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RESPONSES TO SOMATOSTATIN-14 IN COLD-EXPOSED SHEEP

BY

RIAL AUBREY CHRISTENSEN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

IN

ANIMAL PHYSIOLOGY

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

FALL 1988

ISBN 0-315-45542-X

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: RIAL AUBREY CHRISTENSEN

TITLE OF THESIS: RESPONSES TO SOMATOSTATIN-14 IN COLD-EXPOSED SHEEP

DEGREE: MASTER OF SCIENCE

YEAR THIS DEGREE GRANTED: FALL 1988

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled RESPONSES TO SOMATOSTATIN-14 IN COLD-EXPOSED SHEEP submitted by RIAL AUBREY CHRISTENSEN in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ANIMAL PHYSIOLOGY.

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Supervisor

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Date: 10th August 1988

ABSTRACT

The effects of somatostatin-14 (SS-14) on homeothermy, gastrointestinal motility, and plasma hormone and metabolite concentrations were studied in acutely (0°C for 120 min) and chronically cold-exposed (32-52 days at -0.9°C) sheep. In addition, responses to feeding and to an injection of SS-14 during chronic cold exposure were determined. SS-14 decreased ($P< 0.05$) oxygen consumption in both the thermoneutral (TN) and chronic cold (CC) environments but had no effect ($P> 0.05$) on the decreases ($P< 0.05$) in hip, leg, and rectal temperatures in response to chronic or acute cold exposure. Continuous infusion of SS-14 (6.7 ng min^{-1} kg^{-1}) had no effect on reticular or duodenal motility during CC exposure. SS-14 (8.9 ng min^{-1} kg^{-1}) had no effect on duodenal motility in the acute cold experiment, while higher doses (18.4 and 37.3 ng min^{-1} kg^{-1}) decreased ($P< 0.05$) the duration of the MMC. The half-life ($P<0.05$) of somatostatin was increased in the cold environment, resulting in a slower turnover rate ($P<0.004$). This was offset by a decrease ($P< 0.01$) in secretion rate in cold adapted sheep, which resulted in no differences in basal plasma somatostatin-like immunoreactivity (SLI) across environments. A pulse injection of SS-14 increased ($P< 0.05$) free fatty acid concentrations, but not glucose. Minimal values for both insulin and glucagon concentrations occurred 5 min after the pulse injection of SS-14. By 30 min postinjection, insulin ($P<0.09$) and glucagon values were higher than ($P<0.001$) the suppressed 5 min sample. Over the entire day insulin concentration was higher ($P< 0.05$) in the

TN environment. Feeding had no effect on plasma somatostatin or glucagon concentrations, but increased insulin and glucose concentrations, and decreased free fatty acid and growth hormone concentrations. The decline in growth hormone concentration was larger in the TN environment. Plasma growth hormone, glucagon and insulin concentrations were not altered by infusion of somatostatin during either acute or chronic cold exposure. In conclusion, cold exposure alters the plasma somatostatin kinetics and may alter plasma substrate availability for metabolism.

ACKNOWLEDGEMENTS

This research was supported by grants provided by Natural Sciences and Engineering Research Council of Canada and Alberta Farming for the Future program.

The author would like to express his appreciation to Drs. R.J. Christopherson, J.J. Kennelly, and G. de Boer for their thoughtful guidance and assistance throughout this study. I would also like to thank Dr. G. Mears for his friendship and encouragement over the past several years and for his supervisory participation in this study.

I wish to thank Mr. Jack Francis and Ms. Charlaine Shellian for their assistance in the management of the animals for these experiments and to Ms. Angela Bork, Mrs. Brenda Kozak, Mr. Brain Dolan, and Mr. Paul Gregory for their help during sampling periods.

Sincere appreciation is also extended to Mr. Ray Weingardt for his assistance in statistical analysis of data and to Susan Brydon for her help in typing this manuscript. I would also like to thank all my colleagues and friends for their support and encouragement.

This thesis is dedicated to my parents and family for their love, concern, patience, and understanding.

To these people and the many others who have touched my life during the past several years - Thank you.

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After preparation for recording, lambs were left undisturbed for 90 min prior to the start of each experiment at 0900 h. Ten mL blood samples were drawn every 30 min starting at 0900 h. Blood sampling catheter patency was maintained between samples with heparinized (40 IU mL^{-1}) saline (0.9% NaCl) and catheter dead space was cleared by a 3 mL discard prior to each sample. Blood samples were immediately transferred into 16 x 100 mm glass tubes containing heparin (40 IU mL^{-1} of blood) and NaF (4% w/v, 15 μL per mL of blood), mixed by inversion, and kept refrigerated until centrifuged for 15 min at 2280 x g. Aliquots for somatostatin and glucagon contained Trasylol (aprotinin, Miles Laboratories, Mississauga, Ontario; 500 IU mL^{-1} plasma).

Analytical Techniques:

Concentrations of plasma free fatty acids and glucose were determined by enzyme specific kits (acyl CoA synthetase, WAKO Pure Chemical Industry, Dallas, Texas; glucose oxidase, Sigma Chemical Co., Mississauga, Ontario). Somatostatin-like immunoreactivity (SLI), glucagon, insulin, and growth hormone concentrations were determined by double antibody radioimmunoassays as outlined by Christensen et al., (1988), Harris et al. (1979), and Mears et al., (1988). Tri-iodothyronine and thyroxine concentrations were determined by Coat-a-count kits (Intermedico, Markharm, Ontario). Samples were analyzed in a single assay for each of the hormone assays. Intra- and inter-assay coefficients of variation for somatostatin, glucagon, insulin, and growth hormone were 7.0%, 8.9, 8.3, and 10.2%; 11.0%, 11.1%, 11.0%, and 12.3% for samples averaging 0.097, 0.24, 2.19, and 13.53 ng mL^{-1}, respectively.

Statistical Analysis:

Data for hip, average leg, and rectal temperatures, metabolic parameters, and frequency of reticular contractions were averaged across preinfusion and infusion periods and were analyzed by analysis of variance where the main effects were treatment, phase (preinfusion vs infusion periods), environment, and animal as the error term (since group was not significant). Mean duration and number of contractions within the components of the migrating myoelectric complex (MMC) and for the MMC were tabulated within infusion period. Duration, number of contractions associated with each component of the MMC per MMC, and frequency of contraction of each of the MMC components (mean number of contractions associated with each component of the MMC/ mean duration of MMC) were analyzed by analysis of variance where the main effects (i.e. treatment and environment) were tested against main effect by sheep interaction. Treatment by environment interaction was tested against treatment by environment by sheep. Hormone and metabolite concentrations were analyzed by analysis of variance for a repeated measures design with the main effects being treatment, time, environment, and animal as the error term. Means were compared by Student-Newman-Keuls' multicomparison test (Steele and Torrie, 1980).

RESULTS

Rectal and Skin Temperature:

Chronic cold exposure decreased ($P < 0.001$) both hip and average leg skin temperatures, while having no effect on rectal temperature (Table III-1). SS-14 had no effect on rectal or skin temperatures.

Metabolic Parameters:

Oxygen consumption, carbon dioxide production, respiratory quotients, and heat production were similar for the saline and SS-14 treatment groups during the preinfusion period and, with the exception of respiratory quotient, these parameters decreased ($P< 0.05$) over the experimental recording period. Treatment comparisons were, therefore, made within the period of infusion. Chronic cold exposure increased ($P< 0.05$) oxygen consumption, carbon dioxide production, and heat production by 41.4%, 24.8%, and 41.4%, respectively (Table III-2). SS-14 infusion decreased ($P< 0.05$) oxygen consumption and heat production by a similar amount in both TN and CC environments (4.1% and 3.2%, respectively), while SS-14 had no effect on carbon dioxide production. Respiratory quotient was not significantly affected by either cold exposure or SS-14 infusion, although respiratory quotient was somewhat lower in the CC group.

Gastrointestinal Motility:

Frequency of reticular contractions was not significantly altered by chronic cold exposure (52 vs 59 number of contractions/h; TN vs CC, respectively SEM= 4), or SS-14 infusion (56 vs 55 number of contractions/h; saline vs SS, respectively SEM= 0.5).

SS-14 infusion had no effect on either the duration or number of contractions within an MMC or for any of its components. Cold exposure increased ($P< 0.05$) the total number of contractions within an MMC, which was a reflection of a nonsignificant increase in the number of contractions associated with both irregular spiking activity (ISA) and regular spiking activity (RSA).

Hormonal and Metabolite Concentrations:

Plasma somatostatin-like immunoreactivity concentration was elevated prior to infusion for animals receiving the SS-14 treatment in the TN environment in comparison to the other treatments, due to one animal having concentrations four times the mean value for any of the other animals. This resulted in a significant ($P< 0.05$) treatment, time, treatment by time, and treatment by environment by time interaction. The reason for the elevated SLI concentration is unclear. After removal of this animal from the analysis (n=4), SS-14 infusion increased ($P< 0.01$) plasma SLI concentration (0.296 vs 0.341 ng mL^{-1}; saline vs SS, respectively, SEM= 12.7) (Figure III-1), although by the end of the infusion period plasma SLI concentrations were similar across all treatments.

Growth hormone concentrations were not affected by SS-14 infusion or environmental temperature (Figure III-2).

Plasma insulin (Figure III-3) and glucagon concentrations were not affected by SS-14 infusion or environmental temperature. However, the average glucagon concentration for the 330 min blood sample was significantly ($P< 0.007$) different from the 30, 60 120 and 210 min samples. This was due to an increase in both treatments for the TN environment, thus resulting in a treatment by time interaction ($P< 0.008$) for glucagon (Figure III-4). There was no significant change in insulin:glucagon ratio as the result of either environmental temperature or SS-14 infusion (Figure III-5).

Plasma concentration of free fatty acids was significantly higher ($P< 0.004$) in the cold environment (Figure III-6), while there was a trend ($P<0.06$) for glucose concentration to be higher in the cold

environment (Figure III-7). The effects of SS-14 infusion on plasma free fatty acid levels were opposite across the two environments, thus producing a significant ($P< 0.05$) environment by treatment interaction (0.30, 0.33, 0.55, and 0.48 mM; saline-TN, SS-TN, saline-CC, and SS-CC, respectively SEM= 0.02). These differences became evident between 90 and 150 min after initiating the infusion (treatment by environment by time interaction $P< 0.008$) (Figure III-6). Overall glucose values decreased ($P< 0.05$) by the end of the experimental period, while overall glucose rose ($P< 0.05$) between 30-60 min after initiating the infusions. The latter was primarily observed in the saline infused lambs in the TN environment, although the increase was seen to some extent in all treatment by environment combinations (Figure III-7). SS-14 infusion had no effect on glucose concentration.

Overall basal tri-iodothyronine (T_3) concentration was elevated ($P< 0.02$) (122.4 vs 86.0 ng dL^{-1}; CC vs TN, SEM=6.8) during cold exposure, whereas thyroxine (T_4) concentration (10.7 vs 9.5 μg dL^{-1}; CC vs TN, SEM=0.4) was not significantly affected ($P< 0.09$) by environment. Overall, T_3 and T_4 plasma concentrations decreased ($P< 0.02$) during the day and represented a 25% and 6% decrease in T_3 and T_4 concentrations, respectively. A significant environment by time interaction resulted from a smaller decline ($P< 0.05$) in T_3 concentration across the entire day in cold adapted lambs (Figure III-8). SS-14 infusion decreased T_4 concentration across the entire day in both environments, while saline infusion produced a small transient rise for T_4 in the cold and thus resulted in a treatment by time interaction ($P< 0.05$) (Figure III-9).

DISCUSSION

Somatostatin infusion had no effect on rectal and skin temperature responses to environmental temperature, even though heat production decreased slightly in both environments. This indicates that on a short-term basis the sheep could maintain their body temperature in the cold despite the 3-4% decrease in metabolic rate.

Isolation of receptors for somatostatin, D-cells which release somatostatin, or somatostatinergic neurons has not been established in the ruminoreticulum. However, exogenous somatostatin at low doses did not seem to have any effect on the ruminoreticulum suggesting that somatostatin has no direct effect. Barry et al. (1985) also reported that prolonged continuous infusion somatostatin at physiological doses has almost no effect on rumen retention time, while somatostatin significantly increases retention time in the abomasal to caecal region.

Somatostatin infusion had no effect on the number of contractions within a migrating myoelectric complex or the duration of any of the components of the migrating myoelectric complex. The work of Barry et al. (1985) suggests that the dynamics of digesta movement through this region is altered by somatostatin. However, the rate of passage of digesta is the result of several factors including type and frequency of contraction waves, viscoelastic properties of chyme, and volume fluxes of the digesta (Meyers, 1987). Thus the type of diet and feeding practices may have lead to different results. It is also possible that at the low doses used in these studies, a longer period of infusion is required before the effects are evident on the lower digestive tract.

Somatostatin may have indirect effects on heat production and thermoregulation via the CNS and by inhibiting peripheral release of several metabolic hormones. Within the CNS, somatostatin is thought to inhibit sympathetic outflow from the brain to the adrenal medulla and thus reduce epinephrine release (Brown and Fisher, 1985). Although it is possible that somatostatin could cross the blood-brain barrier within regions of the hypothalamus (Ermisch et al., 1985), intravenous infusion of somatostatin probably indirectly modifies heat production by alteration of metabolic hormones. This has resulted in an alteration of free fatty acid mobilization and utilization at higher doses (Gray et al., 1980).

Large doses of somatostatin have been reported to inhibit TSH release from the pituitary, as well as T_3 and T_4 from the thyroid gland (see M^{c}Quillan, 1980). We found a decrease in both T_3 and T_4 levels across the experimental period, although a decline in T_3 was also found in the saline treated group, particularly in the TN environment. This may be indicative of a diurnal rhythm for thyroid hormones possibly in response to the increasing length of time after feeding. However, somatostatin infusion decreased T_4 concentration in both environments. Barry et al. (1985) also found similar results (i.e. the decline in T_3 concentration was not significant, while the decrease in T_4 levels was). This is in agreement with the finding that somatostatin immunoneutralization increases both basal and cold induced TSH release in rats (Ferland et al., 1976). These findings would imply that thyroid hormones may be important in the decrease in metabolic rate.

For the thermoneutral environment the results of this experiment were similar to Barry et al. (1985) who observed no effect of somatostatin infusion on growth hormone, insulin, glucagon, free fatty acids, and glucose. A similar rise in plasma somatostatin concentration during infusion was also observed, although the basal concentrations in this experiment were was about 5 times higher.

Chronic cold exposure increased free fatty acid levels while there was only a trend for glucose levels to increase. Plasma glucose concentrations are usually, but not always, increased during cold exposure (Sasaki and Weekes, 1986) a response which may depend on the type of diet and level of feed intake (Horton, 1981). Fasting has also been shown to alter the relative concentrations of free fatty acids and glucose (Karihaloo et al., 1970) and could help to explain the increase in free fatty acid concentrations in the cold environment rather than glucose, since our lambs had been without feed for 19-23 h. However, the respiratory quotient was not significantly altered in the cold environment in contrast to the findings of M^{c}Kay et al. (1974). This may indicate that free fatty acids were not preferentially oxidized in the cold environment.

Basal insulin concentration was not significantly reduced by cold exposure; an observation that is consistent with findings of Sasaki et al. (1982), but contrary to Christensen et al. (1988) and to the more generally observed response to cold (Sasaki and Weekes, 1986). Cold exposure had no significant effect on glucagon concentrations, although plasma glucagon concentrations increased in the thermoneutral environment by the end of the experiment, probably due to increased time after feed. Although basal concentrations of these hormones were

not altered, this does not preclude the possible alteration of the metabolic clearance rate or secretion rate of these hormones.

Plasma growth hormone was not affected by cold exposure. Possibly the cold stress was not severe enough, since more severe cold exposure elevates growth hormone concentration in sheep (Blom et al., 1976) and cattle (Olsen and Trenkle, 1973).

In summary, chronic cold exposure stimulated increased plasma free fatty acid and glucose concentrations to meet increased metabolic needs, though these changes could not be attributed to changes in plasma growth hormone, insulin, or glucagon concentration. Increasing plasma somatostatin concentration by continuous infusion, within the physiological range, decreased oxygen consumption in both environments, while having no effect on plasma concentration of free fatty acids or glucose. Body core and peripheral skin temperatures were not altered by somatostatin. Neither cold exposure or somatostatin infusion at low doses had an effect on either reticular or duodenal motility.

BIBLIOGRAPHY

Alexander, G. 1979. Cold Thermogenesis. Pages 46-155. *In* D. Robertshaw ed. Environmental Physiology III. University Park Press, Baltimore, Ma.

Barry, T.N., Faichney, G.J., and Redekopp, C. 1985. Gastro-intestinal Tract Function in Sheep Infused with Somatostatin. Aust. J. Biol. Sci. 38: 393-403.

Bell, A.W. 1980. Lipid Metabolism in Liver and Selected Tissues and in the Whole Body of Ruminant Animals. Prog. Lipid Res. 18: 117-164.

Blom, A.K., Hove, K., and Nedkvitne, J.J. 1976. Plasma Insulin and Growth Hormone Concentrations in Pregnant Sheep II: Post-absorptive Levels in Mid- and Late Pregnancy. Acta Endocrinologica 82: 553-560.

Brockman, R.P. and Laarveld, B. 1986. Hormonal Regulation of Metabolism in Ruminants; A Review. Livest. Prod. Sci. 14: 313-334.

Brown, M.R. and Fisher, L.A. 1984. Brain Peptide Regulation of Adrenal Epinephrine Secretion. Am. J. Physiol. 247 (Endocrinol. Metab. 10):

Ferland, L., Labrie, F., Jobin, M., Arimura, A., and Schally, A.V. 1976. Physiological Role of Somatostatin in the Control of Growth Hormone and Thyrotropin Secretion. Biochem. Biophys. Res. Commun. 68(1): 149-156.

Gray, D.E., Lickley, H.L.A., and Vranic, M. 1980. Physiologic Effects of Epinephrine on Glucose Turnover and Plasma Free Fatty Acid Concentrations Medicated Independently of Glcuagon. Diabetes 29: 600-608.

Harris, V., Faloona, G.R., and Unger, R.H. 1979. Glucagon. Page 643. *In* B.M. Jaffe and H.R. Behrman eds. Methods of Hormone Radioimmunoassay. 2nd ed. Academic Press Inc., New York, NY.

Horton, G.M.J. 1981. Responses of Shorn and Full-Fleeced Lambs Given Two Levels of Feed Intake and Exposed to Warm and Cold Temperatures. Am. J. Vet. Res. 42(12): 2151-2154.

Karihaloo, A.K., Webster, A.J.F., and Combs, W. 1970. Effects of Cold, Acute Starvation, and Pregnancy on Some Indices of Energy Metabolism in Lincoln and Southdown Sheep. Can. J. Anim. Sci. 50: 191-198.

Laarveld, B., Chaplin, R.K., and Kerr, D.E. 1986. Somatostatin Immunization and Growth of Lambs. Can. J. Anim. Sci. 66(1): 77-83.

McKay, D.G., Young, B.A., and Milligan, L.P. 1974. Energy Substrates for Cold Induced Thermogenesis. Pages 39-42. *In* K.H. Menke, H.-J., Lantzsch, and J.R. Reichl eds. 6th Sumposium on Energy Metabolism of Farm Animals. European Association for Animal Production Publ. 14. Universitat Hohenheim, Dokumentationsstelle, Stuttgart.

McLean, J.A. 1972. On the Calculation of Heat Production from Open-Circuit Calorimetric Measurements. Br. J. Nutr. 27: 597-600.

McQuillan, M.T. 1980. Somatostatin. Volume 2. Pages 52-53. *In* Annual Research Reviews. Eden Press Inc. Westmount, Quebec.

Mears, G.J., Vesely, J.A., and Cheng, K.-J. 1988. Plasma Insulin and Growth Hormone in Growing Lambs Fed Monensin. Can. J. Anim. Sci. 68: 165-171.

Meyers, J. 1987. Relationship Between Small Intestinal Transit and Small intestinal Motility. Pages 327-338. *In* J.H. Szurszewski ed. Cellular Physiology and Clinical Studies of Gastrointestinal Smooth Muscle. Excerpta Medica, Elsevier Science Publ., New York, N.Y.

Olsen, J.D., and Trenkle, A. 1973. Exposure of Cattle to Controlled Subzero Temperature: Growth Hormone, Glucose, and Free Fatty Acid Concentrations in Plasma. Am. J. Vet. Res. 34(6): 747-751.

Reichlin, S. 1986. Somatostatin: Historical Aspects. Scand. J. Gastroenterol. 21(Suppl. 119): 1-10.

Reichlin, S. 1983. Somatostatin (First of Two Parts). New England J. Med. 309(24): 1495-1501.

Sasaki, Y. and Weekes, T.E.C. 1986. Metabolic Responses to Cold. Pages 326-346. *In* L.P. Milligan, W.L. Grovum, and A. Dobson, eds. Control of Digestion and Metabolism in Ruminants. Proceedings of the VI International Symposium on Ruminant Physiology. Prentice-Hall, Englewood Cliffs, NJ.

Sasaki, Y., Takahashi, H., Aso, H., Hikosaka, K., Hagino, A., and Oda, S. 1982. Insulin Response to Glucose and Glucose Tolerance Following Feeding in Sheep. Br. J. Nutr. 52: 351-358.

Schusdziarra, V. 1980. Somatostatin - A Regulatory Modulator Connecting Nurient Entry and Metabolism. Horm. Metab. Res. 12: 563-577.

Steele, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics. A Biometrical Approach. McGraw-Hill Book Co., New York, NY.

Young, B.A., Fenton, T.W., and McLean, J.A. 1984. Calibration Methods in Respiratory Calorimetry. J. Appl. Physiol. Respirat. Environ. Exercise Physiol. 56(4): 1120-1125.

Young, B.A., Kerrigan, B., and Christopherson, R.J. 1975. A Versatile Respiratory Pattern Analyzer for Studies of Energy Metabolism of Livestock. Can. J. Anim. Sci. 55: 17-22.

Table III-1. Effect of primed continuous infusion of somatostatin-14 (6.7 ng $min^{-1} kg^{-1}$) on skin and rectal temperature (°C) in five lambs adapted to either thermoneutral (TN) or chronic cold (CC) environment.

	Experimental Treatment					
	Environment			Infusion		
Parameter	TN	CC	SEM	Saline	SS	SEM
Hip Temperature	35.3[a]	27.6[b]	0.6	32.1	30.9	0.4
Leg Temperature	29.5[a]	6.3[b]	1.6	17.5	18.3	0.9
Rectal Temperature	39.2	38.9	0.3	39.2	38.9	0.2

a,b Means within a parameter and within an experimental treatment are different ($P < 0.05$).

