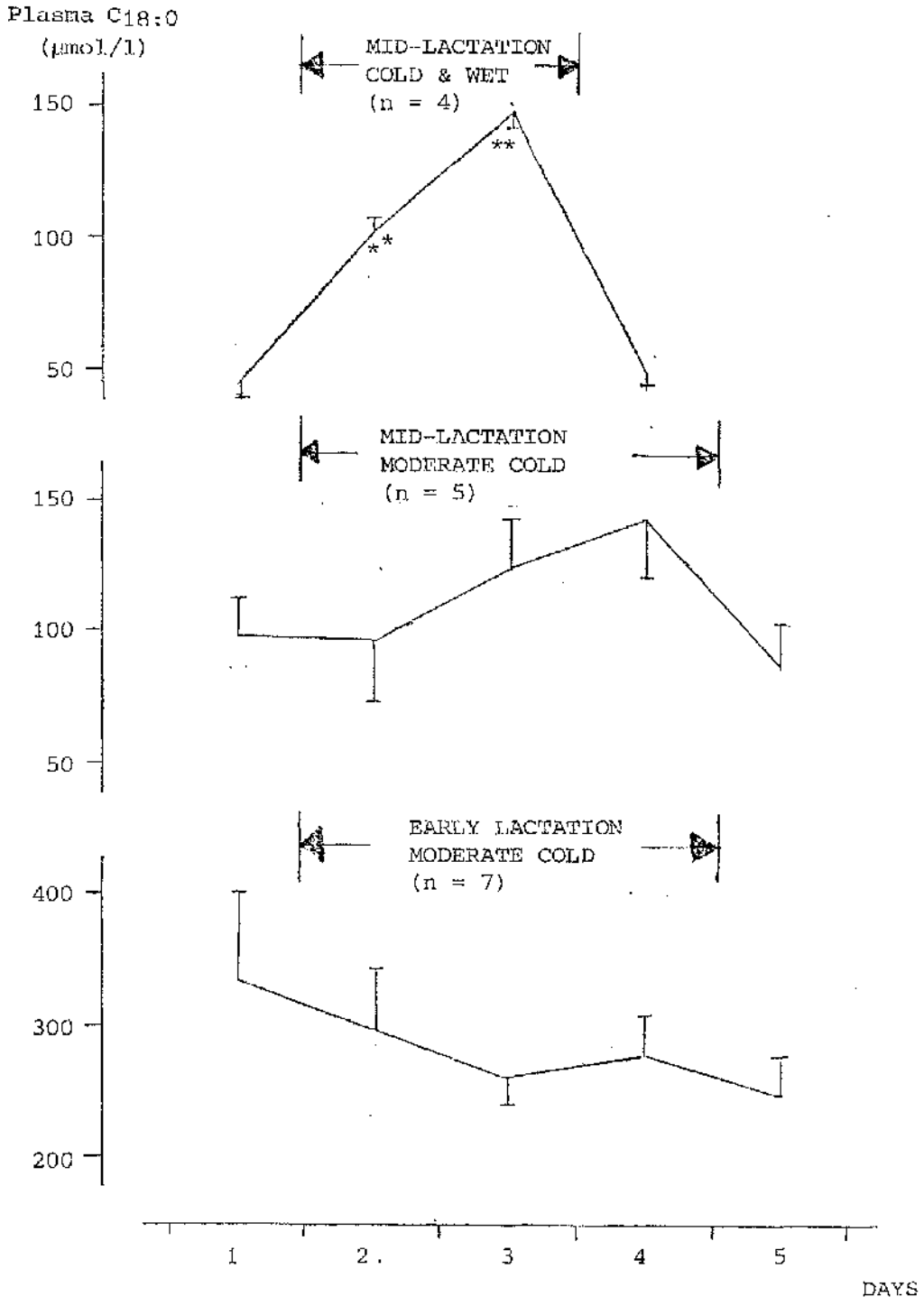

Fig 6.5. Effect of different cold environments on plasma free stearic acid ($C_{18:0}$) in the goat.
(mean ± SE in μmol/l)
See text for details of climatic conditions.
** $P < 0.01$ significance of difference from previous concentration

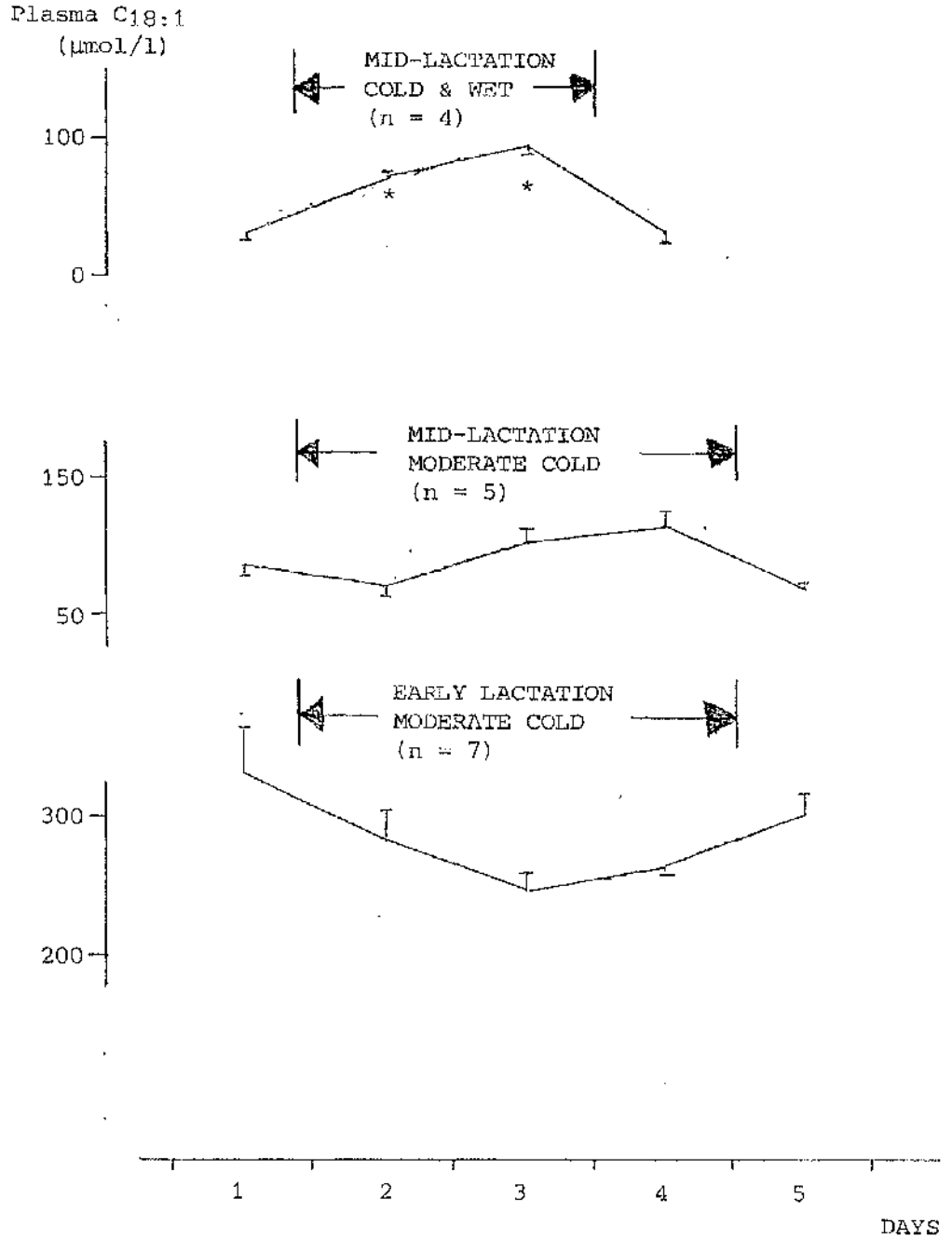

Fig 6.6. Effect of different cold environments on plasma free oleic acid ($C_{18:1}$) in the goat.
(mean ± SE in μmol/l)
See text for details of climatic conditions.
* $P < 0.05$ significance of difference from previous concentration

environment at that stage of lactation caused no significant changes.