LIST OF FIGURES

I. INTRODUCTION

A major constraint on animal production in Canada and other cold temperate regions results from increased energy demands imposed by exposure to cold environments. Animals normally maintain blood metabolite concentrations within narrow limits, since prolonged deviations away from normal limits have unfavourable consequences (Yousef, 1985). However, animals are capable of rapid physiological, biochemical, and behavioural responses to environmental stress, which often result in marked changes in blood concentrations or flux of metabolites used as substrates to meet changing energy demands. Numerous regulatory mechanisms mediate the responses to such stressors. These mechanisms include chemical and physical sensors which can orchestrate both neural and hormonal responses capable of altering metabolism and function of several organs and tissues (Szabo and Szabo, 1986).

In the past 15 years a number of bioactive peptides have been isolated and characterized from regulatory tissues within the body. Somatostatin, which is a family of closely related peptides, has received considerable attention because of its ubiquitous nature and its importance as a chalone, an internal secretion that depresses activity (Reichlin, 1986). Somatostatin has been found in the central nervous system including the hypothalmus, peripheral nervous system, retina, cerebrospinal fluid, blood, thyroid gland, pancreas, and gastrointestinal tract (Dileepan and Wagle, 1985). This study centers on the dynamics of somatostatin-14 and its effects on growth hormone,

insulin, glucagon secretion, free fatty acid, and glucose concentrations and gastrointestinal motility.

Importance of Somatostatin in Ruminants:

Although the emphasis of recent research has been on the role of somatostatin in human physiology and pathophysiology, it was first isolated from ovine hypothalami and identified in vitro as an inhibitor of growth hormone secretion (Brazeau et al., 1973). Since then, growth hormone, insulin, and glucagon in vivo secretory responses to numerous secretogagues have been shown to be inhibited by somatostatin, while basal growth hormone, insulin, glucagon and thyrotropin levels are not consistently affected by large doses of somatostatin (Brockman and Laarveld, 1986; Davis, 1975), or by physiological doses of somatostatin (Schusdziarra, 1980; Barry et al., 1985). This implies that somatostatin acts over a relatively short distance.

Recently, it has been observed that immunoneutralization of somatostatin can improve milk production in mature goats, and growth rate and feed efficiency in growing animals (Spencer, 1987). In one study Laarveld et al. (1986), noted a possible interaction between immunoneutralization of somatostatin and environmental temperature in the growth response of lambs. Lambs immunized against somatostatin maintained a similar growth rate, while growth rate in the control animals was reduced during the period of cold exposure. The improvements in animal production by immunoneutralization of somatostatin has renewed interest in understanding the mechanisms by which this hormone controls metabolite flux in ruminants.

Direct Effects of Somatostatin on Metabolite Concentrations:

Very little emphasis has been placed on the direct effects of somatostatin on plasma metabolite concentrations, possibly because the indirect (neuropeptide-hormonal, paracrine, and true hormone) effects are thought to be the prime acute regulators of nutrient flux Schusdziarra, 1980; Brockman and Laarveld, 1986).

Several lines of evidence suggest that the prime site of direct somatostatin action is on the rate of nutrient entry into the body from the digestive tract (Schusdziarra, 1980), which is released in response to protein, fatty acids, and carbohydrates (Schusdziarra, 1980). Somatostatin inhibits exocrine secretions from both the gut and the pancreas, including gastric acid, pepsinogen, bicarbonate, and digestive enzymes. Secondly, it alters motor activity, including inhibition of the gastric migrating myoelectric complexes (MMC), while stimulating intestinal MMC (Yamada, 1987). Finally, somatostatin acts on the digestive tract to reduce absorption rate of calcium, glucose, amino acids, and triglycerides from the gut, and alters blood flow, possibly by stimulating release of renin (Reichlin, 1986; Yamada, 1987). Somatostatin also affects digestive function by suppressing secretion of all known gut peptides including gastrin, cholecystokinin, vasoactive intestinal peptide, gut glucagon, and secretin (Schusdziarra, 1980).

Somatostatin may also increase plasma glucose concentration by either stimulating renal gluconeogenesis, possibly by increasing cellular Ca^{++} influx (Dileepan and Nagle, 1985), or by depressing glucose utilization as seen during cold exposure (Minaire et al., 1981). However, the reduction in glucose utilization may have been

secondary to insulin deficiency (Lowry et al., 1981). Somatostatin has also been shown to potentiate epinephrine-induced lipolysis, but it is unclear whether this is due to a direct or indirect mechanism (Gray et al., 1980).

Indirect Effect of Somatostatin on Metabolite Concentrations:

Intermediary metabolism and substrate supply may be altered by somatostatin through its effects on pancreatic, thyroid, and pituitary hormones. The most consistent effect of somatostatin is on glucagon secretion, where it inhibits not only basal secretion (Brockman and Greer, 1980), but also secretory responses to propionate, arginine (Bryce et al., 1975), and exercise (Brockman, 1979). The major function of glucagon is to stimulate hepatic gluconeogenesis, primarily from alanine, and glycogenolysis (Szabo and Szabo, 1987), while having very little effect on the release of glucogenic precursors from muscle and adipose tissue (Cherrington and Vranic, 1987).

Basal insulin secretion has not been consistently inhibited by somatostatin (Brockman and Laarveld, 1986), while somatostatin has been found to suppress the secretory response to propionate, glucose, arginine, and glucagon (Bryce et al., 1975). Suppression of insulin concentration favours the release of glucogenic precursors from muscle and the release of free fatty acids and glycerol from adipose tissue. The free fatty acids may be oxidized and the glucogenic substrates transported to the liver where they are converted to glucose by increased gluconeogenesis (Cherrington and Vranic, 1986).

The principal effect of thyroid hormones is to increase the metabolic activities of most tissues of the body, while increasing substrate availability by enhancing glucose and free fatty acid uptake by cells; increasing glycogenolysis and gluconeogenesis; stimulating lipolysis; and increasing rate of absorption from the gastrointestinal tract (Guyton, 1977). Somatostatin inhibits the release of thyroid stimulating hormone from the pituitary (Davis, 1975) and at physiological doses results in reduced tri-iodothyronine and thyroxine concentrations (Barry et al., 1985).

Intravenous infusion of somatostatin has not been shown to decrease basal growth hormone secretion in adult sheep (Bryce et al. 1985; Davis, 1975), although it does reduce the growth hormone response due to propionate, arginine, and glucose (Bryce et al., 1975).

Environmental Temperature and Somatostatin Release:

Very little work has been done to examine the effects of thermal stress on plasma somatostatin concentrations or secretion rates in ruminants. During mild heat exposure, plasma somatostatin concentration increased in sheep (Faichney and Barry, 1986) and the increase was associated with a negative effect on the blood concentration of several metabolic hormones. It could be postulated that cold exposure would have the opposite effect; i.e. decrease plasma somatostatin concentration or somatostatin secretion. As mentioned previously, Laarveld et al. (1986) noted a possible interaction between growth rate of lambs immunized against somatostatin and acute cold exposure, in which immunized lambs continued to gain weight more rapidly than controls during cold stress. Somatostatin would be expected to suppress

the metabolic rate of animals. This could be potentially beneficial in a warm environment, but detrimental in a cold environment where a higher metabolic rate would favour survival and perhaps growth. Prolonged severe cold exposure has been shown to increase thyroid hormones (Kennedy et al., 1985), catecholamines (Christopherson et al., 1978), and growth hormone (Blom et al., 1976), and increase the frequency of reticular contraction and the rate of passage of digesta in sheep (see Kennedy et al., 1985). The fact that many of these variables are inhibited by somatostatin in a thermoneutral environment (Schusdziarra, 1980) would be consistent with the suggestion that somatostatin levels in the blood may be reduced by cold exposure. Contrary to this idea are the observations that basal insulin levels are either not affected (Sasaki et al., 1982) or decreased (Sasaki, et al., 1982; Christopherson and Thompson, 1983) by cold exposure, and that the effects of cold exposure on glucagon secretion are even less clear cut (see Sasaki and Weekes, 1986). Secondly, somatostatin release has been shown to be stimulated by elevated plasma levels of glucose, fatty acids, and amino acids (Schusdziarra, 1980) which could occur during cold exposure (Alexander, 1979). Thirdly, in the hypothalamus, the activity of peptidase enzymes which degrade somatostatin have been found to increase during hypothyroidism, a response which can be reversed by thyroxine administration (Dupont et al., 1978). If the same holds true for peptidases within the blood, then cold-induced elevation of thyroid activity might decrease the clearance rate of somatostatin. This would increase plasma somatostatin concentration unless secretion rate was also reduced.

In view of increased plasma growth hormone and thyroid hormone concentrations which have been observed in response to cold exposure, it seems likely that plasma somatostatin levels in the hypophyseal-portal blood stream would be reduced. However, this may not result in a reduction in peripheral plasma somatostatin levels because somatostatin released from nerve endings, the pancreas, and the digestive tract could be stimulated by increased neural activity, elevation of plasma metabolite concentrations, increased passage rate of digesta in the gastrointestinal tract, or by reducing the degradation rate of somatostatin in the plasma. Therefore, in the absence of direct experimental observations, it is difficult to predict the direction and extent of change (if any) in somatostatin release and blood concentrations in response to cold environmental temperature exposure.

The overall objectives of this study were:

1. to determine the effect of exposure to cold environments on plasma concentration of somatostatin and the relationship to concentrations of other major metabolic hormones and metabolites in sheep.
2. to assess the physiological responsiveness to exogenous somatostatin in sheep exposed to thermoneutral and cold environments. The physiological responsiveness was assessed in terms of plasma hormone and metabolite concentrations, gastrointestinal motility, metabolic rate, and body temperature.

3. to determine the effect of environmental temperature on metabolic clearance and turnover rate of plasma somatostatin in sheep.

Three experiments, one involving acute (3 h) cold exposure, and two involving chronic (≥ 4 wk) cold exposure, were conducted in an attempt to achieve the above objectives.

BIBLIOGRAPHY

Alexander, G. Cold Thermogenesis. Pages 46-155. *In* D. Robertshaw ed. Environmental Physiology III. University Park press, Baltimore, Ma.

Barry, T.N., Faichney, G.J., and Redekopp, C. 1985. Gastro-intestinal Tract Function in Sheep Infused with Somatostatin. Aust. J. Biol. Sci. 38: 393-403.

Blom, A.K., Hove, K., and Nedkvitne, J.J. 1976. Plasma Insulin and Growth Hormone Concentrations in Pregnant Sheep II: Post-absorptive Levels in Mid- and Late Pregnancy. Acta Endocrinologica 82: 553-560.

Brazeau, P., Vale, W., Burgus, R., Ling, N., Butcher, M., Rivier, J., and Guillemin, R. 1973. Hypothalamic Polypeptide that Inhibits the Secretion of Immunoreactive-Growth Hormone. Science 179: 77-79.

Brockman, R.P. 1979. Glucagon Responses to Exercise in Sheep. Aust. J. Biol. Sci., 32: 215-220.

Brockman, R.P. and Greer, C. 1979. Effects of Somatostatin and Glucagon on the Utilization of [2-^{14}C]Propionate in Glucose Production in vivo in Sheep. Aust. J. Biol. Sci. 33: 457-464.

Brockman, R.P. and Laarveld, B. 1986. Hormonal Regulation of Metabolism in Ruminants; A Review. Livest. Prod. Sci. 14: 313-334.

Bryce, D., Yeh, M., Funderburk, C., Todd, H., and Hertelendy, F. 1975. Studies on Growth Hormone Secretion VII. Effects of Somatostatin on Plasma GH, Insulin, and Glucagon in Sheep. Diabetes 24: 842-850.

Cherrington, A.D. and Vranic, M. 1986. Hormonal Control of Gluconeogenesis in vivo. Pages 15-38. *In* N. Kraus-Friedmann ed. Hormonal Control of Gluconeogenesis Vol 1. Function and Experimental Approaches. CRC Press Inc., Boca Raton, Florida.

Christopherson, R.J., Thompson, J.R., Hammond, V.A., and Hills, G.A. 1978. Effects of Thyroid Status on Plasma Adrenaline and Noradrenaline Concentrations in Sheep during Acute and Chronic Cold Exposure. Can. J. Physiol. Pharmacol. 56: 490-496.

Christopherson, R.J. and Thompson, J.R. 1983. Endocrine Responses of Young Bulls Kept Outdoors during Winter. Can. J. Anim. Sci. 63: 1019.

Davis, S.L. 1975. Somatostatin: Its Influence on Plasma Levels of Growth Hormone, Prolactin and Thyrotropin in Sheep. J. Anim. Sci. 40(5): 911-916.

Dileepan, K.N. and Wagle, S.R. 1985. Somatostatin: A Metabolic Regulator. Life Sciences 37: 2335-2343.

Dupont, A., Merand, Y., and Barden, N. 1978. Effect of Propylthiouracil and Thyroxine on the Inactivation of Somatostatin by Rat Hypothalamus. Life Sci. 23: 2007-2012.

Faichney, G.J. and Barry, T.N. 1986. Effects of Mild Heat Exposure and Suppression of Prolactin Secretion on Gastro-intestinal Tract Function and Temperature Regulation in Sheep. Aust. J. Biol. Sci. 39: 85-97.

Gray, D.E., Lickley, H.L.A., and Vranic, M. 1980. Physiologic Effects of Epinephrine on Glucose Turnover and Plasma Free Fatty Acid Concentrations Mediated Independently of Glucagon. Diabetes 29: 600-608.

Guyton, A.C. 1977. Basic Human Physiology: Normal Function and Mechanisms of Disease 2nd edition. W.B. Saunders Co., Toronto, Ontario.

Kennedy, P.M., Christopherson, R.J., and Milligan, L.P. 1985. Digestive Responses to Cold. Pages 285-306. *In* L.P. Milligan, L.W. Grovum, and A. Dobson eds. Control of Digestion and Metabolism in Ruminants. Reston Publ. Co., Reston, Va.

Laarveld, B., Chaplin, R.K., and Kerr, D.E. 1986. Somatostatin Immunization and Growth of Lambs. Can. J. Anim. Sci. 66(1): 77-83.

Lowry, S.F., Burt, M.E., and Brennan, M.F. 1981. Glucose Turnover and Gluconeogenesis in a Patient with Somatostatinoma. Surgery 89(3): 309-313.

Minaire, Y., Forichon, J., Dallevet, G., and Jomain, M.J. 1981. Combined Effects of Cold and Somatostatin on Glucose Kinetics in Dogs. Eur. J. Appl. Physiol. 46: 249-259.

Reichlin, S. 1986. Somatostatin: Historical Aspects. Scand. J. Gastroenterol. 21(Suppl. 119): 1-10.

Sasaki, Y. and Weekes, T.E.C. 1985. Metabolic Responses to Cold. Pages 326-343. *In* L.P. Milligan, L.W. Grovum, and A. Dobson eds. Control of Digestion and Metabolism in Ruminants. Reston Publ. Co., Reston, Va.

Sasaki, Y., Takahashi, H., Aso, H., Ohneda, A., and Weekes, T.E.C. 1982. Effects of Cold Exposure on Insulin and Glucagon Secretion in Sheep. Endocrinol. 111: 2070-2076.

Schusdziarra, V. 1980. Somatostatin - A Regulatory Modulator Connecting Nutrient Entry and Metabolism. Horm. Metab. Res. 12: 563-577.

Spencer, G.S.G. 1987. Biotechnology in the Potential Practical Application of Somatotrophic Hormones for Improving Animal Performance. Reprod. Nutr. Develop. 27(2B): 581-589.

Szabo, A.J. and Szabo, O. 1986. CNS Involvement in the Regulation of Blood Glucose. Pages 39-65. *In* N. Kraus-Friedmann ed. Hormonal Control of Gluconeogenesis Vol 1. Function and Experimental Approaches. CRC Press Inc., Boca Raton, Florida.

Yamada, T. 1987. Gut Somatostatin. Pages 221-228. *In* S. Reichlin ed. Somatostatin Basic and Clinical Status. Plenum Press, New York, NY.

Yousef, M.K. 1985. Stress Physiology: Definition and Terminology. Pages 3-8. *In* M.K. Yousef ed. Stress Physiology in Livestock. Vol. I Basic Principles. CRC Press Inc., Boca Raton, Florida.

II. GASTROINTESTINAL MOTILITY AND PLASMA HORMONE AND METABOLITE RESPONSES TO ACUTE COLD STRESS AND VARIOUS DOSES OF SOMATOSTATIN-14.

INTRODUCTION

Animals rely on peripheral vasomotor responses, behavioral and postural modifications, substrate mobilization, increased metabolism, digestive responses, and ultimately increased feed intake to overcome the effects of cold stress. These modifications presumably result from altered neural and hormonal activity. Somatostatin, a tetradecapeptide, which is recognized as a neuromodulator, neurotransmitter, neurohormone, endocrine hormone, as well as a paracrine hormone, (Reichlin, 1983) may alter the response to stress. Immmunoneutralization of somatostatin has sometimes resulted in increased growth rates and improved feed efficiency in animals (see Spencer, 1987 for review). However, in a recent study (Laarveld et al., 1986) the growth advantage in lambs immunized against somatostatin was only evident during the coldest period of winter, suggesting an interaction with cold stress.

The endocrine organs having major effects during acute cold stress are the anterior pituitary, the pancreas, and the adrenal glands (Brockman, 1986). Somatostatin, at physiological doses, often has no effect on basal insulin (Brockman, 1979), growth hormone (Davis 1975), or thyrotropin (TSH) (Davis, 1975) secretion, but reduces their secretory responses to various stimuli in both nonruminants and ruminants (Schusdziarra and Schmid, 1987; Brockman and Laarveld, 1986). On the other hand, the glucagon responses to various stimuli, as well as its basal secretion, are decreased (Brockman and Laarveld,

1986). Therefore, somatostatin may diminish stress-induced responses of many of the metabolic hormones.

Somatostatin inhibits gastrointestinal motility in numerous species (Schusdziarra, 1980). Prolonged infusion of somatostatin did not influence mean rumen retention time of liquid or particulate digesta markers in sheep, although there was a significant increase in mean retention time in the postruminal digestive tract (Barry et al., 1985). However, contradictory to the latter study, passive immunoneutralization of somatostatin also prolonged digesta retention time in lambs (Fadlalla et al., 1985). Since there is conflicting evidence for a role of somatostatin in regulating digesta passage rate in ruminants, and since there is an increase in passage rate of digesta and reduced digestibility of feed associated with cold stress (see Kennedy et al., 1986), it was of interest to determine whether somatostatin might modify the rate of contraction of either the ruminoreticulum or the duodenum in sheep during cold exposure.

The objectives of this study were to determine the effects of primed continuous infusions of somatostatin on gastrointestinal motility, peripheral and core temperatures, and hormonal and metabolite concentrations during acute cold stress in sheep.

MATERIALS AND METHODS

Animals and Their Management:

Three crossbred ewe lambs (39.8 ± 1.8 kg body weight (BW)) with ruminal and duodenal cannulae were housed in metabolic crates in controlled environmental chambers. They were fed once daily at 1600 h at 90% ad libitum. The diet consisted of a 20.4% crude protein

barley:soybean diet, supplemented with vitamin A, D, E and limestone (Appendix Table 1), and was fed at a rate of 25.6 g DM d^{-1} kg^{-1} body weight (BW). Lambs were shorn biweekly. One week prior to experiment sheep were accustomed to the experimental recording procedures. Experimental measurements were simultaneously carried out on two lambs each day over a period of six days and treatments were given in a Youden square design with each lamb receiving each treatment.

Experimental Protocol:

Sixteen h prior to the first experimental period a chronic indwelling polyvinyl chloride tubing (1.68 mm ID, Argyle, St. Louis, Mu.) was inserted into each external jugular vein and filled with physiological saline solution containing heparin (100 IU mL^{-1}). One catheter was used for blood sampling and the other for infusion. Skin temperature was recorded using copper constantan type T thermocouples (Thermo Electric, Edmonton) adhered to a closely sheared patch of skin with a 4 x 4 cm^2 surgical tape patch and contact cement. One thermocouple was placed over the biceps femoris muscle, while the second was placed on the lateral surface of the leg (in the hollow between the metatarsal bone and flexor digitorium brevis) 15 cm inferior to the hock. Lambs were then placed in the environmental chamber overnight with access to water, cobalt iodized salt, and their daily allotment of feed.

Two h prior to the start of the experiment animals were placed in the respiration hoods, and skin thermocouples and the copper constantan rectal thermocouple were connected to an Apple II plus computer and a DT100 datataker (Dycor Industrial Research, Edmonton), although due to

technical problems with the calorimetry equipment, calculations on respired gases have not been included. Polyvinyl chloride open-tipped catheters (1.68 mm ID) were inserted into the duodenum and reticulum via duodenal and ruminal cannulae, respectively. A small weight fastened to the tip of the reticular catheter kept it from becoming displaced from the reticulum. The reticular and duodenal catheter patency was maintained by continuous infusion of distilled water at 0.5 mL min^{-1}. Reticular and duodenal contractions were simultaneously monitored continuously by a pair of inline P23 Series pressure transducer (Gould Instruments Inc., Hato Rey, Puerto Rico), amplified, and charted by a Beckman R-612 Dynograph Recorder (Electronic Instruments Division, Schiller Park, Illinois). Reticular and duodenal spiking activity was manually tabulated.

Following preparation, lambs were allowed approximately 90 min without disturbance prior to the start of experimental recording at 0900 h. At 1000 h a primed continuous infusion of somatostatin-14 (SS-14) (8.9, 18.4, or 37.3 ng min^{-1} kg^{-1} BW) or saline control was initiated and lasted for 300 min. The priming doses were 0.46, 0.86, and 1.39 μg kg^{-1}, respectively, and were given to shorten the length of time required to established the new somatostatin levels. One hour after initiation of SS-14 or saline infusions the environmental chamber was cooled from 20 ± 2.2°C to 0 ± 2°C within 30 min, and was maintained at this temperature for 2 h, before rapidly rewarming (within 30 min). By the end of the 30 min warming period the room temperature always exceeded 16°C. Continuous infusions were terminated 1 h later and lambs were monitored for a further 30 min.

Ten mL blood samples were drawn every 30 min starting at 0900 h. No attempt was made to return the red blood cells after separation of plasma. Catheters were kept patent with heparinized (40 IU mL^{-1}) saline (0.9% NaCl) and catheter dead space was cleared by a 3 mL discard prior to each sample. Blood samples were immediately transferred to 16 x 100 mm glass tubes containing heparin (12 IU mL^{-1} of blood) and NaF (4% w/v, 15 μL per mL of blood), mixed by inversion, and kept on ice until centrifuged at 4°C for 15 min at 2280 x g. Aliquots of plasma were frozen at -20°C until analyzed. The aliquot for somatostatin and glucagon contained Trasylol (aprotinin, Miles Laboratories, Missassauga, Ontario; 500 IU mL^{-1} plasma).

Analytical Techniques:

Concentrations of free fatty acids and glucose were determined by enzyme specific kits (acyl CoA synthetase, WAKO Pure Chemical Industry, Dallas, Texas; glucose oxidase, Sigma Chemical Co., Mississauga, Ontario). Somatostatin-like immunoreactivity (SLI), glucagon, insulin, and growth hormone concentrations were determined by double antibody radioimmunoassays as outlined by Christensen et al., (1988), Harris et al., (1979), and Mears et al., (1988).

Statistical Analysis:

Hormone data were initially plotted for 30 min intervals, and since preliminary inspection suggested there were no short-term effects, blood samples were aggregated into 8 timeslots: preinfusion (0, 30, 60 min); infusion in thermoneutral (TN) environment (90, 120 min); at end of cooling phase (150 min); during the first hour of cold (180, 210 min); during the second hour of cold (240, 270 min); 30 min after starting rewarming chamber (300 min); infusion in the postcold period

(330, 360 min); and postinfusion (390 min) prior to analysis of variance. Mean rectal and skin temperatures were averaged for the same timeslots as blood samples. Reticular motility was tabulated for each timeslot, except the cooling and rewarming timeslots, where increased sheep movement artifacts obscured the motility records. To assess the effect of somatostatin on migrating the myoelectric complex (MMC) and its components, spiking activity was tabulated across the entire day and was averaged to obtain a mean duration and number of contractions per MMC. Data was analyzed by analysis of variance for a repeated measures design. The model included the main effects of infusion treatment, timeslot, and treatment by timeslot with sheep being a random factor. Means were compared by Student-Neuman-Keuls' multicomparison test (Steele and Torrie, 1980).

RESULTS

Rectal and Skin Temperatures:

Two h of cold exposure decreased ($P< 0.05$) mean rectal temperature (39.6 vs 38.9°C, SEM= 0.1), hip temperature (32.7 vs 22.4°C, SEM= 0.1), and leg temperature (29.4 vs 11.3°C, SEM= 1.0). Rectal temperature remained depressed even after rewarming, while hip temperature returned to precold values (31.5°C) within 2 h. A larger decline in leg temperature at the 8.9 ng min^{-1} kg^{-1} BW dose of SS-14, when compared to either saline or two highest doses of SS-14 followed by a slower postcold recovery, lead to a significant ($P< 0.05$) treatment by time interaction. SS-14 infusion had no effects on hip or rectal temperature.

Gastrointestinal Motility:

Frequency of reticular contractions were expressed as a change from the preinfusion period, since preinfusion values were variable across treatments (43, 51, 36 and 40 contractions h^{-1} for saline, SS-8.9, SS-18.4, and SS-37.3, respectively). Reticular motility was not affected by SS-14. Cold stress transiently, but significantly ($P< 0.001$) increased reticular motility across all treatments during the first hour, while rewarming decreased ($P< 0.001$) reticular motility (Figure II-1).

SS-14 decreased ($P< 0.05$) the duration of irregular spiking activity at higher doses of SS-14 (SS-18.4 and SS-37.3) (Table II-1), and resulted in a decrease ($P< 0.02$) in the duration of the entire MMC. However, SS-14 had no effect on the number of contractions associated with any of the components of the MMC or the total number of contractions within the MMC (Table II-1).

The priming injection at all doses of SS-14 induced an ectopic burst of spiking activity within 15 min in 7 of 9 trials, whereas no such responses were observed in the saline treated animals.

Hormonal and Metabolite Profiles:

Continuous infusion of SS-14 produced mean plasma SLI concentrations which were 160%, 246%, and 396% of the preinfuston values. SLI concentrations over the entire infusion period were on average 0.277, 0.483, 0.771, and 1.654 ng mL^{-1} for saline, SS-8.9, SS-18.4, and SS-37.3 treatments, respectively. Acute cold stress had no effect on plasma SLI concentrations (Figure II-2).

Overall mean plasma insulin concentrations were not affected by SS-14 infusion, although there was a trend ($P< 0.06$) for a treatment by timeslot interaction. This was primarily due to a larger increase in insulin values for saline and lowest dose of SS-14 during the initial 90 min of cold exposure (Figure II-3). Across treatments, this resulted in a higher ($P< 0.007$) insulin concentration during the first h of cold exposure than for any of the TN timeslots (1.48 vs 0.96, 0.90, and 0.85 ng mL^{-1}; SEM= 0.11, initial h of cold vs precold infusion, rewarming, and rewarmed periods, respectively). Plasma glucagon concentration was not influenced by SS-14 infusion or acute cold stress, although there was a significant ($P< 0.03$) treatment by timeslot interaction (i.e. glucagon concentration increased during the initial 90 min of cold exposure in the saline treatment showed a delayed response at the lowest dose of SS-14 and did not increase at the two higher doses of SS-14) (Figure II-4). Insulin:glucagon ratios were not different across treatments, although mean values across treatments increased ($P<0.005$) during the initial 90 min of cold exposure.

Mean preinfusion growth hormone concentrations across treatments ranged from 6.0 to 7.2 ng mL^{-1}, were slightly suppressed (28.5%) during initial cold exposure and increased significantly ($P<0.005$) prior to the end of the experiment. SS-14 infusion diminished the rise in growth hormone concentration during the rewarming phase, although this was not significant (Figure II-5).

The highest dose of SS-14 elevated ($P< 0.04$) plasma glucose concentration (76.9, 78.7, 77.5, vs 83.9 mg dL^{-1}; saline, SS-8.9, SS-18.4, vs SS-37.3, SEM= 1.6), while lower doses of SS-14 had smaller effects. SS-14 infusion at the two highest doses increased ($P< 0.03$)

plasma glucose concentration prior to cooling and during initial 90 min of cold exposure, while glucose concentration decreased slightly in the saline treatment resulting in a treatment by timeslot interaction ($P< 0.03$) (Figure II-6).

Cold stress increased ($P< 0.001$) mean plasma FFA concentrations across treatments, especially at the two highest doses of SS-14 (Figure II-7). Upon rewarming, FFA levels returned towards the preinfusion values. Thus, differences in the response to cold exposure during SS-14 resulted in a significant ($P< 0.05$) treatment by timeslot interaction. Mean plasma FFA concentrations across timeslots, when expressed as a change from the initial concentration, were 0.067, 0.027, 0.205, and 0.208 mM for the saline, SS-8.9, SS-18.4, and SS-37.3 treatments, respectively, which were not significantly ($P=0.13$, SEM= 0.06) different.

DISCUSSION

The prime site of somatostatin action in the gastrointestinal tract in this study was in the duodenum. As seen in other species including dogs and humans (Hostein et al., 1984), the priming dose of somatostatin induced ectopic contractile activity in the duodenum. This may indicate either a direct or indirect effect of somatostatin on duodenal spiking activity.

Our results indicate that at twice the infusion dose rate of Barry et al., (1985), somatostatin had no effect on either the duration or the number of contractions within a MMC, but at four times the dose somatostatin could decrease the duration of MMC by decreasing the duration of ISA. The flow rate of digesta through the duodenum is closely associated with ISA contractions, since flow of digesta ceases

during the periods of NSA and RSA (Ruckebusch, 1988). This suggests that at the higher doses of somatostatin, the rate of flow of digesta through the duodenum was increased. However, Barry et al. (1985) found an increase in mean retention time in the post-ruminal segments of the digestive tract and an accumulation of digesta in the abomasum and caecal regions. The reason for the differences are unclear, but may result from inhibition of digesta movement from the abomasum into the duodenum by inhibition of antral motility (Ruckebusch and Merritt, 1985). Thus, somatostatin may reduce the rate of passage of digesta from the abomasum into the duodenum, but the results of the present study would suggest that once the digesta is in the duodenum, somatostatin may increase passage rate of digesta out of the small intestine. Further work needs to be done to confirm this possible relationship.

The cold-induced increase in reticular motility in this study was smaller than has been previously shown during both chronic and short-term cold exposure in sheep and cattle given restricted quantities of feed (see Kennedy et al., 1986). This may have been a result of the pelleted high concentrate diet fed in the present experiment or to the short duration of cold exposure. This type of diet would lead to lower tactile stimulation of mechanoreceptors and since feed was withheld for 17-23 hours in these lambs, there could be reduced stimulation of both mechanoreceptors and chemoreceptors, thus leading to a sluggish vago-vagal reflex (Ruckebusch, 1988).

The two highest doses of somatostatin increased glucose concentrations prior to and during the initial cold exposure. This was associated with little change in both insulin and glucagon

concentrations. Somatostatin may affect substrate mobilization or reduce substrate utilization by tissues and thus alter plasma concentration of glucose. Since somatostatin has been shown to have no effect on hepatic epinephrine-stimulated glycogenolysis in vitro (Oliver and Wagle, 1975), it is unlikely that the increase in glucose was through this mechanism. Glucose utilization has been inhibited by somatostatin in dogs, either directly as seen in cold stressed dogs (Minaire et al., 1981), or indirectly by elevated free fatty acid concentrations reducing glucose utilization (Hendrick et al., 1987).

At the higher doses of somatostatin, the free fatty acid response was enhanced during acute cold stress, while only a small effect was seen prior to or following cold exposure. This increase may have been due to a continuous gradual increase resulting from the somatostatin infusion, but most likely resulted from an enhancement of the free fatty acid response during cold stress. Gray et al. (1979) have shown that, in dogs, somatostatin enhanced the epinephrine-stimulated free fatty acid release, whereas in 7-day fasted dogs somatostatin inhibited free fatty acid release (Hendrick et al., 1987). Since an epinephrine and norepinephrine response to cold exposure would be expected (Christopherson et al., 1978), the present results are consistent with the findings of Gray et al. (1979). Thus, on a short-term basis somatostatin may enhance the effects of other hormones to provide a readily available pool of substrates to overcome an immediate stress.

Acute cold exposure had no effect on glucose concentration, while it stimulated only a small increase in free fatty acid concentrations for the control treatment; whereas other studies have shown significant elevations (Alexander, 1979). These differences may be due to the

duration and severity of the cold exposure or to the type of diets fed. The absence of significant response to acute cold stress may have been due to an initial capacity of the animal to maintain homeostasis by other mechanisms such as reducing blood flow to the peripheral body tissues, or by allowing body core temperature to decline moderately (Horton, 1981). A longer and more severe cold exposure may have caused a greater increase in metabolite concentrations.

Growth hormone was not affected by acute cold stress but increased during rewarming. The lack of response to the cold environment is consistent with results seen in lactating goats (Faulkner et al., 1980), mice (Muller et al., 1971), anesthetized monkeys (Golstein-Golaire et al., 1970), and humans (Glick, 1968). Rewarming also increased plasma growth hormone concentration in humans (Okada et al., 1970). The reason for this increase after rewarming is unclear. However, a more severe and prolonged cold exposure has increased growth hormone concentration in cattle (Olsen and Trenkle, 1973), and sheep (Blom et al., 1976). Regardless of the reason for the rise in growth hormone concentration after cold exposure, somatostatin appeared to dampen the rate of increase during rewarming. This agrees with the concept that somatostatin inhibits the secretory response of growth hormone, but not basal levels (Brockman and Laarveld, 1986).

Reports in the literature on the effects of acute cold exposure on insulin secretion have been somewhat variable (Sasaki and Weekes, 1986). Our results did not clarify this since, in our sheep, insulin concentration increased, while glucagon did not change in response to cold exposure. Unger (1971) has suggested that insulin:glucagon ratio gives an indication of the need for increased endogenous glucose

production. One might expect to see a reduced insulin:glucagon ratio in a cold environment in order to support a higher rate of endogenous glucose production. However, our data for sheep demonstrated a transient increase in this parameter during acute cold exposure, a result that is not consistent with Unger's suggestion.

In conclusion, reticular motility does not seem to be affected by somatostatin infusion, whereas the duration of duodenal migrating myoelectrical complex is reduced. Somatostatin, at very low doses, increases basal free fatty acid levels and enhances cold-induced increases in free fatty acid concentration, while only high doses increased glucose concentration. The 2 h cold exposure had little effect on plasma insulin or glucagon concentration, possibly because the cold stress was too short in duration or not severe enough. Somatostatin did not alter the decrease in rectal or skin temperature during acute cold stress.

BIBLIOGRAPHY

Alexander, G. 1979. Cold Thermogenesis. Pages 43-155. *In* D. Robertshaw Ed. Environmental Physiology III. University Park Press. Baltimore, Ma..

Barry, T.N., Faichney, G.J., and Redekopp, C. 1985. Gastro-intestinal Tract Function in Sheep Infused with Somatostatin. Aust. J. Biol. Sci. 38: 393-403.

Blom, A.K., Hove, K., and Nedkvitne, J.J. 1976. Plasma Insulin and Growth Hormone Concentrations in Pregnant Sheep II: Post-absorptive Levels in Mid- and Late Pregnancy. Acta Endocrinologica 82: 553-560.

Brockman, R.P. 1979. Effect of Somatostatin Suppression of Glucagon Secretion on Glucose Production in Sheep. Can. J. Physiol. Pharmacol. 57: 848-852.

Brockman, R.P. 1986. Pancreatic and Adrenal Hormonal Regulation of Metabolism. Pages 405-419. *In* L.P. Milligan, W.L. Grovum, and A. Dobson eds. Control of Digestion and Metabolism in Ruminants. Prentice-Hall. Englewood Cliffs, NJ.

Brockman, R.P. and Laarveld, B. 1986. Hormonal Regulation of Metabolism in Ruminants; A Review. Livest. Prod. Sci. 14: 313-334.

Christensen, R.A., Christopherson, R.J., Kennelly, J.J., de Boer, G., and Mears, G.J. 1988. Hormonal Responses to Feeding and Somatostatin-14 Injection in Cold Exposed Sheep. Domestic Anim. Endocrinol. (Submitted).

Christopherson, R.J., Thompson, J.R., Hammond, V.A., and Hills, G.A. 1978. Effects of Thyroid Status on Plasma Adrenaline and Noradrenaline Concentrations in Sheep during Acute and Chronic Cold Exposure. Can. J. Physiol. Pharmacol. 56: 490-496.

Davis, S.L. 1975. Somatostatin: Its Influence on Plasma Levels of Growth Hormone, Prolactin, and Thyrotropin. J. Anim. Sci. 40(5): 911-916.

Fadlalla, A.M., Spencer, G.S.G., and Lister, D. 1985. The Effect of Passive Immunization against Somatostatin on Marker Retention Time in Lambs. J. Anim. Sci. 61(1): 234-239.

Faulkner, A., Thomson, E.M., Bassett, J.M., and Thomson, G.E. 1980. Cold Exposure and Mammary Glucose Metabolism in the Lactating Goat. Br. J. Nutr. 43: 163-170.

Glick, S.M. 1968. Normal and Abnormal Secretion of Growth Hormone. Ann. New York Acad. Sci. 148: 471-487.

Golstein-Golaire, J., Vanhaelst, L., Bruno, O.D., Leclercq, R., and Copinschi, G. 1970. Acute Effects of Cold on Blood Levels of Growth Hormone, Cortisol, and Thyrotropin in Man. J. Appl. Physiol. 29(5): 622-626.

Gray, D.E., Lickley, L.A., and Vranic, M. 1980. Physiologic Effects of Epinephrine on Glucose Turnover and Plasma Free Fatty Acid Concentrations Mediated Independently of Glucagon. Diabetes 29: 600-608.

Harris, V., Faloona, G.R., and Unger, R.H. 1979. Glucagon. Page 643. *In* B.M. Jaffe and H.R. Behrman eds. Methods of Hormone Radioimmunoassay. 2nd ed. Academic Press Inc., New York, NY.

Hendrick, G.K., Frizzell, R.T., and Cherrington, A.D. 1987. Effect of Somatostatin on Nonesterified Fatty Acid Levels Modifies Glucose Homeostatis during Fasting. Am. J. Physiol. 253 (Endocrinol. Metab. 16): E443-E452.

Horton, G.M.J. 1981. Responses of Shorn and Full-Fleeced Lambs Given Two Levels of Feed Intake and Exposed to Warm and Cold Temperatures. Am. J. Vet. Res. 42(12): 2151-2154.

Hostein, J., Schippers, E., Janssens, J., Vantrappen, G., Vandeweerd, M., Leman, G., and Peeters, T.L. 1984. Somatostatin Induces Ectopic Activity Fronts of the Migrating Motor Complexes via a Local Intestinal Mechanism. Pages 239-241. *In* C. Roman ed. Gastrointestinal Motility. Proceedings of the 9th International Symposium on Gastrointestinal Motility. MTP Press Ltd. Boston, Ma.

Kennedy, P.M., Christopherson, R.J., and Milligan, L.P. 1986. Digestive Responses to Cold. Pages 285-306. *In* L.P. Milligan, W.L. Grovum, and A. Dobson Eds. Control of Digestion and Metabolism in Ruminants. Prenctice-Hall Englewood Cliffs, NJ.

Laarveld, B., Chaplin, R.K., and Kerr, D.E. 1986. Somatostatin Immunization and Growth of Lambs. Can. J. Anim. Sci. 66(1): 77-83.

Mears, G.J., Vesely, J.A., and Cheng, K.-J. 1988. Plasma Insulin and Growth Hormone in Growing Lambs Fed Monensin. Can. J. Anim. Sci. 68: 165-171.

Minaire, Y., Forichon, J., Dallevet, G., and Jomain, M.J. 1981. Combined Effects of Cold and Somatostatin on Glucose Kinetics in Dogs. Eur. J. Appl. Physiol. 46: 249-259.

Muller, E.E., Miedico, D., Giustina, G., and Cocchi, D. 1971. Ineffectiveness of Hypoglycemia, Cold Exposure and Fasting in Stimulating GH Secretion in the Mouse. Endocrinol. 88: 345-395.

Okada, Y., Miyai, K., Iwatsubo, H., and Kumahara, Y. 1970. Human Growth Hormone Secretion in Normal Adult Subjects During and After Exposure to Cold. J. Clin. Endocrinol. 30: 393-395.

Oliver, J.R. and Wagler, S.R. 1975. Studies on the Inhibition of Insulin Release, Glycogenolysis and Gluconeogenesis by Somatostatin in the Rat Islets of Langerhans and Isolated Hepatocytes. Biochem. Biophys. Res. Commun. 62(3): 772-777.

Olsen, J.D., and Trenkle, A. 1973. Exposure of Cattle to Controlled Subzero Temperature: Growth Hormone, Glucose, and Free Fatty Acid Concentrations in Plasma. Am. J. Vet. Res. 34(6): 747-751.

Reichlin, S. 1983. Somatostatin (First of Two Parts). New Engl. J. Med. 309(24): 1495-1501.

Ruckebusch, Y. 1988. Motility of the Gastro-Intestinal Tract. Pages 64-107. *In* D.C. Church Ed. The Ruminant Animal Digestive Physiology and Nutrition. Prentice Hall, Englewood Cliffs, N.J.

Ruckebusch, Y. and Merritt, A.M. 1985. Pharmacology of the Ruminant Gastroduodenal Junction. J. Vet. Parmacol. Therap. 8: 339-351.

Sasaki, Y. and Weekes, T.E.C. 1986. Metabolic Responses to Cold. Pages 326-343. *In* L.P. Milligan, W.L. Grovum, and A. Dobson eds. Control of Digestion and Metabolism in Ruminants. Prenctice-Hall, Englewood Cliffs, NJ.

Schusdziarra, V. 1980. Somatostatin - A Regulatory Modulator Connecting Nutrient Entry and Metabolism. Horm. Metab. Res. 12: 563-577.

Schusdziarra, V. and Schmid, R. 1987. Physiological and Pathophysiological Aspects of Somatostatin. Scand. J. Gastroenterol. 21(Suppl. 119): 29-41.

Spencer, G.S.G. 1987. Biotechnology in the Potential Practical Application of Somatotrophic Hormones for Improving Animal Performance. Reprod. Nutr. Develop. 27(2B): 581-589.

Steele, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics. A Biometrical Approach. McGraw-Hill Book Co., New York, NY.

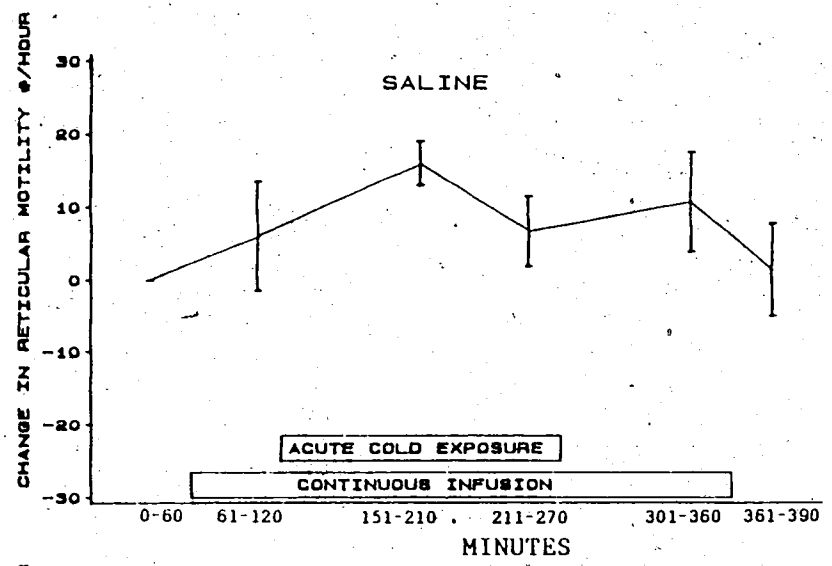
Unger, R.H. 1971. Glucagon and the Insulin:Glucagon Ratio in Diabetes and Other Catabolic Illness. Diabetes 20(12): 834-838.

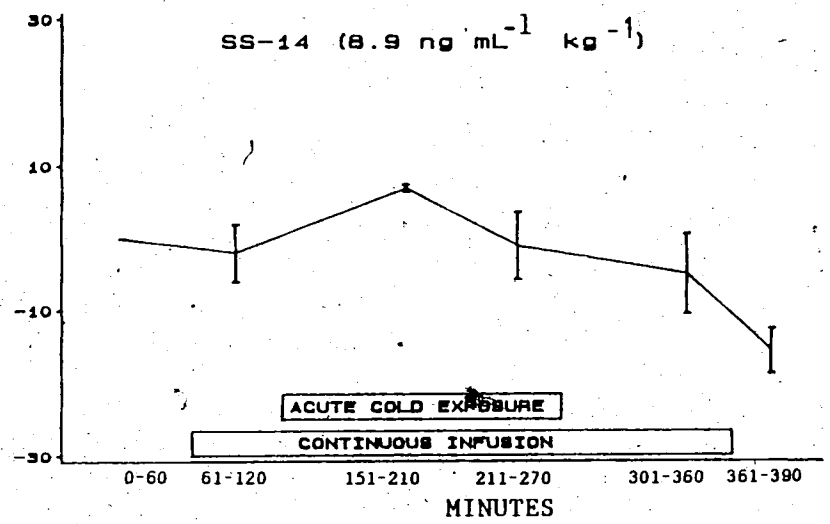
Table II-1. Effect of primed continuous infusion of somatostatin-14 (8.9, 18.4, and 37.3 ng min^{-1} kg^{-1}) on the number of contractions associated with irregular spiking activity (ISA) and regular spiking activity (RSA) and the duration of ISA, RSA, nonspiking activity (NSA) within a migrating myoelectric complex (MMC), and for the entire MMC in three acutely (2 h) cold-exposed lambs.