When plasma levels of free oleic acid were followed during exposure of goats to the moderate cold environment during mid-lactation no changes were observed. Exposing the goats to the cold and wet environment at that stage of lactation, however, lead to significant rises ($P < 0.05$) in free oleic acid to 95.02 µmol/l after 48 hours. On return of the animals to their normal environment the plasma levels of free oleic acid returned to the previous level.

Plasma free linoleic acid concentration

Plasma concentrations of free linoleic acid found in the lactating goats during this study ranged from 10.57 to 35.51 µmol/l (see Fig 6.7 and Appendix 5, Table 5 for data).

During early lactation the basal level of plasma free linoleic acid was higher than in mid-lactation (30.40 - 35.51 compared with 10.57 to 17.53 µmol/l). There was no significant effect of exposure to the moderate cold environment during early lactation, but exposure to the cold and wet environment in mid-lactation did cause a significant increase. The initial mean level of 10.57 µmol/l rose to 14.57 ($P < 0.01$) after 24 hours and again to 17.53 ($P < 0.01$) after 48 hours. On return of the goats to their normal environment there was a decrease in plasma concentration of free linoleic acid to their previous levels.

Plasma glycerol concentration

Levels of free glycerol found in the plasma of goats in this study ranged between 27.51 and 46.26 µmol/l (see Table 6.3 and Fig 6.8).

Owing to a failure of the glycerol assay system, reliable values for plasma free glycerol were only obtained for the group of five goats exposed to the moderate cold environment during mid-lactation;

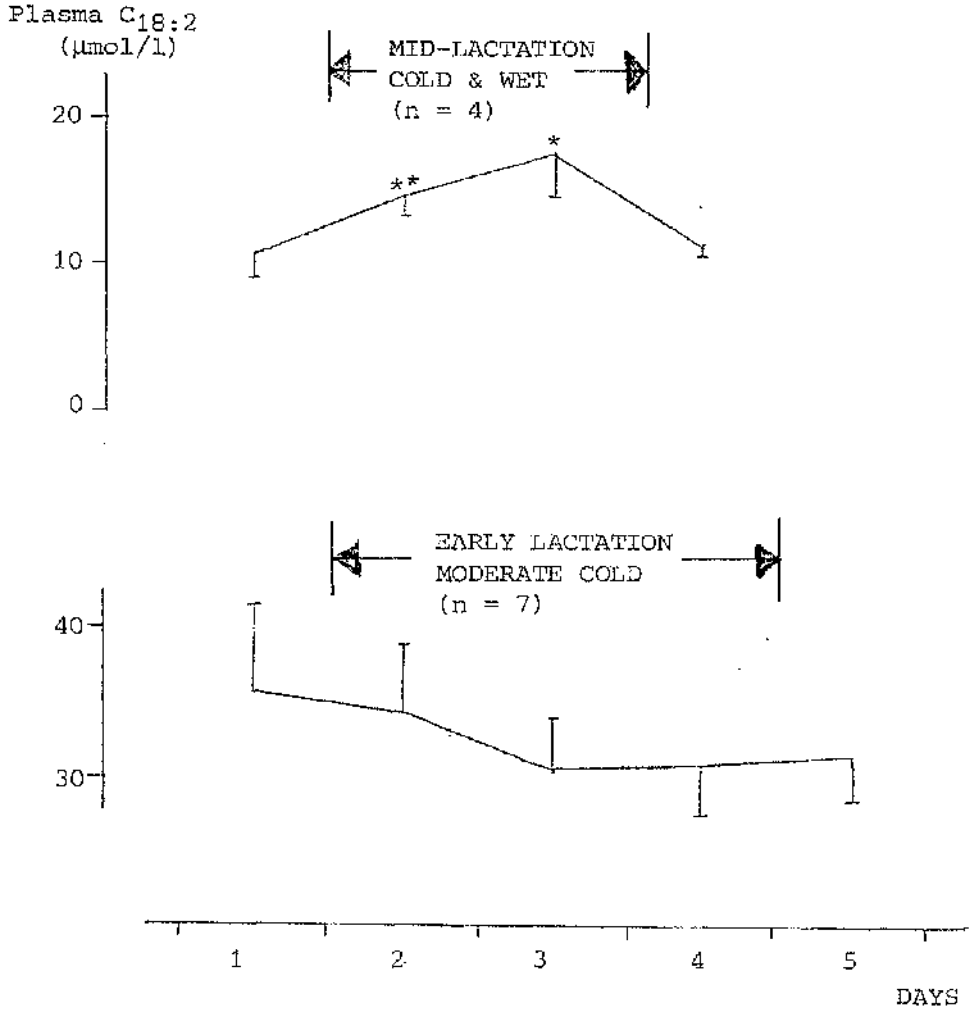

Fig 6.7. Effect of different cold environments on plasma free linoleic acid ($C_{18:2}$) concentration in the goat.
(mean ± SE in µmol/l)
See text for details of climatic conditions.
* $P < 0.05$)
** $P < 0.01$) significance of difference from previous concentration

TABLE 6.3 EFFECT OF MODERATE COLD EXPOSURE DURING EARLY LACTATION ON ARTERIAL PLASMA FREE GLYCEROL CONCENTRATION in μmol/l

GOAT	G24	G32	G22	G34	G16	mean ± SE
DAY 1	71.62	56.08	22.78	47.31	9.64	41.49 ± 11.20
2	77.99[a]	39.43	11.39	3.50	61.33	38.73 ± 14.19
3	61.33	28.04	17.52	27.16	57.83	38.38 ± 8.85
4	92.00	54.32	21.03	23.66	35.92	45.39 ± 13.03
5	52.57	63.09	2.63	45.56	64.84	45.74 ± 11.32

(a - figures within boxes represent results obtained during exposure to the test environment)