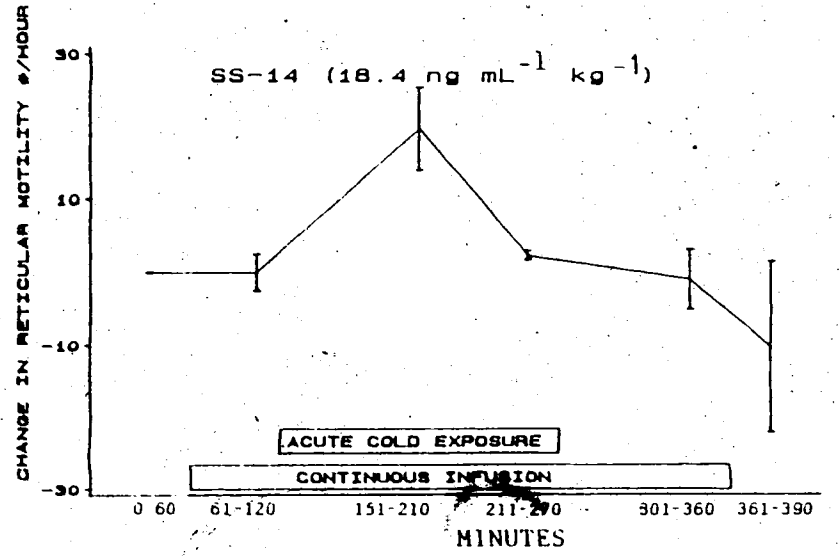
	Experimental Treatment				
	Saline	SS-8.9	SS-18.4	SS-37.3	SEM
Number of contractions/MMC:					
During ISA	51.1	67.6	38.3	26.5	18.5
During RSA	18.5	16.7	14.3	19.6	2.3
Total	69.6	84.3	52.6	46.1	19.0
Duration (Min):					
ISA	68.9[a]	79.4[a]	46.8[b]	39.1[b]	4.5
RSA	5.0	4.3	3.9	3.9	0.7
NSA	8.9	5.8	4.7	5.8	0.6
MMC	82.8[a]	89.5[a]	55.4[b]	48.8[b]	4.9

a,b Means within the same row with different subscripts are different ($P < 0.05$).

Figure II-1. Effect of primed continuous infusion of somatostatin-14 (8.9, 18.4, and 37.3 $ng.min^{-1}$ kg^{-1}) on reticular motility in three acutely (2 h) cold stressed lambs. Somatostatin infusion was initiated at 60 min and terminated at 360 min; cold exposure was introduced at 120 min and terminated at 270 min (mean ± SEM).


SALINE
CHANGE IN RETICULAR MOTILITY #/HOUR
30
20
10
0
-10
-20
-30
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
151-210
211-270
301-360
361-390
MINUTES


SS-14 (8.9 ng mL-1 kg-1)
30
10
-10
-30
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
151-210
211-270
301-360
361-390
MINUTES


SS-14 (18.4 ng mL-1 kg-1)
CHANGE IN RETICULAR MOTILITY #/HOUR
30
10
-10
-30
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0 60
61-120
151-210
211-270
301-360
361-390
MINUTES

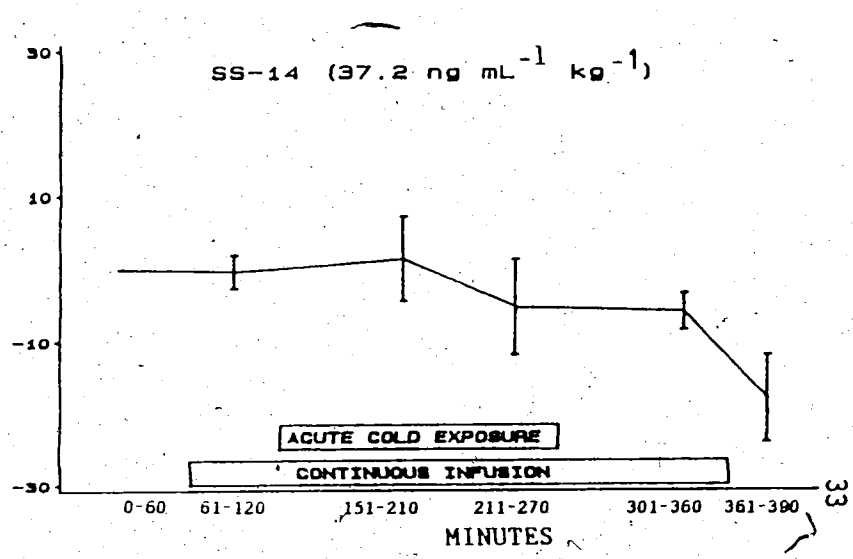
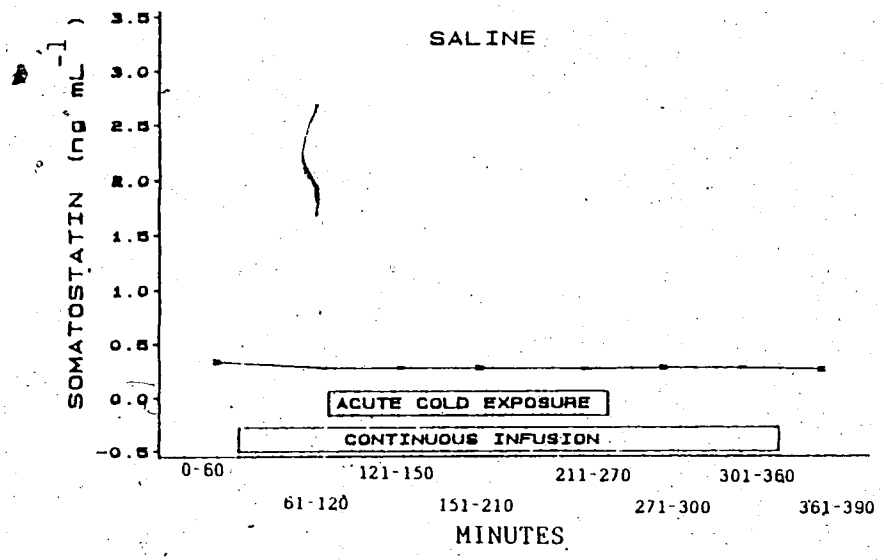
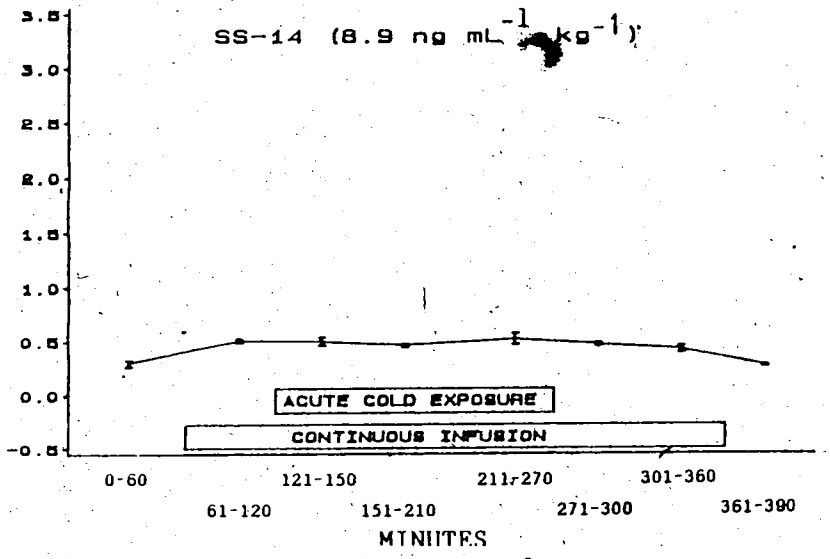
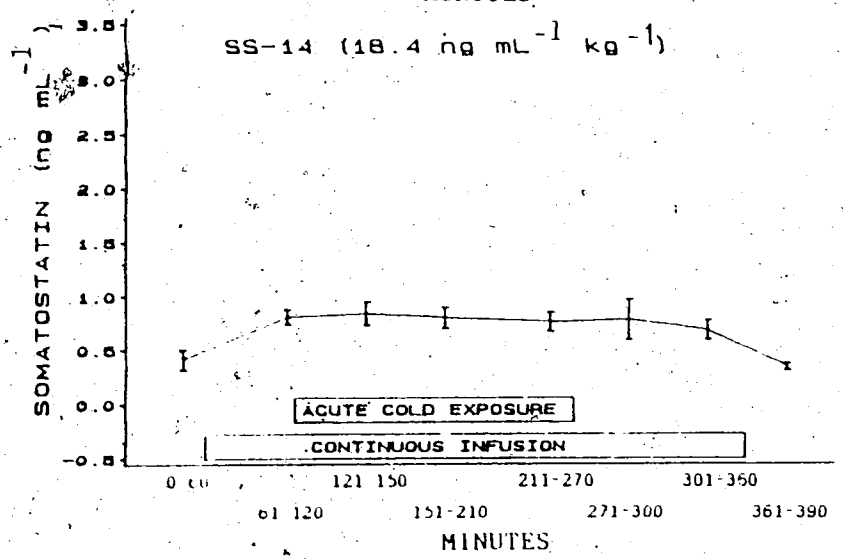

SS-14 (37.2 ng mL-1 kg-1)
30
10
-10
-30
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
151-210
211-270
301-360
361-390
MINUTES

Figure II-2. Effect of primed continuous infusion of **somatostatin**-14 (8.9, 18.4, and 37.3 ng min^{-1} kg^{-1}) on **concentration** of plasma somatostatin-like immunoreactivity in three **acutely** (2 h) cold stressed lambs. Somatostatin was initiated at 60 **min** and terminated at 360 min; acute cold exposure was introduced at 120 min and terminated at 270 min (mean ± SEM).


SALINE
SOMATOSTATIN (ng mL-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES


SS-14 (8.9 ng mL-1 kg-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES


SS-14 (18.4 ng mL-1 kg-1)
SOMATOSTATIN (ng mL-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES

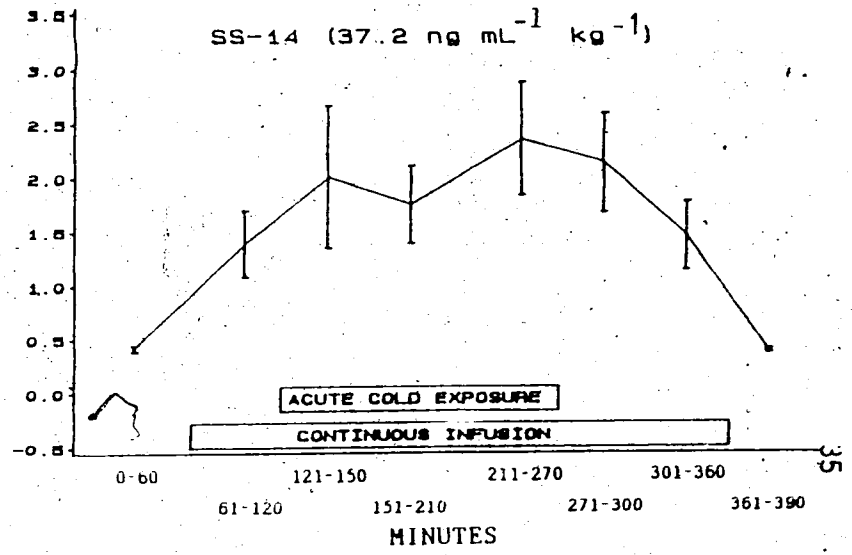

SS-14 (37.2 ng mL-1 kg-1)
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES

Figure II-3. Effect of primed continuous infusion of somatostatin-14 (8.9, 18.4, and 37.3 ng min^{-1} kg^{-1}) on plasma insulin concentration in three acutely (2 h) cold stressed lambs. Somatostatin was initiated at 60 min and terminated at 360 min; acute cold exposure was introduced at 120 min and terminated at 270 min (mean ± SEM).

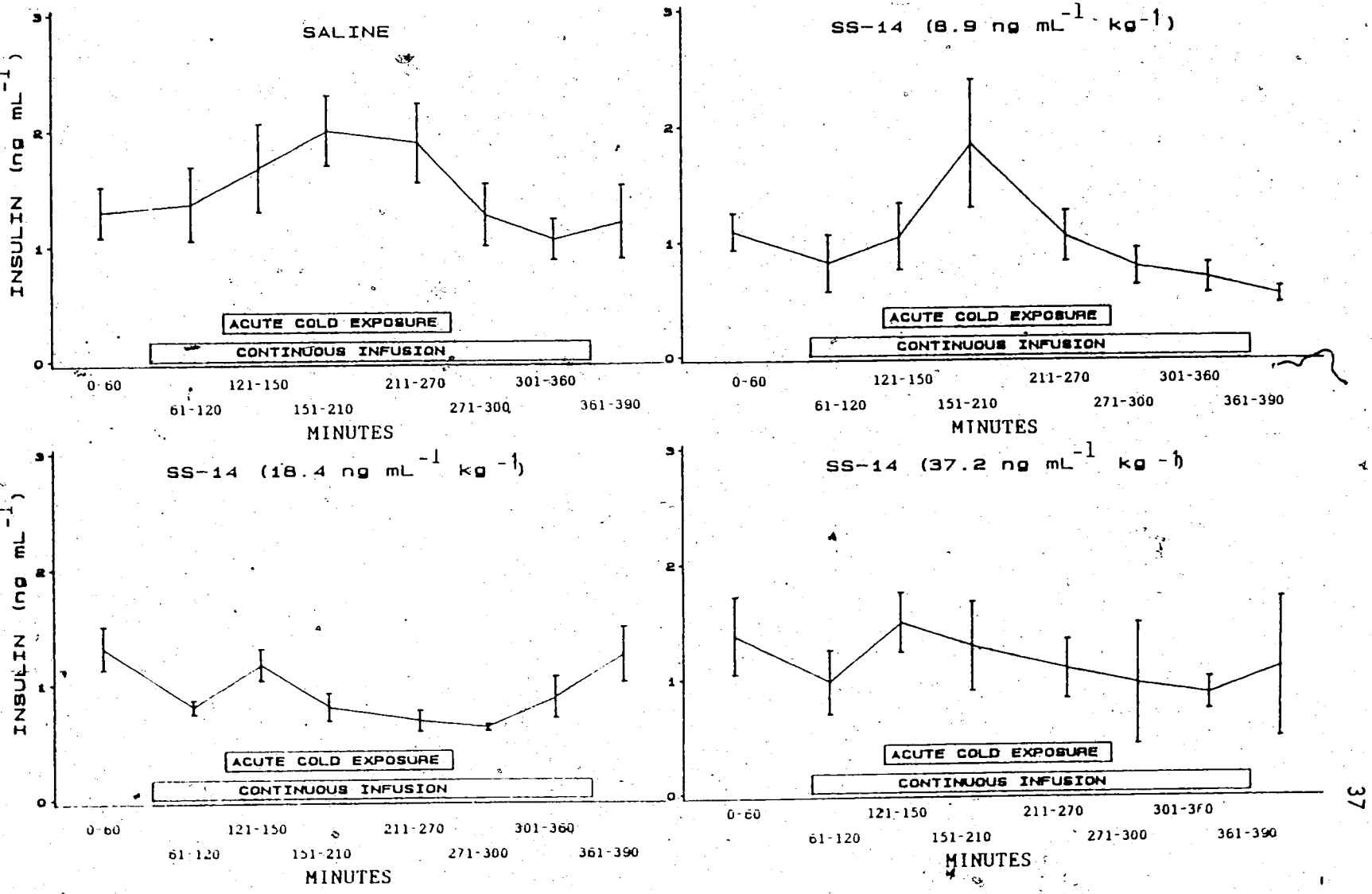
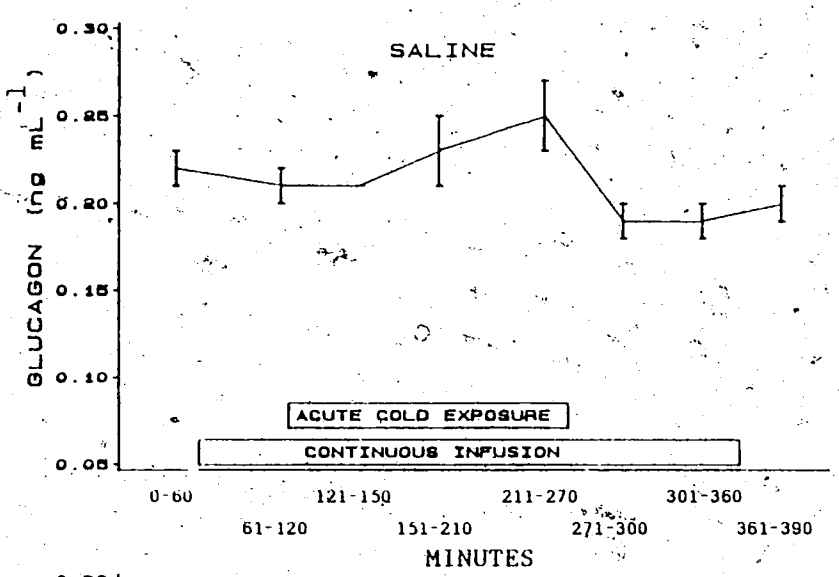
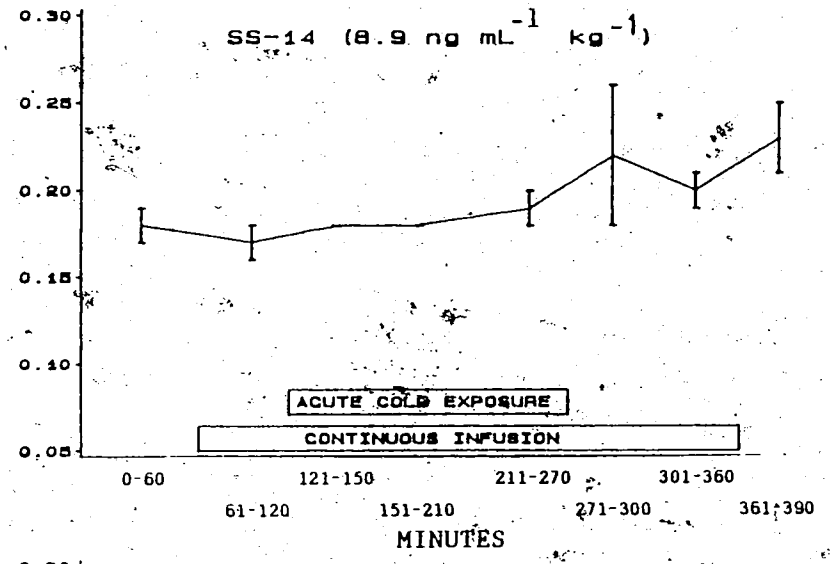
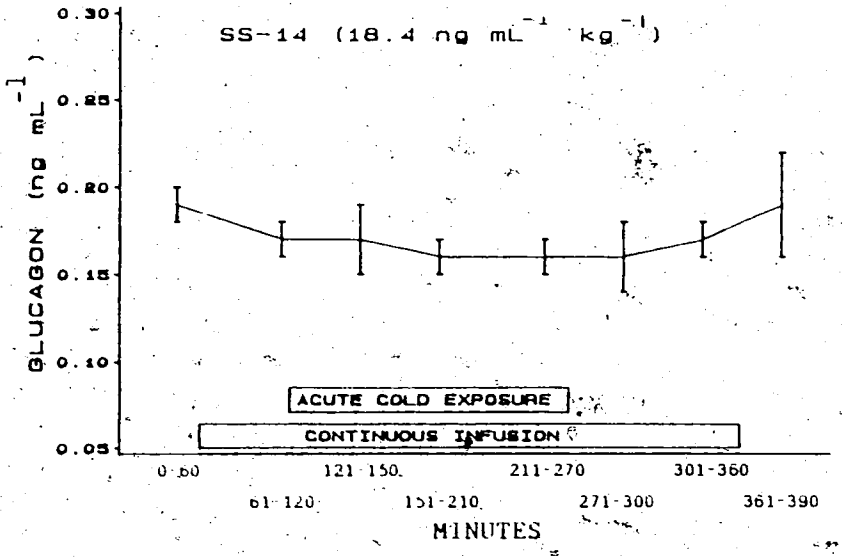

SALINE
INSULIN (ng mL^{-1})
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES
SS-14 (8.9 ng mL^{-1} kg^{-1})
SS-14 (18.4 ng mL^{-1} kg^{-1})
SS-14 (37.2 ng mL^{-1} kg^{-1})

Figure II-4. Effect of primed continuous infusion of somatostatin-14 (8.9, 18.4, and 37.3 ng min^{-1} kg^{-1}) on plasma glucagon concentration in three acutely (2 h) cold stressed lambs. Somatostatin was initiated at 60 min and terminated at 360 min; acute cold exposure was introducted at 120 min and terminated at 270 min (mean ± SEM).


SALINE
GLUCAGON (ng mL^{-1})
0.30
0.25
0.20
0.15
0.10
0.05
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES


SS-14 (8.9 ng mL^{-1} kg^{-1})
0.30
0.25
0.20
0.15
0.10
0.05
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES


SS-14 (18.4 ng mL^{-1} kg^{-1})
GLUCAGON (ng mL^{-1})
0.30
0.25
0.20
0.15
0.10
0.05
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES

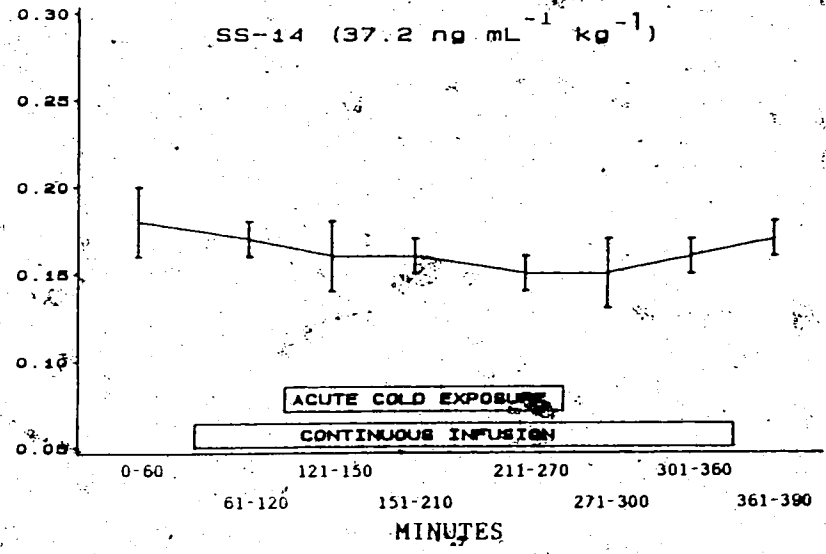

SS-14 (37.2 ng mL^{-1} kg^{-1})
0.30
0.25
0.20
0.15
0.10
0.05
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES

Figure II-5. Effect of primed continuous infusion of somatostatin-14 (8.9, 18.4, and 37.3 ng min^{-1} kg^{-1}) on plasma growth hormone concentration in three acutely (2 h) cold stressed lambs. Somatostatin was initiated at 60 min and terminated at 360 min; acute cold exposure was introduced at 120 min and terminated at 270 min (mean $\pm$ SEM).

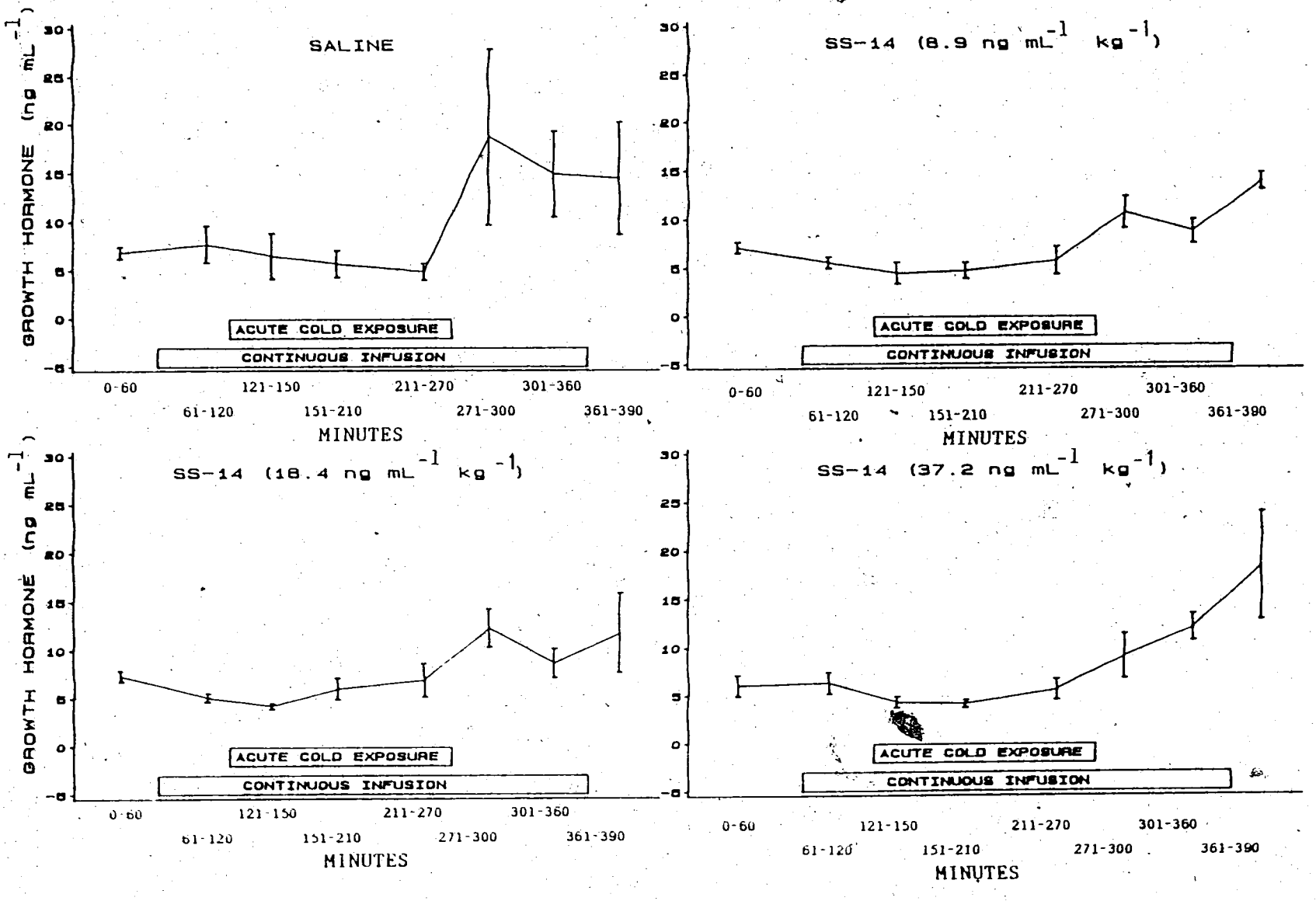
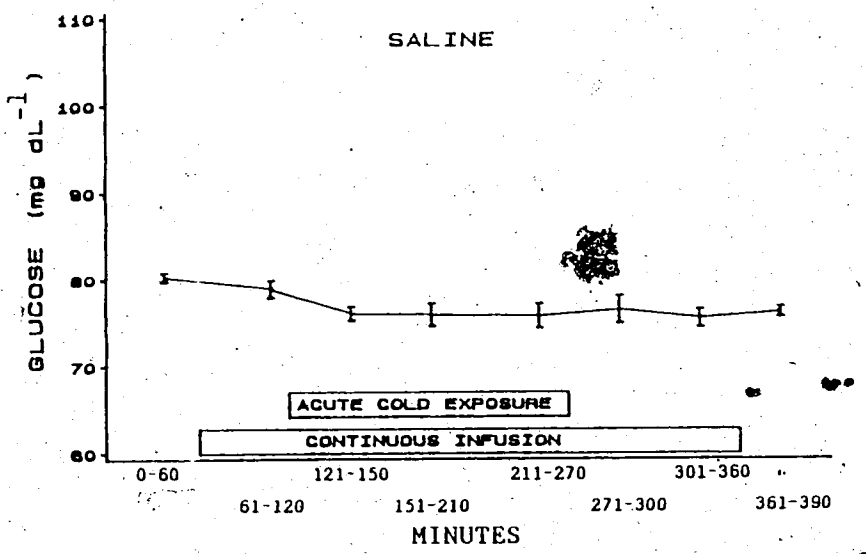
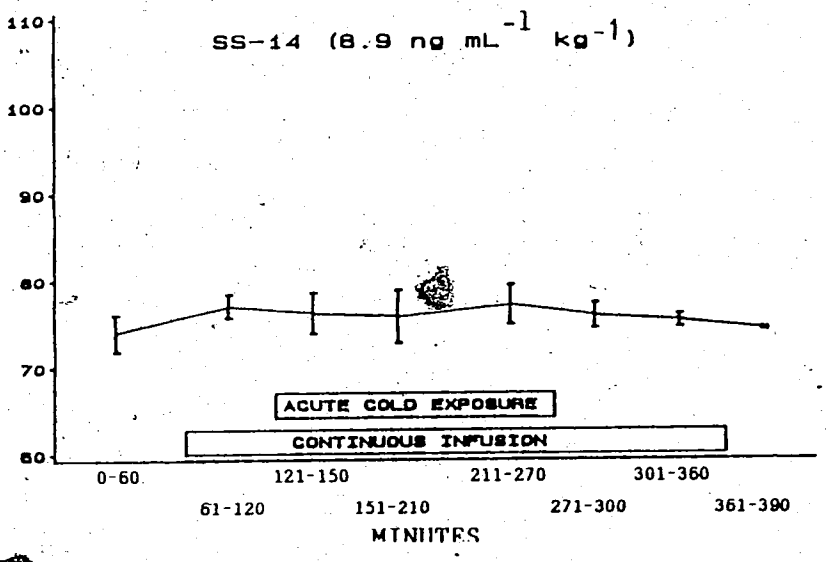
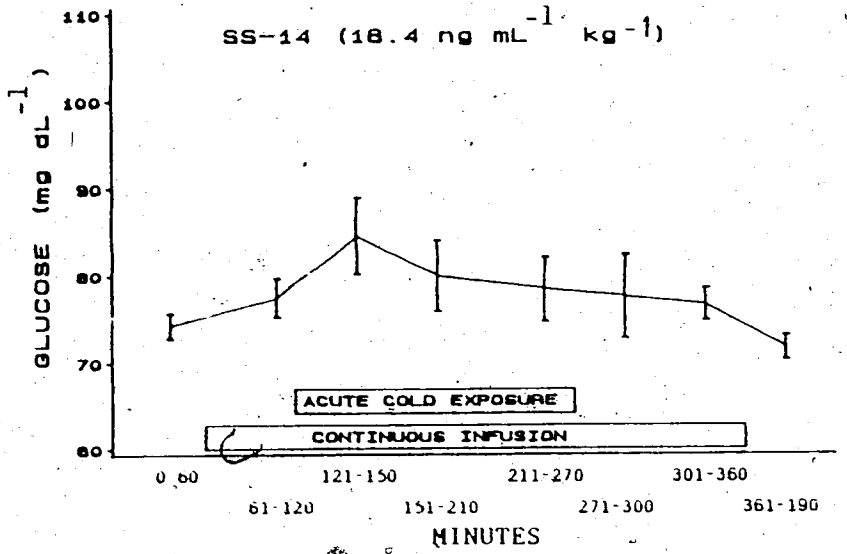
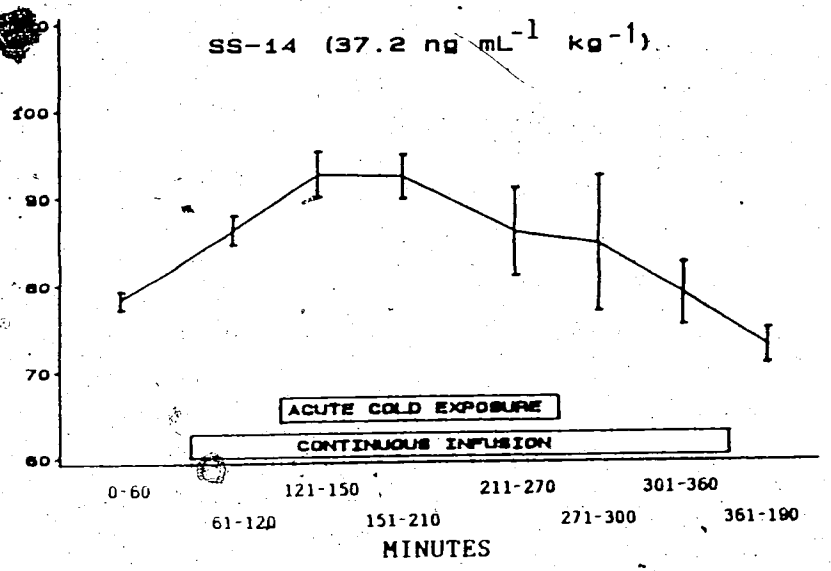

SALINE
SS-14 (8.9 ng mL-1 kg-1)
SS-14 (18.4 ng mL-1 kg-1)
SS-14 (37.2 ng mL-1 kg-1)
GROWTH HORMONE (ng mL-1)
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES

Figure II-6. Effect of primed continuous infusion of somatostatin-14 (8.9, 18.4, and 37.3 ng min^{-1} kg^{-1}) on concentration of plasma glucose in three acutely (2 h) cold stressed lambs. Somatostatin was initiated at 60 min and terminated at 360 min; acute cold exposure was introduced at 120 min and, terminated at 270 min (mean ± SEM).


SALINE
GLUCOSE (mg dL^{-1})
110
100
90
80
70
60
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES


SS-14 (8.9 ng mL^{-1} kg^{-1})
110
100
90
80
70
60
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES


SS-14 (18.4 ng mL^{-1} kg^{-1})
GLUCOSE (mg dL^{-1})
110
100
90
80
70
60
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES


SS-14 (37.2 ng mL^{-1} kg^{-1})
100
90
80
70
60
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES

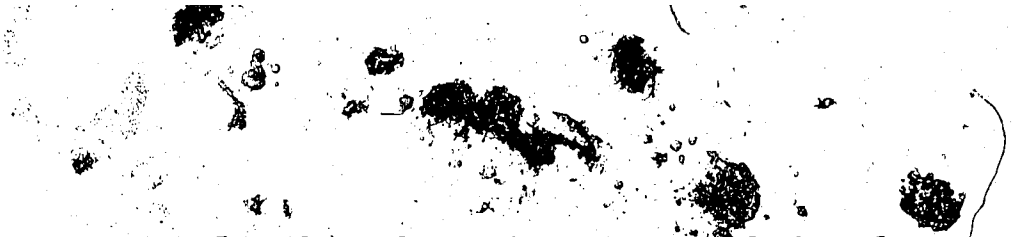
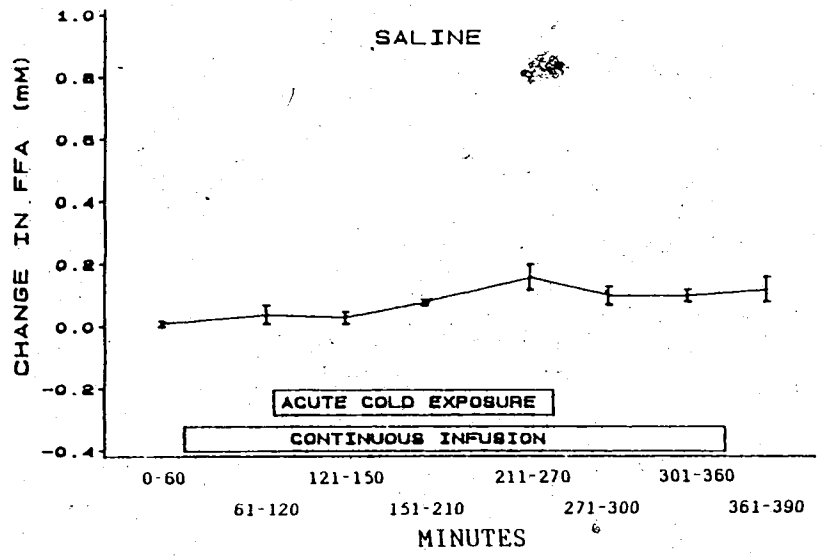
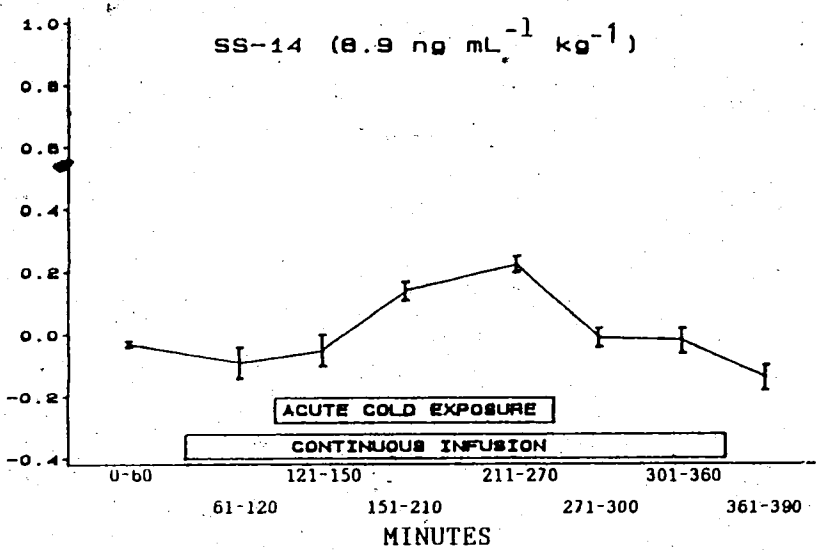
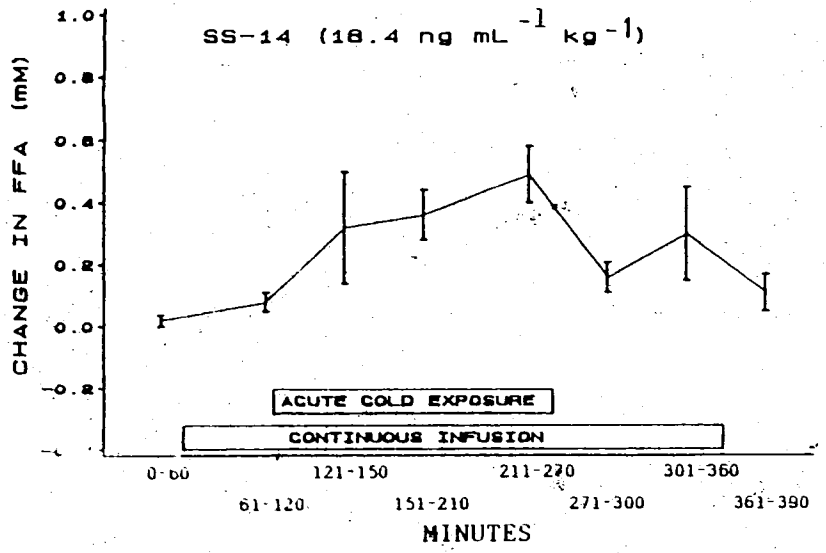
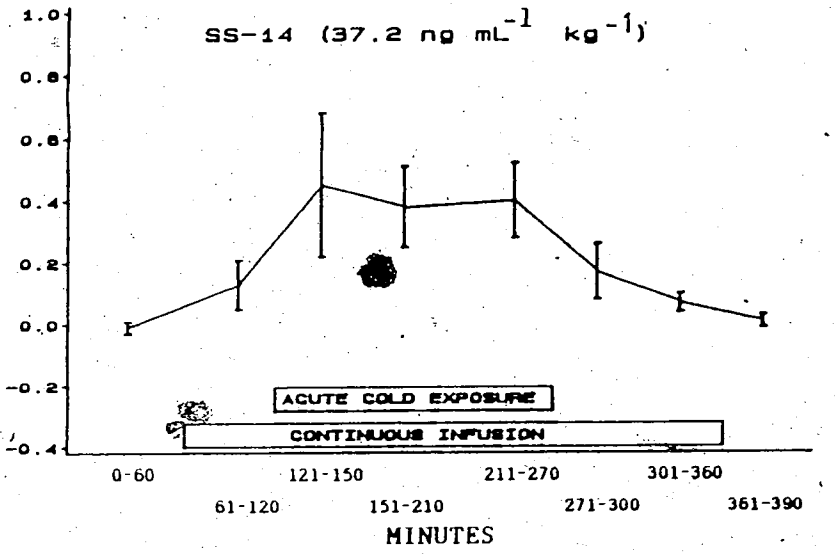


Figure II-7. Effect of primed continuous infusion of somatostatin-14 (8.9, 18.4, and 37.3 ng min^{-1} kg^{-1}) on concentration of plasma free fatty acids in three acutely (2 h) cold stressed lambs. Somatostatin was initiated at 60 min and terminated at 360 min; acute cold exposure was introduced at 120 min and terminated at 270 min (mean ± SEM).


SALINE
CHANGE IN FFA (mM)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES


SS-14 (8.9 ng mL^{-1} kg^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES


SS-14 (18.4 ng mL^{-1} kg^{-1})
CHANGE IN FFA (mM)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES


SS-14 (37.2 ng mL^{-1} kg^{-1})
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
ACUTE COLD EXPOSURE
CONTINUOUS INFUSION
0-60
61-120
121-150
151-210
211-270
271-300
301-360
361-390
MINUTES

III. EFFECT OF SOMATOSTATIN ON HORMONAL AND METABOLITE CONCENTRATION, METABOLIC RATE, THERMOREGULATION, AND GUT MOTILITY IN CHRONICALLY COLD EXPOSED SHEEP.

INTRODUCTION

Regulation of the supply of substrates for thermogenesis during cold exposure is controlled by a variety of mechanisms. These include neural and neurohormonal and neuropeptide-hormonal alteration of nutrient assimilation (Alexander, 1979). Somatostatin, a tetradecapeptide, plays an important role as a chalone in many of these regulatory pathways (Reichlin, 1986), and may be important in the response to stressors such as cold exposure.

One recent study showed that the growth potential of young lambs was maintained during acute cold stress by active immunization against somatostatin (Laarveld et al., 1986), thus suggesting an interaction between somatostatin and response to cold exposure. Numerous metabolic hormones, including thyroid hormones, growth hormone, insulin, glucagon, gut hormones or adrenal hormones, play an important role in altering intermediary metabolism or substrate mobilization and are altered by infusion of somatostatin (Brockman and Laarveld, 1986; Schusdziarra, 1980).

Somatostatin inhibits release of thyroid stimulating hormone (TSH) from the pituitary (Reichlin, 1983), and possibly tri-iodothyronine (T_3) and thyroxine (T_4) from the thyroid gland (M^{c}Quillan, 1980). Passive immunization against somatostatin not only increases basal plasma TSH, but also potentiates the TSH response following exposure to cold stress in rats (Ferland et al., 1976).

At high doses of somatostatin both basal glucagon and glucagon response to numerous secretatogues is inhibited in rats, humans, dogs, and sheep (Schusdziarra, 1980) and as a consequence, glucagon-dependent gluconeogenesis and glycogenolysis is reduced, thus producing a decline in plasma glucose concentration. However, somatostatin also inhibits insulin secretion and thus would stimulate lipolysis and release of free fatty acids and release of substrates for gluconeogenesis from muscle and adipose tissue (Bell, 1980).

Barry et al. (1985) have proposed that the prime site of somatostatin action in lambs infused with somatostatin for a prolonged period was within the lower digestive tract where it could be involved in regulation of nutrient absorption. Similar findings have been reported for humans, dogs, and rats (Schusdziarra, 1980).

The objectives of this study were to examine the effect of somatostatin-14 at physiological doses, on gastrointestinal motility, hormone and metabolite concentrations, whole body metabolic rate, and thermoregulation in response to chronic cold exposure.

MATERIALS AND METHODS

Animals and Their Management:

Six ewe lambs (45.8± 2.8 kg body weight (BW)) fitted with ruminal and duodenal cannulae were housed in individual metabolism crates in controlled environment chambers. During each of two periods, three lambs were adapted for 32 d or longer (average of 45.6 ± 7.3 d) to either a thermoneutral (TN) (17.4°C) or a chronic cold (CC) (-0.9°C) environment prior to experimental measurements. During the second period one sheep was inadvertently removed from the cold chamber for 24 h at day 45 and was subsequently placed back in the cold for 11 d

before measurements began. One sheep died between periods and was removed from the analysis of the data. Measurements on individual animals were taken on consecutive days within each environment for 90 min prior to and during a 300 min primed continuous infusion of either saline or somatostatin-14 (SS-14) (Sigma Chemical Co., St. Louis, Mo.) (6.7 ± 0.5 ng min^{-1} kg^{-1} BW). The priming dose (0.35 ± 0.06 μg kg^{-1}) of SS-14 was given to reduce the length of time required to reached the new equilibrium. The order of treatments was alternated between animals and across environments.