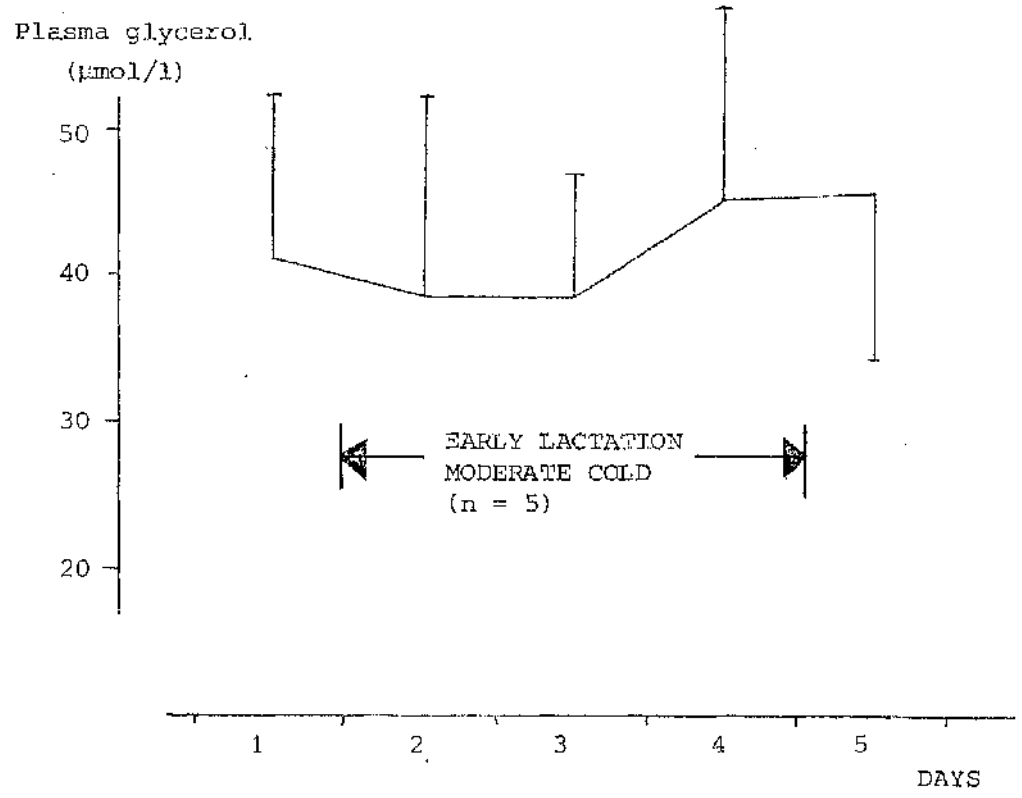

Fig 6.8. Effect of moderate cold exposure on plasma glycerol concentration in the goat.
(mean ± SE in µmol/l)
See text for details of the climatic conditions.

no significant changes were found.

Packed cell volume

Packed cell volume (PCV) or haematocrit ranged from 22.1 to 26.2% in the blood of goats studied in this project (see Table 6.4 and Fig 6.9).

During early lactation the initial PCV was 25.5%; exposure to the 'moderate cold' environment at that stage had no significant effect. On return of the goats to their normal environment there was a small but significant decrease in PCV to 25.3% ($P < 0.05$ with respect to original level and $P < 0.01$ with respect to mean level during cold exposure). In mid-lactation, the initial packed cell volume was lower, 21.7%, and the effect of exposure to the moderate cold environment was to cause a significant increase ($P < 0.05$ after one day and $P < 0.01$ after two and three days exposure). On return to the control environment the packed cell volume fell significantly ($P < 0.05$) to 22.1 which was not significantly different from the original level. Exposure of goats to the 'cold and wet' environment during mid-lactation caused a slightly significant increase in packed cell volume ($P < 0.05$) to 24.6 after 2 days. Subsequent return of the animals to their normal environment again caused a decrease in packed cell volume to 23.0 ($P < 0.05$), the original level.

Discussion

The results of this section illustrate the changes in certain plasma constituents accompanying the decrease in rate of milk secretion during cold exposure. The extent of the reduction in milk secretion were an 8% decrease in early lactation, and a 19% decrease in mid-lactation due to the moderate cold environment as in this chapter, section 2. Exposure to the cold and wet environment in mid-lactation had no statistically significant effect on milk yield

TABLE 6.4 EFFECT OF VARIOUS CLIMATIC CONDITIONS ON PACKED CELL VOLUME IN THE LACTATING GOAT (mean ± SE in %)

ENVIRONMENT		MODERATE COLD	MODERATE COLD	COLD & WET
STAGE OF LACTATION		EARLY	MID	MID
	n	8	5	4
DAY	1	25.5 ± 1.1	21.7 ± 0.6	23.0 ± 1.4
	2	26.0 ± 0.8[a]	23.7 ± 1.3	23.6 ± 1.5
	3	26.2 ± 1.1	23.9 ± 0.7	24.6 ± 1.2
	4	25.6 ± 1.0	23.1 ± 0.8	23.0 ± 0.7
	5	25.3 ± 0.9	22.1 ± 0.6	

(a - figures in boxes represent results obtained during exposure to the test environments)

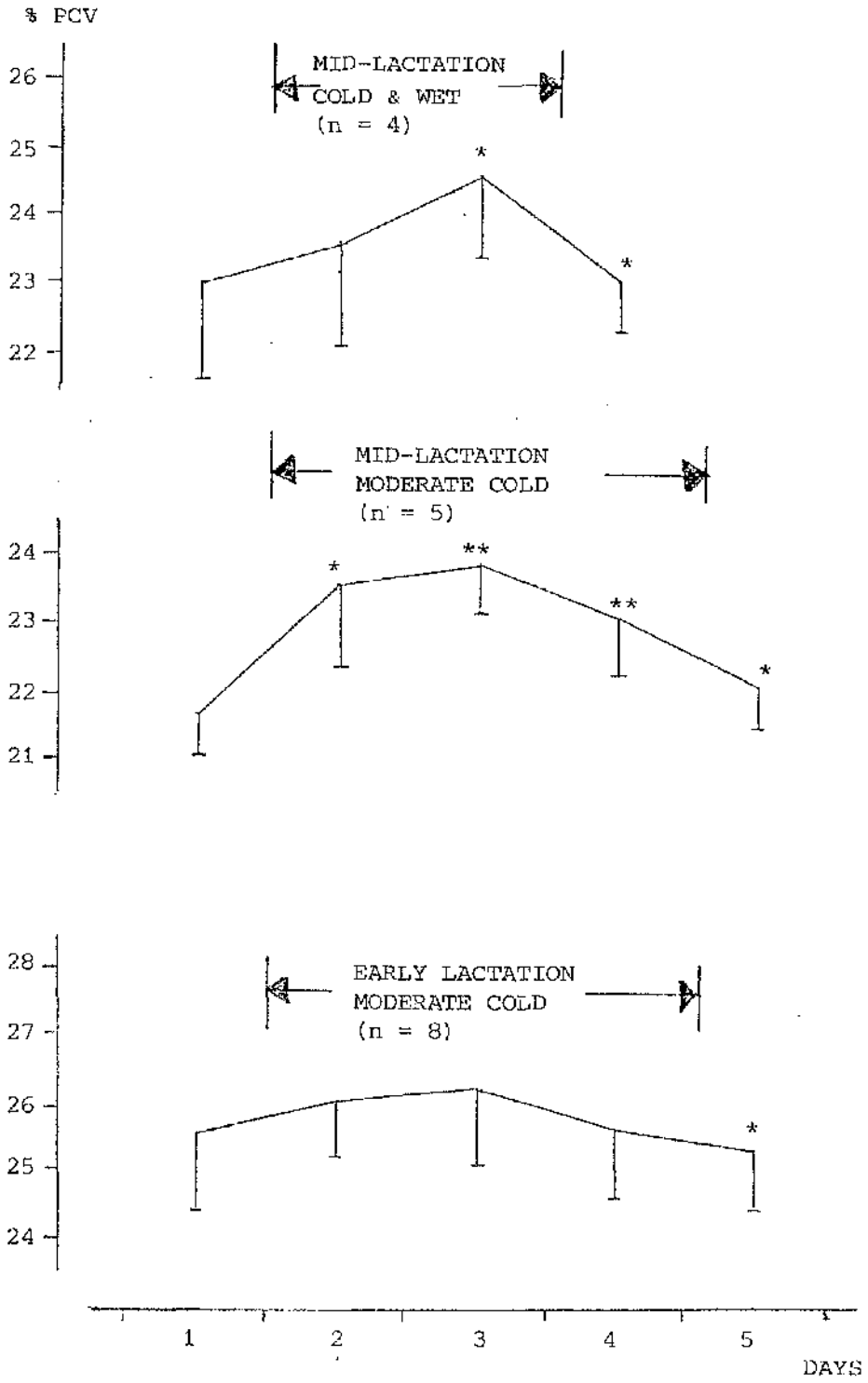

Fig 6.9. Effect of different cold environment on packed cell volume (%) in the goat

(mean ± SE)

See text for details of climatic conditions

* P < 0.05)
** P < 0.01) significance of difference from previous concentration

although the goats were visibly more stressed by the procedure (see section 1).

The levels of plasma glucose recorded are in the same range as reported by previous workers (Davis et al., 1979, Chaiyabutr et al., 1982) for lactating goats, although slightly higher possibly due to the time between feeding and sampling. Cold exposure during mid-lactation, both the moderate and the cold and wet conditions, lead to a significant rise in plasma glucose levels, the highest rise being associated with the largest decrease in milk yield. In early lactation where there had been only a slight decrease in milk yield, there was no change in plasma glucose level. Plasma glucose concentration has been shown to rise during cold exposure in lactating goats (Thomson, 1978) and in the sheep (Thompson et al., 1978). Arterial concentrations may increase due to increased glycogenolysis under cold stress, but glucose uptake by the mammary gland has been shown to fall under these conditions (Thomson, 1978).

Plasma cortisol concentrations recorded in this study are in the same range as reported by Paterson and Linzell (1971), but there was no significant increase during cold exposure, accompanying the decreased rate of milk secretion. Thomson (1978) found a large increase (six times) in lactating goats exposed to a cold environment, as has been found in non-productive sheep by Panaretto and Vickery (1970), and in the newborn calf by Khan et al. (1970). Cortisol secretion rate is higher in lactating animals (Paterson and Linzell, 1971), and secretion has been found to follow an ultradian rhythm (Fulkerson et al., 1980) in cattle; therefore, any interpretation of results from single samples must be interpreted with caution. Training and experience and temperament of the animals may also affect any response to experimental procedures; it is interesting to note that

in two of the goats exposed to the cold and wet environment in the present study, there was a decrease in plasma cortisol levels - whereas in the other two animals of the group the levels rose.

Total free fatty acid levels in the plasma of lactating goats in this study were increased most significantly by exposure of the animals to the cold and wet environment. Cold stress will increase both the rate of fatty acid uptake and the rate of liberation of fatty acids from adipose tissue, in lactating ruminants (Thomson et al., 1979), therefore increased levels of free fatty acids in the plasma must be interpreted cautiously. No measures of mammary blood flow were made in this study, in order to minimize non-specific stress to the animals, therefore rates of lipolysis and fatty acid uptake cannot be assessed.

The high levels of free fatty acids found in early lactation reflect the degree of fat mobilisation that takes place at that time, under hormonal control, and it may be significant that at that time no significant further increase in free fatty acid concentration was effected by cold stress. The fact that the largest increases in free fatty acid concentration were not synchronous with the most dramatic reduction in milk yield suggests that lipolysis was stimulated by catecholamine release in response to stress, rather than as a response to the decreased environmental temperature.

One would expect glycerol levels to increase in a like manner to free fatty acid concentration, but unfortunately no reliable results were obtained.

The most significant effects on packed cell volume occurred when the rate of milk secretion was also inhibited. This may have been due to a general short-term dehydration of the animals during cold exposure when milk yield was at its peak.

In summation the results from this section suggest that although cortisol levels did not indicate any difference in the degree of stress resulting from the different environments, the animals studied during mid-lactation showed a more dramatic response in terms of increasing glucose and free fatty acid concentrations. This difference may have been due to the high level of milk production at mid-lactation.

CHAPTER 4

GENERAL DISCUSSION

The experiments described in this thesis aimed to examine the responses of lactating goats to low temperatures under relatively stress free conditions. A single blood sample only was taken from the animals during each day of the experiment; all other handling being that of the normal husbandry routine. The only major stress that could not be avoided was the noise created by the electric fan. Previous studies at this Institute (Thomson, 1978) have involved measurement of metabolic rate, cardiac function and mammary blood flow during cold exposure and have resulted in a greater reduction in milk yield than found in this study.

The condition of any animal is crucial in determining its response to thermal stress; condition is difficult to define and will normally change during the lactation. In early lactation body energy reserves may be depleted following the later stages of pregnancy, and if milk yield is high the animals' condition may decline further. Also at this time mammary cells are not functioning at their maximum potential. At peak lactation, milk yield is high and mammary cells are operating at their highest efficiency. Food intake at this stage is increasing and a state of positive energy balance will be reached especially as yield starts to decrease. In declining lactation as the cells reduce their production of milk, food intake may be maintained and thus energy can be stored in the form of body fat. Therefore, if energy is required for thermogenesis in late lactation, body reserves are available and the rate of milk secretion can be maintained. Early lactation, however, is the most crucial period in terms of the requirement of the offspring; a period of cold stress at that time may lead to a decline in milk yield.

From the results of this study, however, it appears that in early lactation the 'moderate cold' environment can be withstood by the goat with only a small resultant decline in milk yield.

It is possible that in early lactation when the body is in a fat mobilizing state, free fatty acids are more readily available in the plasma to allow the required increase in heat production. From the results of Section 6 it can be seen that in early lactation there was a tendency for the concentration of plasma free fatty acids to drop during cold exposure whereas in mid-lactation levels rose slightly. It may also be significant that exposure to the moderate cold environment during early lactation leads to the highest increase in milk fat levels.

In Section 3 an attempt was made, by calculation, to examine the interaction of energy balance with stage of lactation during cold exposure. In mid-lactation, when milk energy yielded was at its peak, a reduction in food intake lead to a loss in body weight but no further decline in milk yield during cold stress. This result implies that at that stage of lactation the mammary gland has a priority demand for the substrates for milk production and that body reserves are sufficient to meet the requirements of thermogenesis. In early lactation (when goats are more likely to encounter cold weather) a concurrent food shortage may lead to a significant decrease in milk yield.

Changes in milk and plasma composition may indicate the mechanisms involved in the response of the mammary gland to cold stress.

The ionic composition of the milk was only slightly affected by exposure to the cold environments. The ratio of sodium to potassium was maintained at the normal 1:3 level although the concentration of both cations was reduced. This suggests that there was no transport of material into milk via the intercellular route.

The concentration of lactose in milk was found to rise significantly during cold exposure in this study. If lactose is operating as the major osmole in milk, water should be drawn into the milk to maintain lactose at a constant concentration. As the volume of milk secreted was reduced by cold stress and lactose concentration in that milk increased, it appears that the rate of lactose synthesis and secretion was maintained. The results from Section 5, showing no significant changes in milk glucose, galactose or NDP (the precursors and product of lactose synthesis) concentration, it appears that lactose synthesis is unaffected. The difference in concentration of lactose may be the result of decreased water movement into milk as a result of a rise in plasma osmolality. Fluid balance was not measured in this study, in order to minimize handling stress on the animals but the haematocrit readings did show significant increases on cold exposure suggesting a reduced plasma volume. It is possible that drinking rate was reduced during cold expsoure as has been found by other workers (Thomson, 1978; Fregly, Kaplan, Brown, Nelson and Tyler, 1976) as ice sometimes formed in the water buckets within the precision chamber. Also the water buckets were not of the automatic refiller type as used in the goathouse.

Levels of milk citrate found in the goat's milk in this study were very variable but no significant effects were found on cold exposure. The ratio of milk citrate to 2-oxo-glutarate which has been found to rise in starved animals (Chaiyabutr, 1980) indicating an increased rate of de novo synthesis of fatty acids, was unaffected as could be expected if fat mobilization and oxidation are paramount. The decrease in concentration of phosphoenol pyruvate in milk found during cold exposure, indicates a decrease in the rate of oxidation via the pentose phosphate pathway (Chaiyabutr et al., 1981) and would lead to a decreased rate of fatty acid synthesis by limiting the supply of NADPH.