Lambs received a pelleted 18.8% crude protein barley:soybean concentrate diet, supplemented with vitamin A, D, E and limestone (Appendix Table 2), at 25.6 g DM d^{-1} kg^{-1} BW once daily at 1600 h. Lambs had access to water and cobalt iodized salt free choice and were shorn biweekly.

Experimental Protocol:

One week prior to each experimental period lambs were accustomed to metabolic hoods and to experimental recording and sampling procedures. Sixteen h prior to the first experiment a chronic indwelling polyvinyl chloride tubing (1.68 mm ID, Argyle, St. Louis, Mo.) was inserted into each external jugular vein and was filled with dilute heparin solution (100 IU mL^{-1}) to keep cannulae patent. One catheter was used for infusion and one for blood sampling. On two occasions difficulty was encountered catheterizing the second jugular vein and the infusion catheter was inserted into the radial vein. For recording of skin temperature, a copper constantan T type thermocouple (Thermo Electric, Edmonton) enveloped in a 4 x 4 cm^2 surgical tape patch was adhered to a closely sheared patch of skin using contact cement. One thermocouple

was placed over the biceps femoris muscle, while one was placed on the lateral side of each hindleg (in the hollow between the metatarsal bone and flexor digitorum brevis) 15 cm inferior to the hock.

Oxygen consumption and carbon dioxide production were determined from the oxygen and carbon dioxide concentrations in air entering and leaving a continuously ventilated hood with a ventilation rate of 57 L h^{-1}, as described by Young et al. (1975). The oxygen and carbon dioxide concentrations were measured by a Beckman F3 paramagnetic oxygen analyzer and a Beckman Model 864 infrared analyzer (Beckman Instruments, Inc., Fullerton, Ca.), respectively. Calibration of the calorimetry equipment for oxygen measurement was carried out according to Young et al. (1984). Metabolic rate was calculated according to the method of McLean (1972).

Two h prior to the start of each experiment lambs were placed in metabolic hoods, previously calibrated skin thermocouples and a rectal thermocouple were connected to an Apple II plus computer and a DT100 datataker (Dycor Industrial Research, Edmonton) for recording the thermocouple potentials. Also at this time jugular vein catheters were exposed, and the duodenal and weighted reticular polyvinyl chloride open-tipped catheters were inserted into the duodenum and reticulum. Patency of the reticular and duodenal catheters was maintained by continuous infusion of distilled water at 0.5 mL min^{-1}. Reticular and duodenal contractions were simultaneously monitored continuously by a pair of inline P23 Series pressure transducer (Gould Instruments Inc., Hato Rey, Puerto Rico), amplified, and charted by a Beckman R-612 Dynograph Recorder (Electronic Instrument Division, Schiller Park, Illinois). Spiking activity was manually tabulated.

Table III-2. Effect of somatostatin (SS) on carbon dioxide production (L h^{-1}), oxygen consumption (L h^{-1}), heat production (KJ h^{-1}), and respiratory quotient in lambs adapted to thermoneutral (TN) or cold (CC) environment.

	Experimental Treatment					
	Environment			Infusion		
Parameter	TN	CC	SEM	Saline	SS	SEM
Carbon Dioxide Production	12.6[a]	15.7[b]	0.8	14.4	13.9	0.2
Oxygen Consumption	13.5[a]	19.1[b]	0.4	16.6[a]	16.0[b]	0.1
Heat Production	276.0[a]	390.3[b]	4.4	340.0[a]	326.4[b]	0.8
Respiratory Quotient	0.99	0.83	0.06	0.91	0.90	0.02

a,b Means within a metabolic parameter and within an experimental treatment are different ($P < 0.05$).

Table III-3. Effect of primed continuous infusion of somatostatin-14 (6.7 ng min^{-1} kg^{-1}) on number of contractions and duration of irregular spiking activity (ISA), regular spiking activity (RSA), or nonspiking activity (NSA) within a duodenal migrating myoelectric complex (MMC) or on the entire MMC in five lambs exposed to either thermoneutral (TN) or chronic cold (CC) environment.

	Experimental Treatment					
	Environment			Infusion		
Parameter	TN	CC	SEM	Saline	SS	SEM
Number of contraction/ MMC						
During ISA	59	72	4.0	63	69	6.2
During RSA	21	24	3.1	22	22	1.4
Total	80a	96b	2.3	85	91	6.1
Duration (min)						
NSA	5.2	6.2	0.8	5.6	5.8	1.3
ISA	71.5	74.9	12.0	62.1	84.3	7.7
RSA	4.5	4.5	1.1	4.3	4.7	0.2
MMC	81.8	85.5	11.6	72.4	94.8	7.9

a,b Means within a parameter and within an experimental treatment are different ($P < 0.05$).

Figure III-1. Effect of primed continuous infusion of somatostatin-14 (6.7 ng min^{-1} kg^{-1}) on concentration of plasma somatostatin-like immunoreactivity in five lambs adapted to thermoneutral (TN) or cold (CC) environment. (Primed infusion was initiated at 90 min) (mean ± SEM).

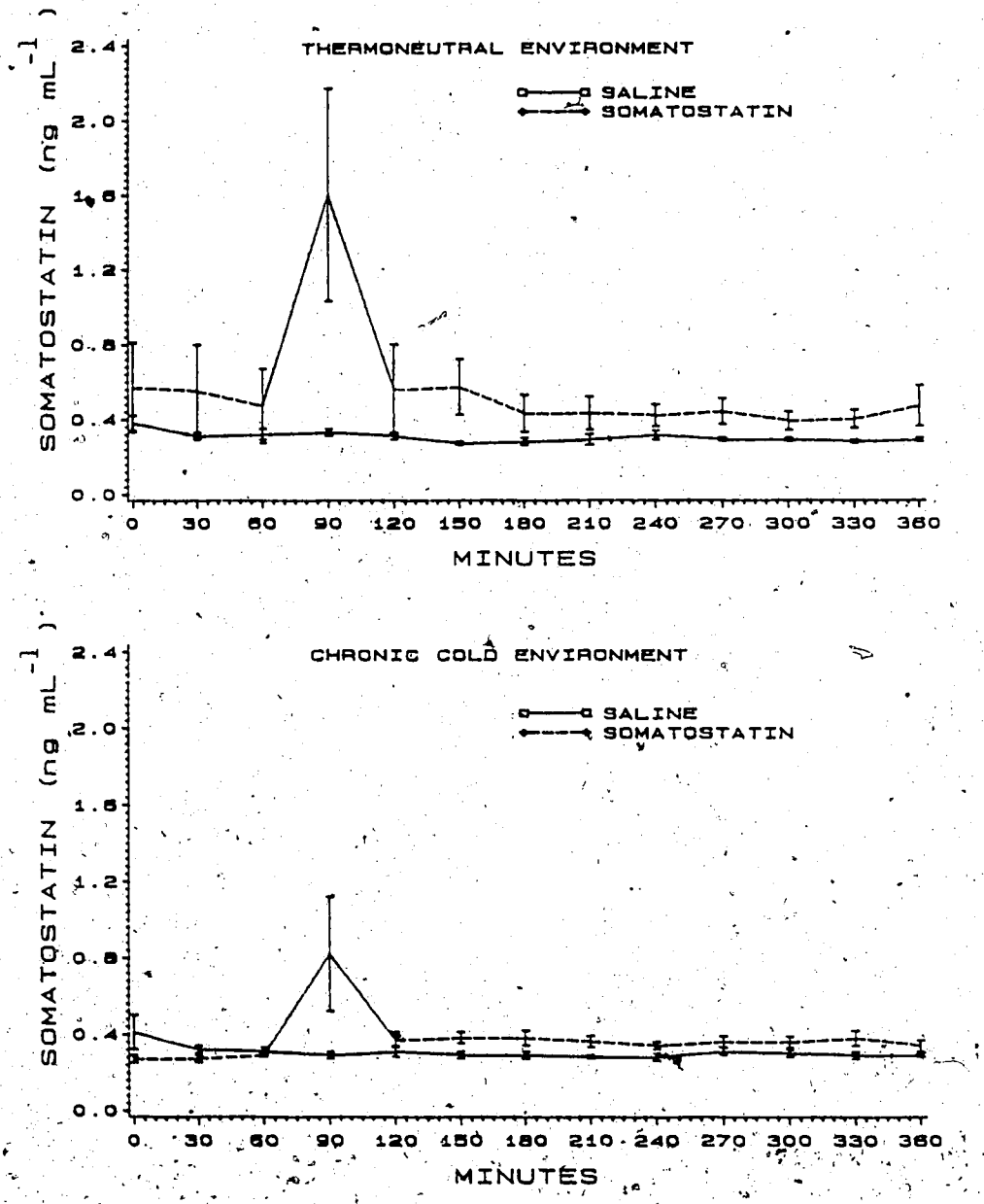


Figure III-2. Effect of primed continuous infusion of somatostatin-14 (6.7 ng min^{-1} kg^{-1}) on plasma growth hormone concentration in five lambs adapted to thermoneutral (TN) or cold (CC) environment. (Primed infusion was initiated at 90 min) (mean ± SEM).

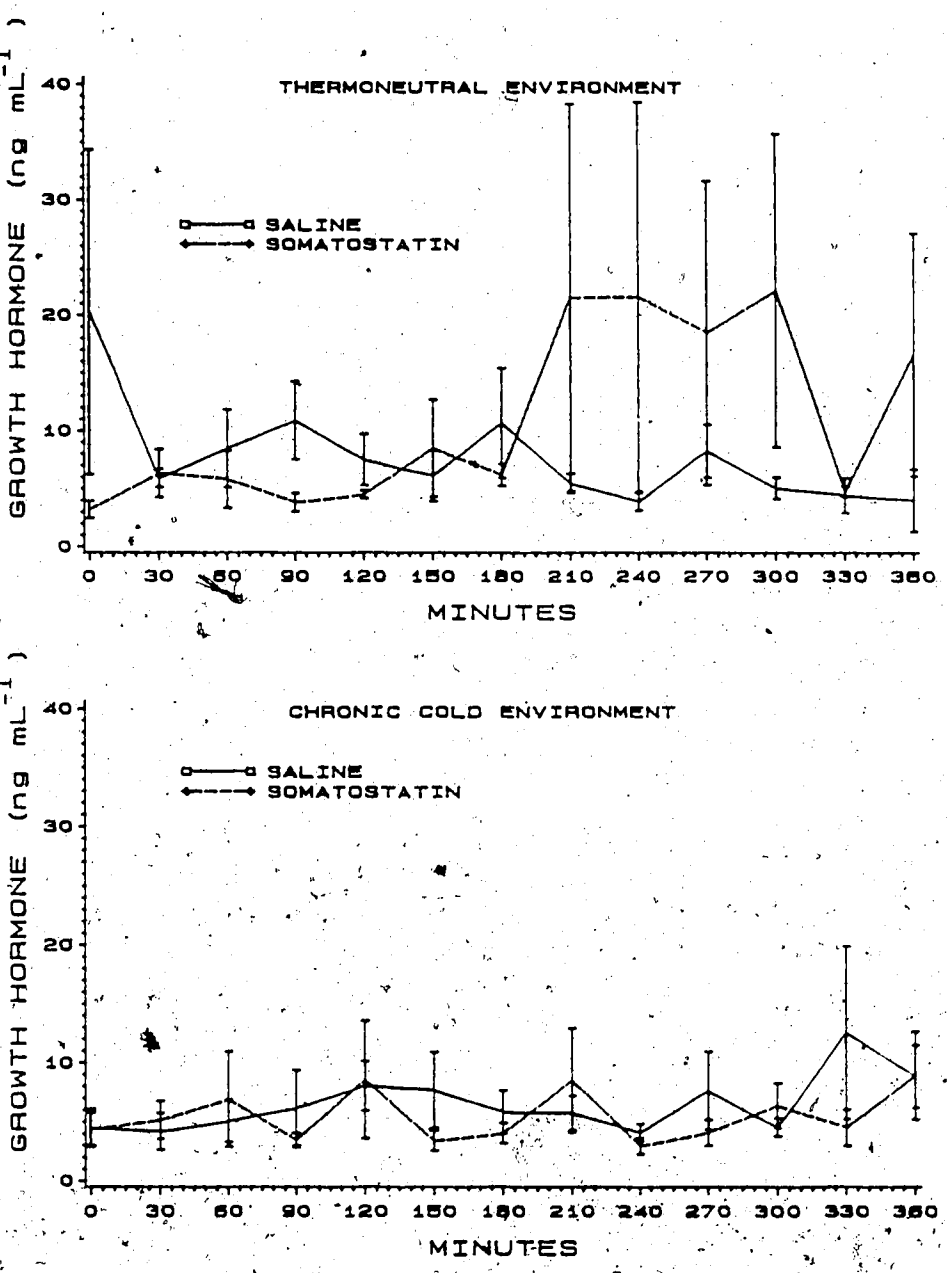
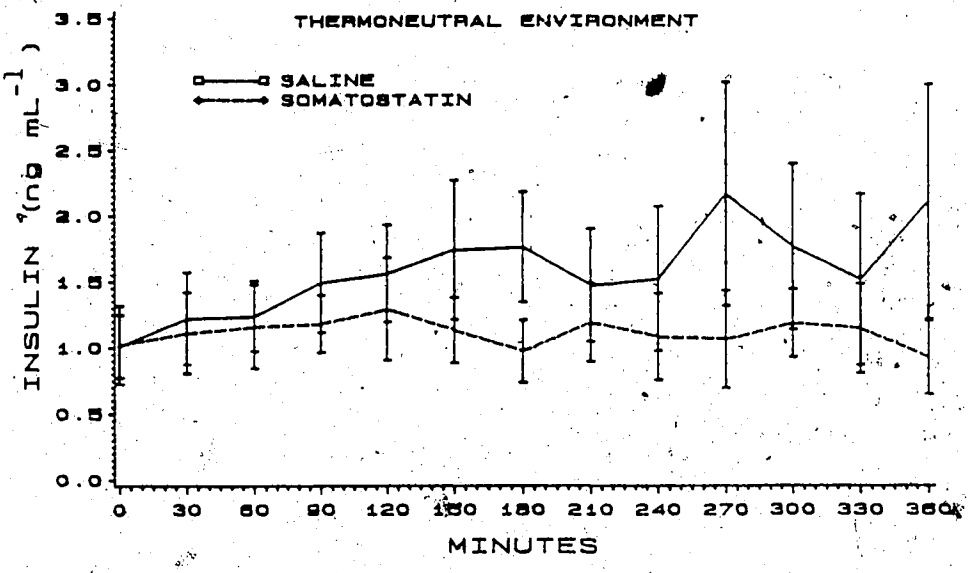


Figure III-3. Effect of primed continuous infusion of somatostatin-14 (6.7 ng min^{-1} kg^{-1}) on plasma insulin concentration in five lambs adapted to thermoneutral (TN) or cold (CC) environment. (Primed infusion was initiated at 90 min) (mean ± SEM).



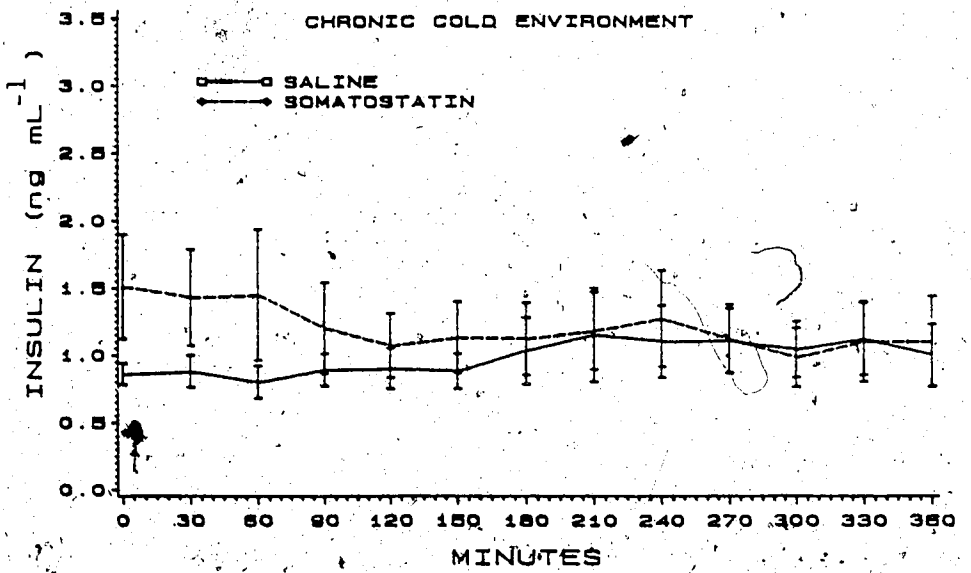


Figure III-4. Effect of primed continuous infusion of somatostatin-14 (6.7 ng $min^{-1} \cdot kg^{-1}$) on plasma glucagon concentration in five lambs adapted to thermoneutral (TN) or cold (CC) environment. (Primed infusion was initiated at 90 min) (mean ± SEM).

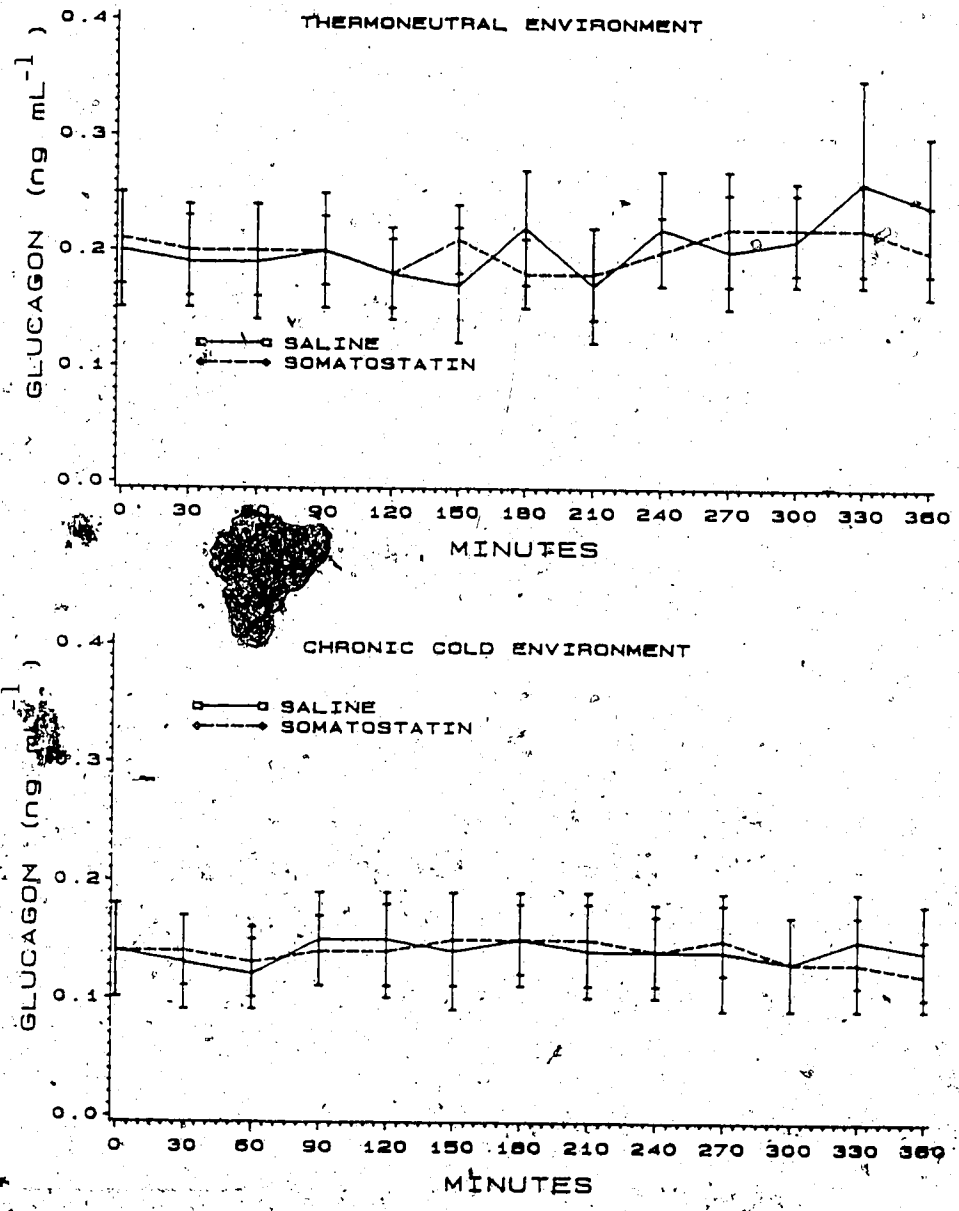


Figure III-5. Effect of primed continuous infusion of somatostatin-14 (6.7 ng min^{-1} kg^{-1}) on insulin:glucagon ratio in five lambs adapted to thermoneutral (TN) or cold (CC) environment. (Primed infusion was initiated at 90 min) (mean ± SEM).