Plasma cortisol, which is thought to be essential to the maintenance of lactation after adrenalectomy (Cowie and Tindal, 1958) did not rise during exposure to the cold environments. In previous studies plasma cortisol has risen in cold stressed lactating goats (Thomson, 1978), and in cold stressed newborn calves (Khan, Dickson and Meyers, 1970). Plasma cortisol levels have also been shown to rise in human subjects exposed to an acute cold stress (Suzuki, 1972) and in animals exposed to other forms of stress (neonatal lambs - maternal separation and handling, Moberg and Wood, 1981; mice exposed to electric fields, Hackman and Graves, 1981). In vivo administration of corticosteroids has been shown to inhibit milk yield (Brush, 1960; Head, Thatcher, Wilcox and Bachman, 1976). In the present study there were increases of around 100% during cold exposure but these increases were not statistically significant. The smaller changes in plasma cortisol than found by Thomson (1978) may be a reflection of the lower degree of stress (cold or other).

As stated previously no calorimetric or cardiovascular measurements were made during this series of experiments, but the visible response of the animals to this level of cold stress was piloerection and shivering. Appetite was maintained in the goats studied, and only during exposure to the cold and wet treatment did the animals' behaviour alter. Body weight loss occurred during early lactation (as would normally occur) and when food intake was restricted at peak milk yield. Milk yield was most significantly reduced during exposure to the 'moderate cold' conditions during mid-lactation. It is possible that had extra food been eaten by the goats, milk yield would have been maintained.

The changes in milk composition studied indicate that lactose synthesis and secretion were maintained during cold stress and the

epithelial structure of the secretory tissue was not disrupted. Fat levels were raised during cold exposure, especially in early lactation, although detailed analysis of milk fat composition was not carried out.

The absence of significant increases in plasma cortisol when milk yield was reduced suggests that the reduction is due to the cold environment rather than non-specific stress. The increased levels of plasma free fatty acids during exposure to the cold and wet environment may be a better indicator of stress.

It is apparent that the lactating goats used for this particular study were able to withstand the described environmental conditions for three days; whilst the volume of milk secreted dropped to 81% of the previous level, milk energy yielded was unaffected.

The work reported in this thesis suggests that a sudden short term exposure to a cold environment of lactating goats previously kept in favourable conditions, will lead to a slight depletion of body reserves and the production of a slightly more concentrated milk. There appear to be no specific effects in the mammary gland; the reduced water movement into the milk is probably related to the dehydrating effects of the environment.

Unfortunately, facilities did not exist for a more severe cold environment to be studied, since in order to study further the mechanism by which the effect of cold is exerted a larger and reproducible effect on mammary function is needed. However, cold per se is not the only problem since the animal's previous thermal and nutritional histories as well as the present state of the animal's reserves are, as suggested, important factors to be taken into account. The problem is not simply one of looking at the effects of cold stress on mammary function in isolation.

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APPENDIX 1

TABLE 1 EFFECT OF CLIMATIC CONDITIONS ON THE CONCENTRATION OF SODIUM IN GOATS MILK (mean ± SE for one gland in mmol/l)

ENVIRONMENT		CONTROL	MILD COLD	MODERATE COLD	COLD & WET
	n	4†	6	5	4
DAY 1	PM	14.5 ± 0.09(3)	15.6 ± 0.54	16.2 ± 1.57	18.3 ± 1.49
	AM	14.4 ± 0.45(3)	15.2 ± 0.59	15.5 ± 1.09	17.7 ± 1.39
2	PM	14.8 ± 0.51	15.2 ± 0.56	16.2 ± 1.79	18.2 ± 1.53
	AM	15.4 ± 0.72	14.9 ± 0.56	15.6 ± 1.24	17.7 ± 1.14
3	PM	14.9 ± 0.15[a]	15.5 ± 0.79	16.9 ± 1.74	18.2 ± 1.47
	AM	14.4 ± 0.45	14.5 ± 0.55	15.7 ± 1.19	18.0 ± 1.58
4	PM	14.0 ± 0.60	14.5 ± 0.44	16.8 ± 1.94	18.0 ± 1.55
	AM	14.8 ± 1.06	14.7 ± 0.66	17.1 ± 2.41	17.9 ± 1.82
5	PM	14.3 ± 0.51	14.8 ± 0.72	16.3 ± 1.42	18.0 ± 2.02
	AM	14.8 ± 1.30(2)	14.8 ± 0.62	16.0 ± 1.24	18.0 ± 1.92
6	PM	14.0 ± 0.30(2)	14.7 ± 0.58	15.6 ± 0.79	18.9 ± 1.97
	AM	20.1 ± 5.06(2)	14.6 ± 0.12	15.3 ± 0.99	17.6 ± 1.27

(† - n = 4 except where indicated in brackets;

a - figures within boxes represent results obtained during exposure to test conditions)

TABLE 2 EFFECT OF CLIMATIC CONDITIONS ON THE CONCENTRATION OF POTASSIUM IN GOATS MILK (mean ± SE for one gland in mmol/l)

ENVIRONMENT		CONTROL	MILD COLD	MODERATE COLD	COLD & WET
	n	4 †	6	5	4
DAY 1	PM	52.8 ± 2.71(3)	45.3 ± 1.08	48.2 ± 0.84	48.1 ± 2.33
	AM	53.0 ± 3.25(3)	47.8 ± 1.43	48.8 ± 1.24	47.4 ± 2.54
2	PM	54.3 ± 3.18	46.6 ± 1.33	48.6 ± 1.69	47.3 ± 2.38
	AM	55.2 ± 2.85	47.4 ± 1.53	49.9 ± 1.22	48.7 ± 2.16
3	PM	53.9 ± 2.87[a]	45.8 ± 1.57	49.0 ± 0.83	48.4 ± 1.99
	AM	53.4 ± 2.65	46.3 ± 1.58	49.3 ± 1.10	48.4 ± 1.91
4	PM	52.0 ± 2.69	45.9 ± 1.45	48.4 ± 0.27	47.9 ± 1.99
	AM	52.6 ± 2.80	44.7 ± 1.46	46.7 ± 0.69	47.5 ± 2.41
5	PM	52.7 ± 3.29	43.8 ± 0.73	45.9 ± 0.63	45.9 ± 2.18
	AM	58.4 ± 3.16(2)	45.6 ± 1.00	48.4 ± 1.10	46.8 ± 2.48
6	PM	58.1 ± 2.61(2)	43.9 ± 1.00	47.8 ± 1.23	45.3 ± 2.37
	AM	56.0 ± 4.81(2)	46.0 ± 1.52	49.1 ± 1.50	48.8 ± 2.26

(† - n = 4 except as indicated in brackets;

a - figures within boxes represent results obtained during exposure to test conditions)

TABLE 3 EFFECT OF CLIMATIC CONDITIONS ON THE CONCENTRATION OF CHLORIDE IN GOATS MILK (mean ± SE for one gland in mmol/l)

ENVIRONMENT		CONTROL	MILD COLD	MODERATE COLD	COLD & WET
	n	4†	6	5	4
DAY 1	PM	41.9 ± 1.21(3)	47.8 ± 1.50	51.5 ± 2.08	45.4 ± 1.72
	AM	42.1 ± 0.86(3)	49.6 ± 1.06	51.9 ± 4.52	44.9 ± 1.70
2	PM	45.5 ± 3.55	47.5 ± 1.20	51.7 ± 2.23	45.4 ± 1.64
	AM	46.3 ± 3.29	48.3 ± 1.13	52.4 ± 1.90	45.6 ± 1.58
3	PM	44.7 ± 2.62[a]	47.6 ± 1.56	53.2 ± 2.01	45.4 ± 1.71
	AM	44.2 ± 2.71	46.8 ± 1.17	51.8 ± 2.03	45.3 ± 2.32
4	PM	43.9 ± 2.36	46.2 ± 1.38	51.8 ± 2.04	44.5 ± 1.70
	AM	44.7 ± 3.00	46.3 ± 1.30	50.7 ± 1.81	43.5 ± 2.43
5	PM	44.4 ± 2.89	45.0 ± 1.26	48.7 ± 1.55	42.7 ± 2.09
	AM	49.1 ± 4.31(2)	46.1 ± 1.34	48.6 ± 1.94	42.4 ± 2.23
6	PM	46.8 ± 2.81(2)	44.6 ± 1.45	47.0 ± 2.06	42.9 ± 2.01
	AM	51.2 ± 1.40(2)	45.2 ± 1.98	46.2 ± 1.97	42.3 ± 1.78

(† - n = 4 except as indicated in brackets;

a - figures within boxes represent results obtained during exposure to test conditions)

TABLE 4 EFFECT OF CLIMATIC CONDITIONS ON THE CONCENTRATION OF LACTOSE IN GOATS MILK (mean ± SE for one gland in mmol/l)

ENVIRONMENT		CONTROL	MILD COLD	MODERATE COLD	COLD & WET
	n	4†	6	5	4
DAY 1	PM	130.7 ± 3.92(3)	136.9 ± 1.63	132.5 ± 3.75	132.4 ± 4.09
	AM	131.8 ± 4.22(3)	136.0 ± 1.59	131.1 ± 3.48	132.0 ± 4.40
2	PM	127.4 ± 5.74	137.0 ± 1.08	133.5 ± 3.69	133.5 ± 4.12
	AM	128.1 ± 4.98	136.3 ± 1.75	131.1 ± 3.14	131.1 ± 3.27
3	PM	131.9 ± 4.55[a]	138.2 ± 2.32	132.8 ± 3.66	131.8 ± 3.23
	AM	130.4 ± 5.47	136.9 ± 1.84	134.1 ± 3.24	132.8 ± 4.15
4	PM	129.3 ± 4.13	142.6 ± 1.92	139.7 ± 3.42	136.3 ± 3.36
	AM	130.3 ± 5.79	142.5 ± 2.11	139.0 ± 4.67	136.4 ± 5.44
5	PM	130.2 ± 5.49	143.5 ± 2.76	141.2 ± 3.62	136.7 ± 4.59
	AM	119.5 ± 3.81(2)	143.1 ± 2.96	138.8 ± 4.26	137.7 ± 5.10
6	PM	123.3 ± 3.81(2)	143.5 ± 2.70	138.8 ± 3.89	131.9 ± 4.17
	AM	117.3 ± 0.65(2)	144.2 ± 1.83	138.8 ± 3.90	133.6 ± 3.60

(† - n = 4 except as indicated in brackets;

a - figures within boxes represent results obtained during exposure to the test conditions)

TABLE 5 EFFECT OF CLIMATIC CONDITIONS ON THE CONCENTRATION OF CITRATE IN GOATS MILK (mean ± SE for one gland in mg/100 ml)

ENVIRONMENT		CONTROL	MILD COLD	MODERATE COLD	COLD & WET
	n	4†	5	5	4
DAY 1	PM	48.4 ± 12.35(3)	118.9 ± 7.72	83.2 ± 3.97	72.9 ± 4.54
	AM	56.4 ± 4.95(3)	116.8 ± 7.72	78.1 ± 2.87	69.2 ± 4.43
2	PM	66.6 ± 11.89	130.0 ± 7.17	83.8 ± 3.71	71.7 ± 2.99
	AM	58.6 ± 6.24	122.6 ± 5.89	81.7 ± 3.91	70.4 ± 4.28
3	PM	74.3 ± 6.28[a]	129.9 ± 11.91	79.6 ± 4.89	63.4 ± 6.84
	AM	70.7 ± 7.80	127.0 ± 7.05	81.1 ± 1.32	64.4 ± 6.95
4	PM	91.5 ± 12.38	133.1 ± 9.17	76.9 ± 9.64	73.0 ± 2.33
	AM	74.8 ± 9.15	123.3 ± 9.07	84.2 ± 5.63	62.8 ± 5.75
5	PM	74.1 ± 8.62	127.3 ± 12.91	94.1 ± 6.85	65.0 ± 2.91
	AM	53.2 ± 7.60(2)	126.1 ± 10.37	87.5 ± 10.10	69.6 ± 6.21
6	PM	68.0 ± 4.45(2)	124.3 ± 11.41	85.9 ± 9.40	79.9 ± 4.62
	AM	59.3 ± 2.00(2)	122.0 ± 13.37	80.7 ± 6.67	72.9 ± 3.03

(† - n = 4 except as indicated in brackets;
a - figures within boxes represent results obtained during exposure to test conditions)

TABLE 6 EFFECT OF CLIMATIC CONDITIONS ON THE CONCENTRATION OF FAT IN GOATS MILK (mean ± SE for one gland in g/100 ml)

ENVIRONMENT		CONTROL	MILD COLD	MODERATE COLD	COLD & WET
	n	4†	6	5	4
DAY 1	PM	5.1 ± 0.27(3)	4.7 ± 0.57	4.0 ± 0.16	4.6 ± 0.12
	AM	4.1 ± 0.13(3)	3.7 ± 0.30	3.5 ± 0.25	4.3 ± 0.39
2	PM	5.2 ± 0.31	4.2 ± 0.55	4.2 ± 0.16	4.8 ± 0.12
	AM	3.9 ± 0.26	3.8 ± 0.33	3.7 ± 0.17	4.5 ± 0.32
3	PM	4.6 ± 0.12[a]	4.3 ± 0.43	4.0 ± 0.16	4.8 ± 0.31
	AM	4.0 ± 0.14	3.9 ± 0.52	3.6 ± 0.22	4.2 ± 0.12
4	PM	5.2 ± 0.37	4.9 ± 0.49	4.2 ± 0.16	4.7 ± 0.35
	AM	4.2 ± 0.25	4.1 ± 0.46	3.7 ± 0.44	4.3 ± 0.31
5	PM	4.9 ± 0.71	5.0 ± 0.48	4.8 ± 0.25	5.2 ± 0.32
	AM	3.9 ± 0.75(2)	4.3 ± 0.53	4.0 ± 0.39	4.7 ± 0.11
6	PM	4.8 ± 0.84(2)	5.1 ± 0.51	4.5 ± 0.23	6.3 ± 0.36
	AM	3.9 ± 0.56(2)	4.5 ± 0.68	4.0 ± 0.38	4.8 ± 0.16

(† - n = 4 except as indicated in brackets;

a - figures within boxes represent results obtained during exposure to test conditions)

APPENDIX 2

TABLE 1 EFFECT OF EXPOSURE TO THE MODERATE COLD ENVIRONMENT IN 3 STAGES OF LACTATION ON SODIUM CONCENTRATION IN GOATS MILK (mean ± SE for one gland in mmol/l)

STAGE OF LACTATION			EARLY	MID	LATE
		n	8	5	5
DAY 1		PM	14.7 ± 1.15	16.2 ± 1.57	16.3 ± 1.41
		AM	13.5 ± 0.53	15.5 ± 1.09	15.3 ± 0.99
	2	PM	13.9 ± 0.47	16.2 ± 1.79	15.0 ± 1.27
		AM	14.0 ± 0.67	15.6 ± 1.24	14.9 ± 0.80
	3	PM	14.5 ± 0.66	16.9 ± 1.74	15.8 ± 1.49
		AM	13.7 ± 0.52	15.7 ± 1.19	15.6 ± 1.30
	4	PM	13.8 ± 0.53[a]	16.8 ± 1.94	15.1 ± 1.13
		AM	14.0 ± 0.76	17.1 ± 2.41	15.2 ± 1.02
	5	PM	13.8 ± 0.58	16.3 ± 1.42	17.2 ± 2.39
		AM	12.9 ± 0.28	16.0 ± 1.24	14.9 ± 0.91
	6	PM	13.1 ± 0.34	15.6 ± 0.79	14.7 ± 0.94
		AM	12.3 ± 0.38	15.3 ± 0.99	15.0 ± 0.93

(a - figures within boxes represent results obtained during exposure to the cold environment)