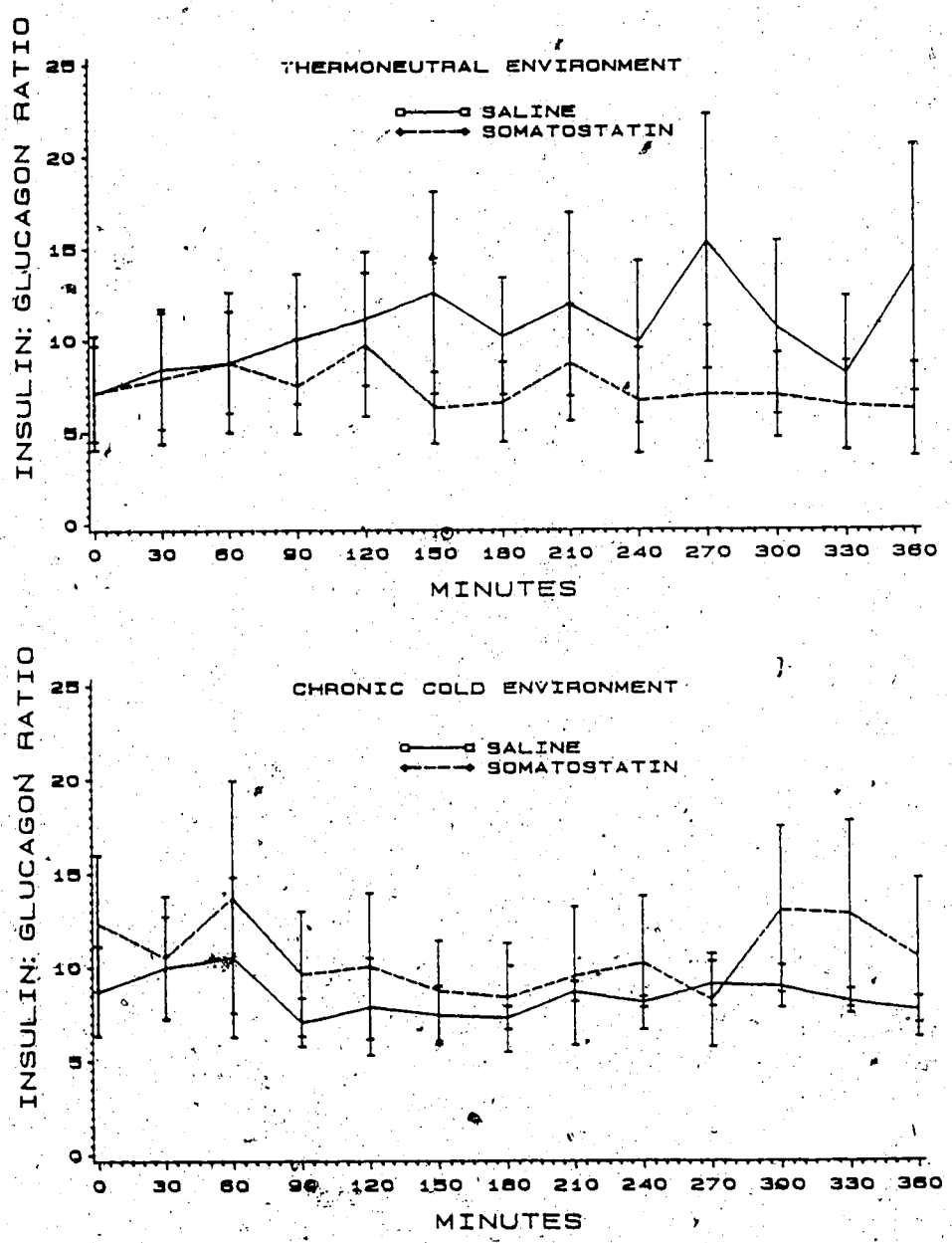
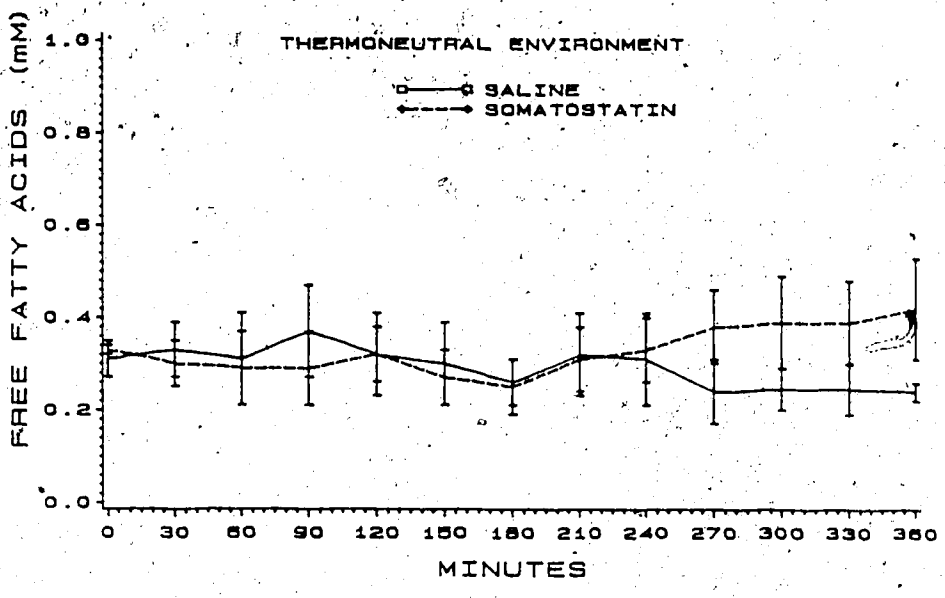


Figure III-6. Effect of primed continuous infusion of somatostatin-14 (6.7 ng min^{-1} kg^{-1}) on plasma free fatty acids concentration in five lambs adapted to thermoneutral (TN) or cold (CC) environment. (Primed infusion was initiated at 90 min) (mean ± SEM).



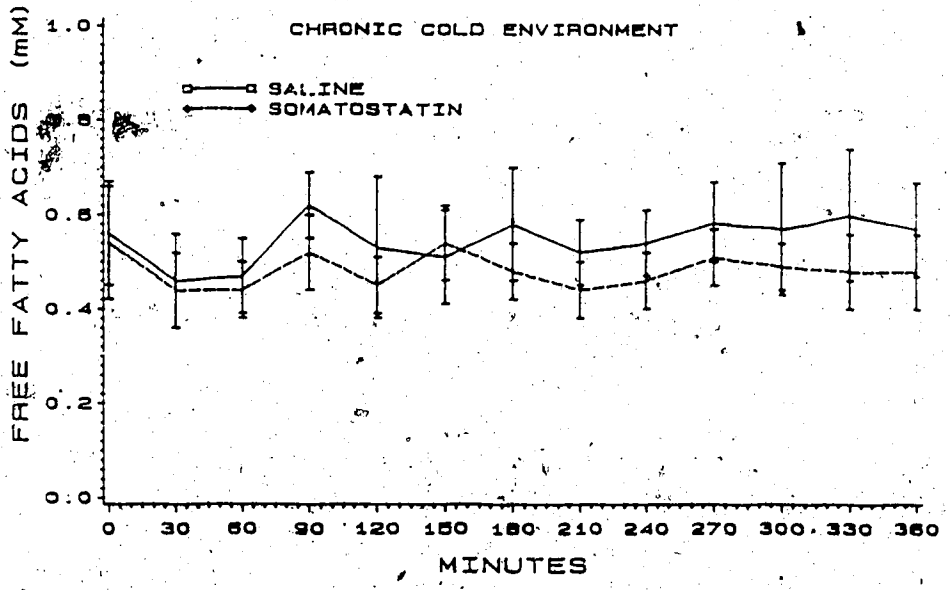


Figure III-7. Effect of primed continuous infusion of somatostatin-14 (6.7 ng min^{-1} kg^{-1}) on plasma glucose concentration in five lambs adapted to thermoneutral (TN) or cold (CC) environment. (Primed infusion was initiated at 90 min) (mean ± SEM).

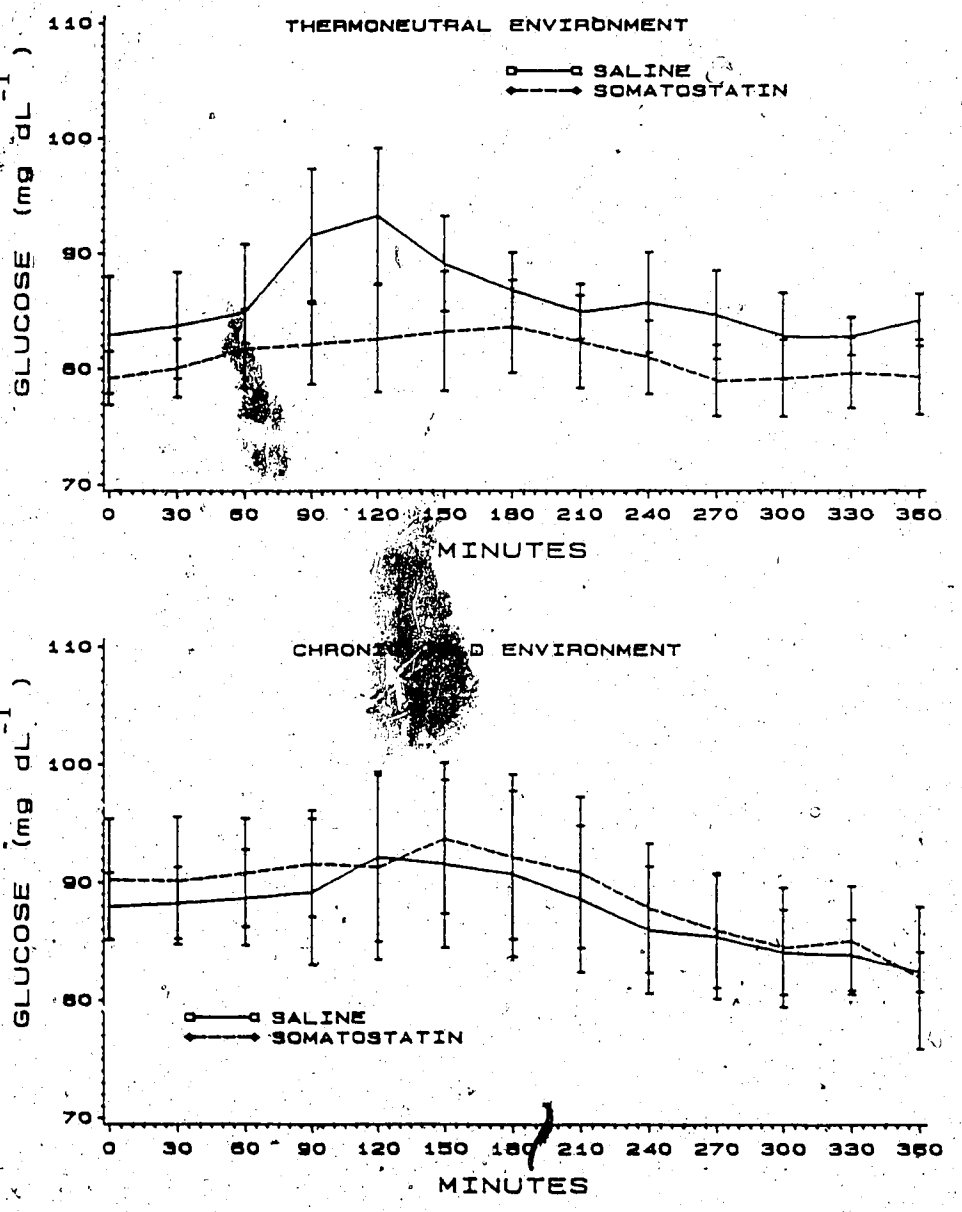
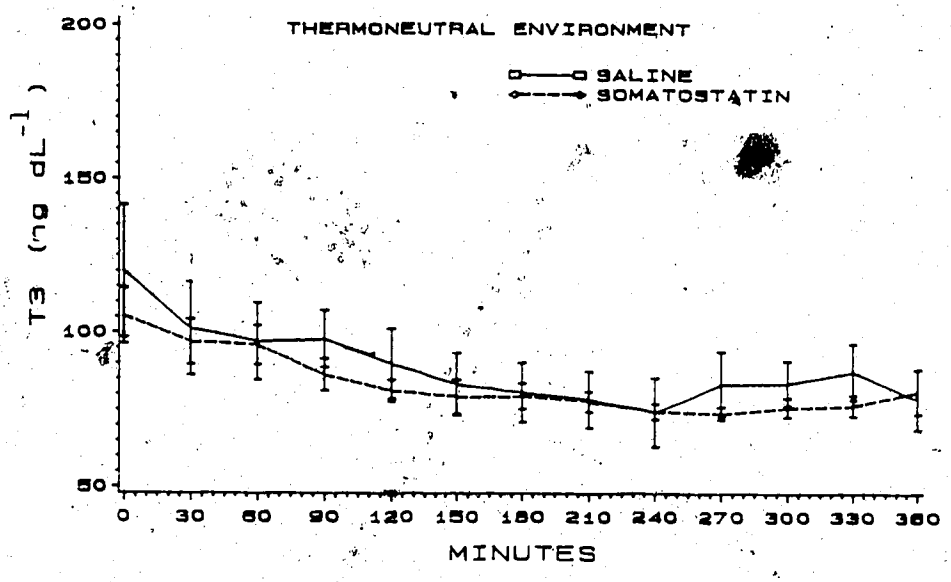


Figure III-8. Effect of primed continuous infusion of somatostatin-14 (6.7 ng min^{-1} kg^{-1}) on plasma tri-iodthyronine (T3) concentration in five lambs adapted to thermoneutral (TN) or cold (CC) environment. (Primed infusion was initiated at 90 min). (mean ± SEM).



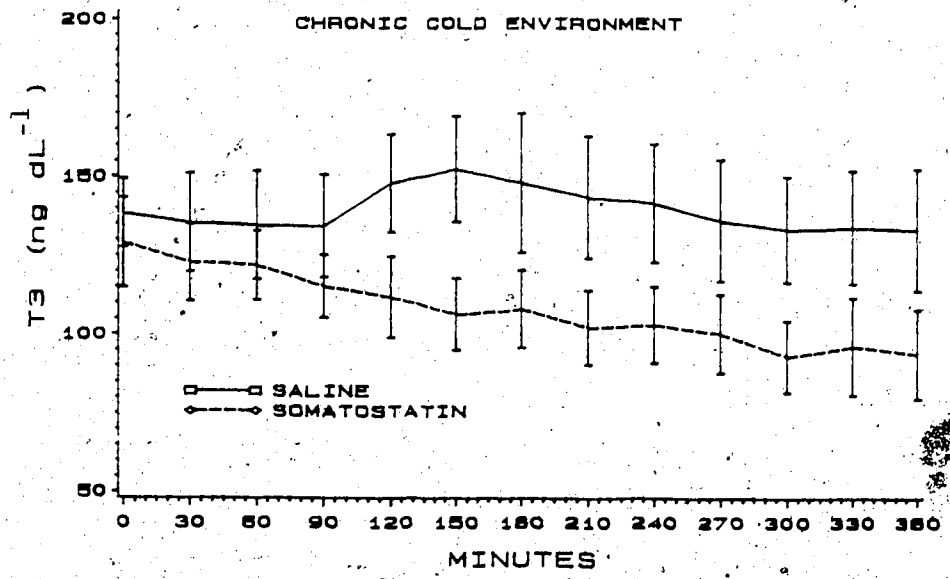
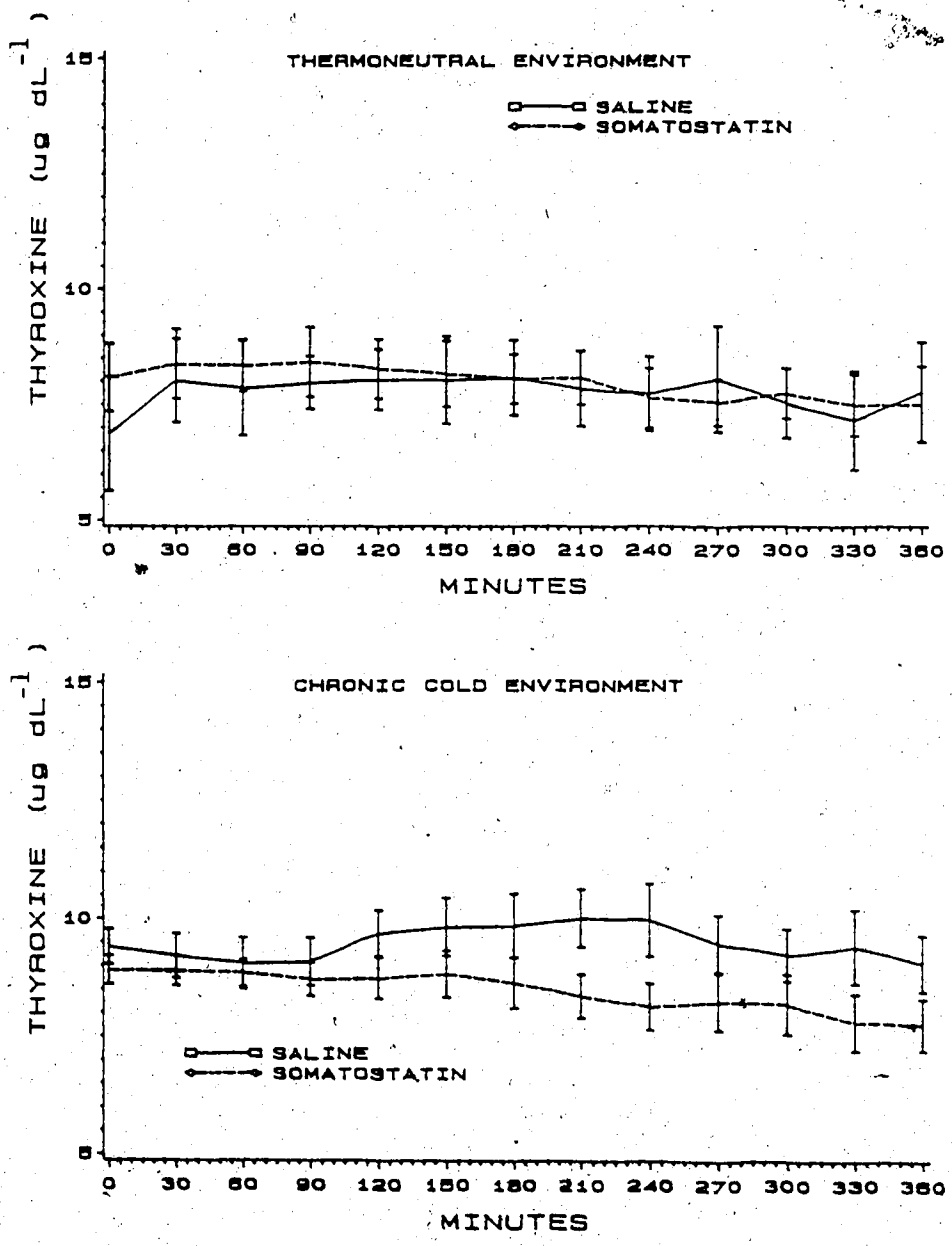


Figure III-9. Effect of primed continuous infusion of somatostatin-14 (6.7 ng min^{-1} kg^{-1}) on thyroxine concentration in five lambs adapted to thermoneutral (TN) or cold (CC) environment. (Primed infusion was initiated at 90 min) (mean ± SEM).



IV. METABOLIC CLEARANCE RATE OF SOMATOSTATIN AND HORMONAL RESPONSES TO FEEDING AND SOMATOSTATIN-14 INJECTION IN CHRONICALLY COLD-EXPOSED SHEEP.

INTRODUCTION

Somatostatin is a tetradecapeptide secreted by the hypothalamus, pancreas, gastrointestinal tract, and peptidergic neurons. In nonruminants it plays an important role in altering nutrient uptake and partitioning through direct and indirect effects on tissues, and by inhibition of other metabolic hormones (Schusdziarra and Schmid, 1986). In ruminants, there is relatively little information on the role played by somatostatin in these events.

Release of somatostatin in response to feeding has not been studied in ruminants, although abomasal infusion of casein plus methionine in growing lambs significantly decreased plasma somatostatin concentrations (Barry et al., 1982). Conversely, increased dietary protein stimulated somatostatin release in preruminant lambs (Reid et al., 1984). Since somatostatin concentrations were not altered by abomasal dilation (Vlaminck et al., 1986), plasma somatostatin concentrations are thought to be responsive to dietary composition rather than to gut distension. These results agree with research done with dogs and humans (see Schusdziarra and Schmid, 1986), in which meal size had no effect on somatostatin secretion, while the nutrient composition of the diet and supply of nutrients to the lower gut appeared to be major factors stimulating somatostatin secretion from D-cells of the gut (Schusdziarra and Schmid, 1986).

Somatostatin, at pharmacological doses, has been shown to reduce or inhibit secretory responses of growth hormone, insulin, and glucagon to numerous secretagogues in ruminants (Bryce et al., 1975; Brockman and Laarveld, 1986) and nonruminants (Brockman and Laarveld, 1986), while having no effect on basal secretion of growth hormone (Varner et al., 1980). Exogenous somatostatin appears to reduce basal concentrations of insulin and glucagon (Brockman and Greer, 1980), although these responses are not always consistent (Brockman and Laarveld, 1986).

The extent to which somatostatin may be involved in modulating physiological responses of ruminants in different thermal environments has not been studied. However, increases in glucagon, catecholamine, glucocorticoid, and growth hormone concentrations are associated with increased energy mobilization during stresses such as acute cold exposure (Bassett and Alexander, 1971), exercise (Brockman, 1979), or hypoglycemia (Brockman et al., 1975).

The objectives of this experiment were to determine the effect of cold exposure, feeding, and a bolus injection of somatostatin-14 on plasma hormone and metabolite concentrations and to determine the influence of environmental temperature on metabolic clearance rate of somatostatin.

MATERIAL AND METHODS

Animals and Their Management:

Ten crossbred wether lambs (44.9 ± 0.96 kg) were housed for 9 [illegible] either a thermoneutral (TN)(20°C) or chronic cold (CC)(0°C) environ[illegible] with 5 lambs in each environment. Lambs were shorn biweekly during th[illegible] adaptation period and 6 d prior to the experiment. Lambs were fed a

19.6% crude protein (CP) pelleted barley:soybean diet (86.1% dry matter (DM), supplemented with vitamin ADE and limestone (Appendix Table 3), at a level of 24.8 g DM kg^{-1} body weight (BW) once daily at 0900 h. Feed intake was adjusted after weekly weighings. On the day of blood sampling, feed not consumed within 75 min postfeeding was removed and weighed.

Experimental Protocol:

During the week prior to blood sampling, lambs were accustomed to receiving their daily diet in individual headstalls and to the blood sampling collection procedure. Polyvinyl chloride catheters were inserted into a jugular vein 18 h prior to collection of blood samples. Blood was collected at 90, 60, 30 min, and immediately prior to feeding (0900 h), and 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, and 210 min postprandially. Catheters were kept patent with heparinized (40 IU mL^{-1}) saline (0.9% NaCl). Two h after feeding, lambs were released from the headstalls for 15 min to permit access to water and exercise.

On the same afternoon, 240 min postprandially, lambs were given a somatostatin-14 (SS-14) (Sigma Chemical Co., St. Louis, Missouri) injection over 15 sec (1.13 ± 0.08 $\mu g\ kg^{-1}$ BW) for determination of kinetic parameters. Blood samples were drawn 15 min before and immediately prior to SS-14 injection. After injection, the three-way stopcock was rinsed in sterile saline and 20 mL of sterile saline was rapidly infused to flush the catheter. The catheter was further cleared by withdrawal of 25 mL of blood prior to the 1 min blood sample. Blood samples were taken at 1, 2, 3, 5, 7, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90, 105, 120, and 150 min postinjection. Catheters were not flushed after the 1 or 2 min blood samples. Between the 3 min and 150 min

samples the catheter dead space was filled with heparinized saline (40 IU mL^{-1}). A 3 mL discard was drawn immediately prior to all blood samples to clear the dead space in the catheter.

Blood samples (8 mL) were immediately transferred to 16 x 100 mm glass tubes containing heparin (40 IU mL^{-1} of blood) and NaF (4% w/v)(15 μL mL^{-1} of blood), mixed by inversion, and kept on ice for up to 2 h until centrifuged for 15 min at 2280 x g. Aliquots of plasma were frozen at -20°C until analyzed. The aliquot for somatostatin-like immunoreactivity (SLI) and glucagon determination contained Trasylol (aprotinin, Miles Laboratories, Mississauga, Ontario; 500 IU mL^{-1} plasma).