TABLE 2. EFFECT OF EXPOSURE TO THE MODERATE COLD ENVIRONMENT IN 3 STAGES OF LACTATION ON POTASSIUM CONCENTRATION IN GOATS MILK (mean ± SE for one gland in mmol/l)

STAGE OF LACTATION		EARLY	MID	LATE
	n	8	5	5
DAY 1	PM	43.0 ± 1.40	48.1 ± 1.51	47.5 ± 0.87
	AM	43.9 ± 1.43	49.0 ± 2.24	47.2 ± 1.21
2	PM	42.9 ± 1.29	48.8 ± 1.36	47.2 ± 1.07
	AM	45.3 ± 1.80	49.5 ± 2.13	47.7 ± 1.16
3	PM	43.7 ± 1.35	47.8 ± 0.58	45.7 ± 1.61
	AM	44.0 ± 1.48	49.7 ± 1.86	46.2 ± 1.64
4	PM	43.1 ± 1.42[a]	48.7 ± 0.10	47.7 ± 1.33
	AM	43.5 ± 1.51	46.3 ± 1.13	46.4 ± 1.37
5	PM	42.6 ± 1.68	46.1 ± 0.94	44.9 ± 2.30
	AM	43.9 ± 1.46	48.8 ± 1.81	46.6 ± 1.39
6	PM	42.3 ± 1.78	48.6 ± 1.87	46.0 ± 1.32
	AM	43.5 ± 1.70	48.8 ± 2.53	46.9 ± 1.58

(a - figures within boxes represent results obtained during exposure to the cold environment)

TABLE 3 EFFECT OF EXPOSURE TO THE MODERATE COLD ENVIRONMENT IN 3 STAGES OF LACTATION ON CHLORIDE CONCENTRATION IN GOATS MILK (mean ± SE for one gland in mmol/l)

STAGE OF LACTATION		EARLY	MID	LATE
	n	8	5	5
DAY 1	PM	39.9 ± 1.47	53.7 ± 2.38	41.7 ± 1.06
	AM	41.0 ± 1.44	54.6 ± 1.94	42.2 ± 1.12
2	PM	40.0 ± 1.51	54.5 ± 2.36	41.8 ± 0.95
	AM	41.6 ± 1.44	54.9 ± 2.04	43.2 ± 0.99
3	PM	40.5 ± 1.45	55.5 ± 2.38	44.0 ± 0.78
	AM	39.6 ± 1.51	54.4 ± 1.84	43.5 ± 0.86
4	PM	37.9 ± 1.62[a]	54.1 ± 2.39	43.3 ± 0.79
	AM	38.4 ± 1.63	52.6 ± 2.21	42.8 ± 1.25
5	PM	37.5 ± 1.40	50.4 ± 1.79	41.0 ± 1.12
	AM	37.5 ± 1.48	50.4 ± 1.73	40.4 ± 2.23
6	PM	36.2 ± 1.48	48.8 ± 1.46	39.2 ± 1.34
	AM	36.4 ± 1.74	47.8 ± 1.73	39.5 ± 1.13

(a - figures within boxes represent results obtained during exposure to the cold environment)

TABLE 4 EFFECT OF EXPOSURE TO THE MODERATE COLD ENVIRONMENT IN 3 STAGES OF LACTATION ON LACTOSE CONCENTRATION IN GOATS MILK (mean ± SE for one gland in mmol/l)

STAGE OF LACTATION		EARLY	MID	LATE
	n	8	5	5
DAY 1	PM	145.7 ± 1.66	128.8 ± 5.40	133.3 ± 0.83
	AM	144.0 ± 1.45	127.3 ± 4.60	134.0 ± 1.30
2	PM	145.1 ± 1.62	130.1 ± 5.50	133.7 ± 0.91
	AM	143.7 ± 1.80	128.5 ± 4.90	136.0 ± 1.78
3	PM	145.7 ± 1.87	129.3 ± 5.40	136.5 ± 2.50
	AM	146.6 ± 2.38	130.9 ± 4.65	138.2 ± 2.05
4	PM	148.6 ± 2.87[a]	137.1 ± 6.21	140.8 ± 1.95
	AM	148.6 ± 1.98	133.8 ± 6.19	138.5 ± 1.15
5	PM	150.9 ± 2.46	139.9 ± 6.01	139.8 ± 1.16
	AM	151.6 ± 2.22	134.7 ± 6.09	140.6 ± 1.09
6	PM	152.2 ± 3.24	136.2 ± 5.87	138.8 ± 1.75
	AM	151.6 ± 2.76	136.2 ± 6.36	139.3 ± 1.16

(a - figures within boxes represent results obtained during exposure to the cold environment)

TABLE 5 EFFECT OF EXPOSURE TO THE MODERATE COLD ENVIRONMENT IN 3 STAGES OF LACTATION ON CITRATE CONCENTRATION IN GOATS MILK

(mean ± SE for one gland in mg/100 ml)

STAGE OF LACTATION		EARLY	MID	LATE
	n	8	5	5
DAY 1	PM	174.3 ± 16.3	81.8 ± 7.02	57.1 ± 6.78
	AM	161.7 ± 20.4	74.6 ± 3.52	68.2 ± 8.54
2	PM	157.8 ± 15.2	83.8 ± 6.30	64.6 ± 8.54
	AM	148.2 ± 15.3	78.1 ± 5.90	63.8 ± 8.60
3	PM	166.1 ± 22.5	77.8 ± 8.68	67.0 ± 8.57
	AM	173.5 ± 25.6	82.1 ± 1.27	71.6 ± 6.14
4	PM	174.4 ± 23.5[a]	74.2 ± 17.28	72.0 ± 7.23
	AM	150.5 ± 24.5	77.93± 6.64	69.7 ± 8.54
5	PM	167.0 ± 17.8	90.2 ± 3.35	63.3 ± 9.62
	AM	158.4 ± 21.6	78.2 ± 7.52	69.5 ± 6.98
6	PM	178.5 ± 27.3	79.3 ± 4.97	73.8 ± 6.71
	AM	172.6 ± 22.4	78.5 ± 8.32	74.3 ± 5.04

(a - figures within boxes represent results obtained during exposure to the cold environment)

TABLE 6 EFFECT OF EXPOSURE TO THE MODERATE COLD ENVIRONMENT IN 3 STAGES OF LACTATION ON FAT LEVELS IN GOATS MILK

(mean ± SE for one gland in g/100 ml)

STAGE OF LACTATION		EARLY	MID	LATE
	n	8	5	5
DAY 1	PM	3.9 ± 0.29	4.0 ± 0.16	4.9 ± 0.82
	AM	3.4 ± 0.26	3.5 ± 0.25	3.6 ± 0.39
2	PM	4.2 ± 0.34	4.2 ± 0.16	4.9 ± 0.53
	AM	3.3 ± 0.24	3.7 ± 0.17	4.3 ± 0.39
3	PM	4.1 ± 0.32	4.0 ± 0.16	5.0 ± 0.82
	AM	4.4 ± 0.31	3.6 ± 0.22	4.6 ± 0.70
4	PM	4.9 ± 0.31[a]	4.2 ± 0.16	4.5 ± 0.50
	AM	4.6 ± 0.38	3.7 ± 0.44	4.4 ± 0.25
5	PM	5.2 ± 0.27	4.8 ± 0.25	5.4 ± 0.75
	AM	4.8 ± 0.36	4.0 ± 0.39	4.8 ± 0.23
6	PM	5.5 ± 0.41	4.5 ± 0.23	5.5 ± 0.49
	AM	4.6 ± 0.29	4.0 ± 0.38	4.3 ± 0.38

(a - figures within boxes represent results obtained during exposure to the cold environment)

APPENDIX 3

TABLE 1 INDIVIDUAL ENERGY BALANCE (MJ/DAY) FOR LACTATING GOATS EXPOSED TO THE 'MODERATE COLD' ENVIRONMENT

GROUP	GOAT	WEEK OF LACTATION	E_{INTAKE}	E_{MILK}		'E'		MEAN % DECREASE IN MILK YIELD
				CONTROL	COLD	CONTROL	COLD	
	G16	3	24.84	8.2	13.8	13.3	5.13	-4.3
	505	3	24.84	10.3	9.9	10.13	10.70	-19.6
1	G24	3	24.84	8.4	10.0	12.84	10.55	-0.3
	G22	4	24.84	8.6	10.0	12.55	10.55	-2.7
	G35	5	24.84	10.6	9.7	9.7	10.98	-5.4
	E19	19	19.84	6.1	5.4	11.13	12.13	-17.1
2	G16	14	19.84	6.3	7.8	10.84	8.70	-8.9
	505	9	19.84	6.9	6.7	9.98	10.27	-19.1
	G24	17	19.84	7.1	6.5	9.70	10.55	-13.9
	W5	17	27.34	8.2	8.0	15.63	15.91	-8.4
	775	20	27.34	4.8	4.2	20.48	21.34	-17.5
3	G34	21	27.34	6.5	6.0	18.05	18.77	-8.2
	G24	22	24.84	7.0	5.5	14.84	16.98	-14.3
	G32	22	24.84	5.9	5.4	16.41	17.13	-23.7
	G34	28	27.34	4.9	5.4	20.34	19.63	-2.7
	B24	28	27.34	4.6	3.9	21.19	21.77	-15.5
4	E19	30	27.34	6.3	5.7	18.34	19.20	-0.4
	786	30	27.34	5.7	5.6	19.20	19.34	-4.7
	G22	33	27.34	4.3	4.6	21.17	19.81	-5.7

TABLE 2 CHANGES IN BODY WEIGHT (kg) ON EXPOSURE OF LACTATING GOATS TO THE 'MODERATE COLD' ENVIRONMENT

GROUP	GOAT	WEIGHT	
		before cold treatment	after cold treatment
1. EARLY LACTATION	G16	61.0	56.5
	505	62.5	64.0
	G24	57.5	53.4
	G34	64.0	61.0
2. MID-LACTATION/ REDUCED DIET	E19	69.0	66.5
	G16	53.5	47.4
	505	58.0	53.0
	G24	53.0	47.6
3. MID-LACTATION	W5	55.0	56.0
	775	53.0	52.5
	G34	62.0	64.0
4. LATE LACTATION	G22	61.0	63.0

APPENDIX 4

EFFECT OF VARIOUS CLIMATIC CONDITIONS ON RATE OF MILK SECRETION IN THE GOAT (mean for one gland in ml/h)

(see Chapter 3, Section 5 for details)

		GROUP 1	GROUP 2	GROUP 3	GROUP 4
	n	3	3	4	2
DAY 1	PM	71	-	47	60
	AM	69	72	43	59
2	PM	74	51	52	56
	AM	70	68	42	41
3	PM	78	59	55	42
	AM	69	66	47	34
4	PM	48[a]	58	52	39
	AM	56	52	42	42
5	PM	63	62	54	36
	AM	60	52	43	41
6	PM	66	62	51	40
	AM	57	47	42	43

(a - figures within boxes represent results obtained during exposure to the test conditions)

APPENDIX 5

TABLE 1 EFFECT OF VARIOUS COLD ENVIRONMENTS ON THE CONCENTRATION OF FREE PALMITIC ACID IN THE PLASMA OF LACTATING GOATS

(mean ± SE for arterial plasma in μmol/l)

ENVIRONMENT		MODERATE COLD	MODERATE COLD	COLD & WET
STAGE OF LACTATION		EARLY	MID	MID
	n	7	5	4
DAY	1	192.06 ± 33.48	56.32 ± 7.49	25.74 ± 3.42
	2	175.69 ± 34.47[a]	50.96 ± 9.16	44.94 ± 2.04
	3	158.55 ± 15.69	69.89 ± 8.10	60.45 ± 5.59
	4	161.31 ± 15.84	76.04 ± 11.59	26.61 ± 1.79
	5	174.16 ± 21.57	44.82 ± 2.69	-

(a - figures within boxes represent results obtained during exposure to test conditions)

TABLE 2 EFFECT OF VARIOUS COLD ENVIRONMENTS ON THE CONCENTRATION OF FREE PALMITOLEIC ACID IN THE PLASMA OF LACTATING GOATS

(mean ± SE for arterial plasma in μmol/l).

ENVIRONMENT		MODERATE COLD	MODERATE COLD	COLD & WET
STAGE OF LACTATION		EARLY	MID	MID
	n	7	5	4
DAY	1	9.94 ± 1.64	4.20 ± 1.16	1.76 ± 0.04
	2	8.96 ± 1.37[a]	3.08 ± 0.53	3.17 ± 0.23
	3	7.48 ± 1.26	4.54 ± 1.38	4.26 ± 0.63
	4	9.33 ± 2.49	5.03 ± 0.87	1.69 ± 0.02
	5	12.01 ± 1.98	2.93 ± 0.34	-

(a - figures within boxes represent results obtained during exposure to test conditions)

TABLE 3 EFFECT OF VARIOUS COLD ENVIRONMENTS ON THE CONCENTRATION OF FREE STEARIC ACID IN THE PLASMA OF LACTATING GOATS

(mean ± SE for arterial plasma in umol/l)

ENVIRONMENT		MODERATE COLD	MODERATE COLD	COLD & WET
STAGE OF LACTATION		EARLY	MID	MID
	n	7	5	4
DAY	1	332.46 ± 68.36	95.97 ± 12.72	43.15 ± 10.34
	2	295.88 ± 48.39[a]	91.49 ± 19.96	102.45 ± 10.57
	3	269.62 ± 30.12	122.42 ± 18.33	145.51 ± 10.11
	4	275.43 ± 28.75	146.34 ± 24.80	43.75 ± 3.92
	5	246.87 ± 27.30	86.11 ± 8.82	-

(a - figures within boxes represent results obtained during exposure to test conditions)

TABLE 4 EFFECT OF VARIOUS COLD ENVIRONMENTS ON THE CONCENTRATION OF FREE OLEIC ACID IN THE PLASMA OF LACTATING GOATS

(mean ± SE for arterial plasma in μmol/l)

ENVIRONMENT		MODERATE COLD	MODERATE COLD	COLD & WET
STAGE OF LACTATION		EARLY	MID	MID
	n	7	5	4
DAY	1	334.10 ± 66.92	86.09 ± 15.75	31.89 ± 7.88
	2	282.89 ± 50.46[a]	71.61 ± 15.16	71.01 ± 3.78
	3	245.34 ± 25.27	102.01 ± 14.30	95.02 ± 8.45
	4	264.67 ± 28.01	116.77 ± 19.09	35.67 ± 0.78
	5	306.96 ± 39.88	69.00 ± 6.24	-

(a - figures within boxes represent results obtained during exposure to test conditions)

TABLE 5 EFFECTS OF VARIOUS COLD ENVIRONMENTS ON THE CONCENTRATION OF FREE LINOLEIC ACID IN THE PLASMA OF LACTATING GOATS

(mean ± SE for arterial plasma in μmol/l)

ENVIRONMENT		MODERATE COLD	COLD & WET
STAGE OF LACTATION		EARLY	MID
	n	7	4
DAY	1	36.51 ± 6.17	10.57 ± 1.58
	2	34.28 ± 4.59[a]	14.57 ± 1.24
	3	30.40 ± 3.32	17.53 ± 3.09
	4	30.91 ± 3.26	11.12 ± 0.54
	5	31.84 ± 3.04	-

(a - figures within boxes represent results obtained during exposure to test conditions)