Blood sample collections were run on concurrent days for the two environmental groups. Concentrations of free fatty acids and glucose were determined by enzyme specific kits (acyl CoA synthetase, WAKO Pure Chemical Industry, Dallas, Texas; glucose oxidase, Sigma Chemical Co., St. Louis, Missouri). Intra-assay and inter-assay coefficients of variation for free fatty acids and glucose were 6.4 and 7.8%; and 1.1 and 2.8%, respectively. Somatostatin-like immunoreactivity, glucagon, insulin, and growth hormone concentrations were determined by double antibody radioimmunoassay.

Somatostatin-Like Immunoreactivity Assay:

Standard curves were constructed with cyclic somatostatin-14 (Sigma Chemical Co., St. Louis, Missouri) in the range of 2.5 to 4000 pg mL^{-1} and when compared to similarily prepared standard curves using cyclic somatostatin-14 from

triplicate (100 μL). Plasma (25 μL) was assayed in duplicate in 12 x 75 mm glass disposable tubes. Samples which did not fall on the assay curve were reassayed and the volume of plasma was reduced. Total assay volume was 700 μL. To 100 μL of standard or diluted plasma was added 200 μL of assay buffer (0.05 M phosphate buffer, pH 7.5, 0.25% BSA), 100 μL of rabbit anti-somatostatin antibody, which was kindly provided by B. Laarveld University of Saskatchewan, Saskatoon, Saskatchewan (1:800,000 final dilution in 700 μL), 100 μL of ^{125}I-1-tyr-somatostatin (10,000 cpm/tube, cat.# NEX-129, Dupont Canada Ltd, Mississauga, Ontario), and 100 μL of normal rabbit serum diluted 1:250 with assay buffer. At this dilution 30-40% of the tracer was bound to antibody in the absence of unlabelled peptide. Tubes were vortexed and incubated for 24 h at 4°C. Separation of bound from free ^{125}I-1-tyr-somatostatin was carried out by double antibody precipitation by adding 0.37 units of goat anti-rabbit gamma globulin (Calbiochem, San Diego, CA.) in 100 μL of assay buffer to each tube. Tubes were vortexed and incubated overnight at 4°C prior to centrifugation at 3000 x g for 15 min. The supernatant was aspirated and the pellet was counted on a Beckman 8400 Gamma Counter for 2 min. Intra- and inter-assay coefficients of variation for a sample averaging 97 pg mL^{-1} were 7.0% and 11.0% and assay sensitivity was 10 pg mL^{-1}. Inhibition curves for a pool of ovine plasma were parallel to somatostatin standard curves (Appendix Figure 1). Recovery on a standard addition from 10 to 200 pg mL^{-1} somatostatin to 10 μL plasma was 94.9% ± 4.1 (mean ± SEM). Displacement of the standard curve by addition of 10 μL of plasma to each standard is shown in Appendix figure 2. Rabbit anti-somatostatin antisera was found to

crossreact 100% with 1-tyr-somatostatin and somatostatin-28, 30-40% with 11-tyr-somatostatin, 0.001% with neurotensin and did not crossreact with IGF-I, bGH, bPRL, insulin, glucagon, oxytocin, TRH, or LHRH (B. Laarveld, personal communication).

Protocols for Other Hormone Assays:

Protocols for glucagon (Harris et al., 1979), insulin (Mears et al., 1988), and growth hormone (Mears et al., 1988) assays were as published by these authors. Rabbit anti-glucagon antisera (04A) was purchased from Dr. R.H. Unger University of Texas, Dallas, Texas, and glucagon (lot # 258-25J-120) was generously provided by Dr. M.A. Root of Eli Lilly Company, Indianpolis, In. Guinea-pig anti-bovine insulin (cat. # 65-101) was purchased from Miles Laboratories, while ovine insulin for standards (cat. I-9254, 23.2 IU mg^{-1}) was purchased from Sigma Chemical Co., St. Louis, Mo. Rabbit anti-ovine-GH2 and ovine GH (NIADDK-oGH-13, 1.5 IU mg^{-1}) was kindly provided by Dr. A.F. Parlow of the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, Torrance, Ca. Bovine GH (NIH-GH-B18, 0.56 IU mg^{-1}) was used for iodination. Samples used in the same comparison tests were analyzed in a single assay and animals within each environment were equally represented throughout glucose, free fatty acid, SLI, and glucagon assays, while insulin and growth hormone were analyzed in a single assay. Intra- and inter-assay coefficients of variation for glucagon, insulin, and growth hormone assays were 8.9%, 8.3%, and 10.2%; 11.1%, 11.0%, and 12.3% for samples averaging 2.24, 2.19, and 13.53 ng mL^{-1}, respectively.

Somatostatin Kinetics:

Disappearance curves for plasma SS-14 concentration expressed as a fraction of dose were fit to a two-component exponential model (Shipley and Clark, 1972) using a non-linear iterative procedure (SAS User's Guide: Statistics, 1982). Since plasma concentrations of SLI had returned to preinfusion levels by 15 min, only blood samples taken over the first 15 min were used in fitting disappearance curves. When plotted on a semi-log (natural) scale, disappearance curves of concentrations expressed as fraction of dose were curvilinear. Equations of the line for the TN and CC environments were $Y=0.000365e^{-1.851t} + 0.0000115e^{-0.0497t}$ and $Y=0.000240e^{-0.847t} + 0.0000112e^{-0.0397t}$, respectively. Kinetic parameters were calculated as described by de Boer et al. (1986).

Statistical Analysis:

Kinetic parameters were subjected to analysis of variance using SAS (SAS User's Guide: Statistics, 1982) where the model included environment tested against sheep within environment. Hormonal data were analyzed using a repeated measures design and the model included environment, sheep within environment, time, environment by time interaction, and time by sheep within environment as the residual. Where significant, variables within the model were tested by Student-Newman-Kuels' multicomparison test (Steele and Torrie, 1980).

RESULTS

Average daily weight gains of the lambs were 94 and 161 g d^{-1}, SEM= 73.0, in the cold and TN environments, respectively. At the restricted level of intake offered, lambs in both environments routinely consumed all of their meal within 2 h.

Effect of Feeding:

Overall plasma SLI concentrations were not influenced by environmental temperature or by feeding (0.336 vs 0.276 ng mL^{-1} for TN and CC environments, respectively) (Figure IV-1). One animal in the TN group had SLI concentrations double those of other animals on that treatment. Eliminating values for this lamb produced an overall mean of 0.289 ± 0.004 ng mL^{-1}.

A significant proportion ($P<0.001$) of the variability in plasma growth hormone concentration could be attributed to individual animal variation and to a pulsatile secretion occurring at 2 - 3 h intervals. Mean plasma growth hormone concentration increased ($P< 0.05$) transiently prior to feeding, was similar in both environments at time 0, and then significantly ($P< 0.001$) fell within 45 min after feeding (Figure IV-2). Growth hormone concentration declined ($P< 0.001$) across environments from an average prefeeding value of 3.0 ng mL^{-1} to 1.6 ng mL^{-1} at 120 min postprandial. A significant ($P< 0.03$) environment by time interaction for growth hormone concentration could be attributed to the larger decline in the TN group. In the cold environment, plasma growth hormone concentrations were stable from 60 until 120 min, whereas values continued to decline in the TN group. Both groups showed a small nonsignificant transient increase over the next 30 min, but returned to minimal values by 210 min. Mean growth hormone concentrations over the whole experiment did not differ between environments.

Plasma glucagon values were higher ($P< 0.05$) in the TN environment (TN-0.27 vs CC-0.19 ng mL^{-1}; SEM- 0.004). None of the postfeeding values for glucagon were significantly different from prefeeding values in either environment (P

Plasma insulin concentrations gradually increased postprandially in the TN environment, were different ($P< 0.001$) from prefeeding values by 75 min after feeding (Figure IV-4) and continued to rise throughout the remainder of the trial. Overall insulin concentration was lower ($P< 0.02$) in the CC lambs (2.34 vs 1.20 ng mL^{-1}, SEM= 0.12), although this did not become evident until 75 min postprandial (environment by time interaction $P< 0.001$). The insulin:glucagon ratio was not influenced by environmental temperature, but was altered by feeding ($P< 0.001$) (Figure IV-5). The insulin:glucagon ratio was significantly elevated at 75 min postprandial in the TN environment, and stabilized over the next 75 min before increasing again to 210 min (environment by time interaction $P< 0.001$).

Plasma glucose concentrations were similar and showed a similar time course after feeding in both environments (Figure IV-6). Glucose declined transiently for 30 min postfeeding and then gradually, but significantly ($P< 0.001$), increased until 180 min postprandial. This represented a 14% rise over average prefeeding values.

Overall concentrations of free fatty acids were higher ($P< 0.02$) in the cold, although this was primarily due to elevated prefeeding values (Figure IV-7). Plasma concentrations of free fatty acids declined after feeding ($P< 0.001$) and within 30 min after feeding animals in both environments had similar free fatty acid concentrations. Plasma free fatty acid concentrations continued to decline slowly throughout the remaining experimental period. Overall, the postprandial free fatty acid concentrations declined 76% in the CC environment, while only declining 57% in the TN group. Due to the larger decline in the CC environment, there was a significant ($P< 0.001$) environment by time interaction.

Somatostatin Challenge:

A curve for mean disappearance of injected SS-14 from plasma is shown in Figure IV-8. One animal in the CC environment developed catheter problems and was removed. SS-14 injection (average 1.13 ± 0.08 $\mu g\ kg^{-1}$ BW) resulted in an increase in SLI concentrations from an average basal concentration across environments of 0.284 ng mL^{-1} to 6.18 ng mL^{-1} in the CC group and 3.38 ng mL^{-1} in the TN group within 1 min of injection (Figure IV-9).

Parameters estimated by curve fitting the two-component exponential equation to the declining plasma somatostatin concentration are summarized in Table IV-1. The slope of the first component (g_1) was greater ($P< 0.05$), for sheep in the TN environment indicating a faster rate of removal of SLI. The first component of the exponential equation (A_1 and g_1) was the dominant part of the equation because it explained the majority of the two-component model (H_1=.961 vs H_2=.039). However, inclusion of the second component significantly improved the description of the disappearance curve compared to use of a single component model (R^2=1.00 vs 0.66, respectively).

Plasma SLI concentration and kinetics, which were derived from the plasma concentration and curve fitting analysis, are summarized in Table IV-2. Basal concentrations of SLI were not different and were comparable to the values observed for effect of feeding experiment. Overall means for half-life of the first component were greater ($P< 0.005$) in the cold environment, while the second component was not affected by environment.

Sheep maintained in the TN environment had a higher ($P< 0.005$) turnover rate of somatostatin. Secretion rates of somatostatin were also significantly greater ($P< 0.02$) for sheep in the TN group. Pool size, distribution space and metabolic clearance rates were not significantly influenced by temperature

During the SS-14 challenge experiment, glucagon concentration was not significantly different in the two environments (0.26 vs 0.19 ng mL^{-1}, SEM= 0.004) (Figure IV-10). Insulin concentration was higher ($P< 0.04$) in the TN compared to the cold environment (3.4 vs 1.0 ng mL^{-1}, SEM= 0.12) (Figure IV-11). Following the pulse dose of SS-14 there were transient decreases in both glucagon and insulin concentrations during the first 5 min, but neither of these decreases was significant. Within 30 min of SS-14 injection concentrations of glucagon were higher ($P< 0.001$) than the 5 min values. The insulin:glucagon ratio was not different across environments or time (Figure IV-12).

Growth hormone concentration was not influenced by environmental temperature (3.4 vs 4.3 ng mL^{-1}, TN vs CC respectively), or by the pulse dose of SS-14 (Figure IV-13).

The pulse dose of SS-14 did not influence plasma glucose concentration nor did glucose concentration differ between environments (80.5 vs 84.3 mg dL^{-1}, TN vs CC respectively) (Figure IV-14).

The pulse dose of SS-14 induced a rapid (within 2 min) increase ($P< 0.001$) in plasma free fatty acid concentration in lambs from both environments (Figure IV-15). Peak concentrations of free fatty acids (166.9% of overall preinjection values) were obtained at 10 min postinjection, which corresponded with the maximum length of time

required for SLI concentrations to return to basal levels for either environment. By 90 min free fatty acid concentrations had returned to preinjection values in both environments. In the CC group free fatty acid levels seemed to remain elevated above those of the TN group for a prolonged period (from 10 to 105 min), but the environment by time interaction was not significant. Overall mean values for free fatty acids in the two environments were not ($P > 0.05$) different (0.33 vs 0.36 mM, TN vs CC, respectively).

DISCUSSION

Basal plasma concentrations of somatostatin were not different across environments. Our basal concentration was 2 to 5 times higher than reported for sheep (Barry et al., 1982) and for rats, dogs, and humans (Schusdziana and Schmid, 1987). However, the concentrations are similar to those for rats (Engelhardt and Schwille, 1981), which used an assay technique that did not involve an extraction procedure.

Plasma hormone concentration is a function of secretion rate and rate of clearance from the blood. Thus, even though changes in plasma hormone concentrations during different physiological states were not observed, secretion and/or clearance rates could have been altered in the same direction. The results of this experiment indicate that this did happen. Higher secretion rates of somatostatin in the TN environment were offset by a shorter half-life. The corrected half-lives for somatostatin in this study (0.693/turnover rate; TN-0.39, CC-0.85 min) are shorter than the values reported for dogs, rats, and humans (Lucey, 1986). This may reflect differences in methods used to determine and calculate half-life, since a comparatively low dose of somatostatin for injection was used. Differences in half-lives

across environments presumably reflects differences in the removal of somatostatin by various tissues and organs and by plasma peptidases. Somatostatin is thought to be metabolized by the liver, kidney, endogenous plasma peptidases and possibly by the pancreas (Lucey, 1986). The rate of degradation of somatostatin by hypothalamic peptidases has been found to be sensitive to thyroid hormones, with increased enzyme activity in hypothyroidism, which can be reversed by thyroxine administration (Dupont et al., 1978). If this principle also holds true for plasma peptidases, this could account for the differences between environmental groups since thyroid hormones have been shown to be elevated during cold stress (Christopherson and Thompson, 1983; Sasaki and Weekes, 1986).

Plasma growth hormone, insulin, glucose, and free fatty acid responses after feeding in the thermoneutral group (direction and time course) were similar to previous findings (Bassett, 1972). However, postprandial increases in glucagon were not observed as reported by Bassett, (1972). Postprandial increases in glucagon are thought to be important for prevention of hypoglycaemia induced by higher insulin concentrations, although glucagon release is less sensitive to most secretagogues than is insulin release (Brockman and Laarveld, 1986). In this study there was no postprandial hypoglycemia so that elevated glucagon may not have been necessary.

Two other theories have been proposed to explain postprandial changes in insulin and glucagon secretion. In the first theory, the flow of protein and acylglycerides to the lower digestive tract may influence pancreatic endocrine secretion indirectly via gut hormones (Schusdziarra, 1987). In ruminants, continuous outflow of rumen

fermentation endproducts may prolong nutrient assimilation and alter the rate of endproduct absorption to such an extent that response of gut hormones is diminished and thus pancreatic endocrine secretion is modified. This concept is supported by the work of Bassett, (1975), where the feeding of protein, protected against ruminal breakdown, resulted in significantly higher insulin concentrations. In this instance glucagon was not measured, so the effects of dietary protein on glucagon could not be ascertained. Alternatively, uptake of propionate by the liver may alter secretion of pancreatic endocrine hormones (Armentano et al., 1984). The work of Bloom et al., (1980) illustrated the vital role of the autonomic nervous system in control of pancreatic endocrine secretion in calves, but they also found that exogenous somatostatin is capable of modifying parasympathetic responses.

The significant decrease in overall basal insulin concentration during cold exposure in this experiment is consistent with responses reported in young bulls under more intense cold exposure (Christopherson and Thompson, 1983). In addition, the insulin secretory response to feeding (Sasaki et al., 1984) and numerous insulin secretotogues (Sasaki and Weekes, 1986) has been shown to be depressed by cold exposure. Since in the present study, somatostatin turnover rate was also lower in the cold, it is likely that some factor other than somatostatin was responsible for the lower plasma insulin concentration in the cold. Catecholamines are a prime candidate because their secretion rate is increased in the cold and they reduce insulin release by α-adrenergic mechanisms (Weekes et al., 1983). Sasaki and Weekes (1986) also observed that tissue sensitivity and

responsiveness to insulin is increased by cold exposure. Therefore, the impact of endogenous insulin concentration on metabolism may be similar despite lower plasma concentrations in the cold environment.

Prior to feeding, free fatty acids play an important role as an energy substrate for cold exposed lambs (Sasaki and Weekes, 1986). Postprandial increases in glucose concentrations were closely associated with a decline in free fatty acids, which is similar to previous findings (Bassett, 1975) and probably results from the antilipolytic effects of insulin. Suppression of insulin concentrations during chronic cold exposure enhanced mobilization of free fatty acids and glucose in response to increased sympatho-adrenomedullary activity and increased thyroid hormone concentrations (Sasaki and Weekes, 1986). It is possible that somatostatin may also act on fat mobilization by altering the lipolytic effect of epinephrine (Gray et al., 1980). Our observation that a large dose of somatostatin increased free fatty acid concentrations within 2 min is consistent with the latter. However, at more physiological doses of somatostatin, Barry et al. (1985) failed to significantly alter plasma free fatty acid concentrations. The results indicate that somatostatin may play a role in regulation of adipose tissue lipolysis, however, conclusive evidence for a direct role requires further study.

In contrast to other studies using sheep (Sasaki and Weekes, 1986) and cattle (Christopherson and Thompson, 1983), glucose concentration was not influenced by environmental temperature. This may be due to the high level of concentrate fed in this experiment.

Limited evidence suggests that prolonged cold exposure increases plasma growth hormone concentration in both sheep (Blom et al., 1976) and cattle (Olsen and Trenkle, 1973). Our data with less severe cold exposure suggests a relacively small environmental temperature effect on plasma growth hormone which is largely manifested as a reduced inhibitory effect of feeding. These results concur with the finding of Faulkner et al. (1980). Although this correlates with the reduced secretion of somatostatin in the cold environment, it is unlikely that a cause and effect relationship exists, since injection of a large dose of somatostatin had little or no effect on plasma growth hormone secretion in either environment. Alternatively, the attenuated postprandial decrease in plasma growth hormone concentration in the cold exposed sheep suggests that temperature somehow modifies the release and/or metabolic clearance of growth hormone following feeding.

In conclusion, plasma concentrations of somatostatin did not change during cold exposure or after feeding. Changes in plasma concentrations of insulin, glucagon, and growth hormone in response to feeding a concentrate diet do not seem to be related to changes in peripheral plasma concentrations of somatostatin, although this does not rule out the possibility of a paracrine action within the pancreas. Alteration of somatostatin kinetics by environmental temperature may reflect changes in somatostatin binding to its receptors or alteration of rates of clearance or degradation. However, the functional consequences of the slower turnover rate of somatostatin in the cold are unclear.

BIBLIOGRAPHY

Armentano, L.E., Mills, S.E., de Boer, G., and Young, J.W. 1984. Effects of Feeding Frequency on Glucose Concentration, Glucose Turnover, and Insulin Concentration in Steers. J. Dairy Sci. 67: 1445-1451.

Barry, T.N., Faichney, G.J., and Redekopp, C. 1985. Gastro-Intestinal Tract Function in Sheep Infused with Somatostatin. Aust. J. Biol. Sci. 38: 393-403.

Barry, T.N., Manley, T.R., Redekopp, C., Davis, S.R., Fairclough, R.J., and Lapwood, K.R. 1982. Protein Metabolism in Growing Lambs Given Fresh Ryegrass (Lolium perenne)-Clover (Trifolium repens) pasture ad lib. 2. Endocrine Changes, Glucose Production, and Their Relationship to Protein Deposition and the Partition of Absorbed Nutrients. Br. J. Nutr. 47: 319-329.

Bassett, J.M. 1975. Dietary and Gastro-Intestinal Control of Hormones Regulating Carbohydrate Metabolism in Ruminants. Pages 383-398. *In* I.W. McDonald and A.C.I. Warner eds. Proceeding of the IV International Symposium on Ruminant Physiology. Univ. New Eng. Publ. Unit, Armidale, NSW, Australia.

Bassett, J.M. 1972. Plasma Glucagon Concentrations in Sheep: Their Regulation and Relation to Concentrations of Insulin and Growth Hormone. Aust. J. Biol. Sci. 25: 1277-1287.

Bassett, J.M. and Alexander, G. 1971. Insulin, Growth Hormone, and Corticosteroids in Neonatal Lambs. Normal Concentrations and the Effects of Cold. Biol. Neonate 17: 112-125.

Blom, A.k., Hove, K., and Nedkvitne, J.J. 1976. Plasma Insulin and Growth Hormone Concentrations in Pregnant Sheep II: Post-Absorptive Levels in Mid- and Late Pregnancy. Acta Endocrinologica 82: 553-560.

Bloom, S.R., Edwards, A.V., and Jarhult, J. 1980. The Effect of Somatostatin on Pancreatic Endocrine Responses Mediated Via the Parasympathetic Innervation in the Concious Calf. J. Physiol. 308: 29-38.

Brockman, R.P. 1979. Effect of Somatostatin on Plasma Glucagon and Insulin, and Glucose Turnover in Exercising Sheep. J. Appl. Physiol. Respir. Environ. Exercise Physiol. 47: 273-278.

Brockman, R.P., Bergman, E.N., Joo, P.K., and Manns, J.G. 1975. Effects of Glucagon and Insulin on Net Hepatic Metabolism of Glucose Precursors in Sheep. Am. J. Physiol. 229: 1344-1350.

Brockman, R.P. and Greer, C. 1980. Effects of Somatostatin and Glucagon on the Utilization of [2-^{14}C] Propionate in Glucose Production in vivo in Sheep. Aust. J. Biol. Sci. 33: 457-464.

Brockman, R.P. and Laarveld, B. 1986. Hormonal Regulation of Metabolism in Ruminants; A Review. Livest. Prod. Sci. 14: 313-334.

Bryce, D., Yeh, M., Funderburk, C., Todd, H., and Hertelendy, F. 1975. Studies on Growth Hormone Secretion VII. Effects of Somatostatin on Plasma GH, Insulin, and Glucagon in Sheep. Diabetes 24(9): 842-850.

Christopherson, R.J. and Thompson, J.R. 1983. Endocrine Responses of Young Bulls kept Outdoors during Winter. Can. J. Anim. Sci. 63: 1019 (Abstract).

de Boer, G., Trenkle, A., and Young, J.W. 1986. Secretion and Clearance Rates of Glucagon in Dairy Cows. J. Dairy Sci. 69: 721-733.

Dupont, A., Merand, Y., and Barden, N. 1978. Effect of Propylthiouracil and Thyroxine on the Inactivation of Somatostatin by Rat Hypothalamus. Life Sci. 23: 2007-2012.

Engelhardt, W. and Schwille, P.O. 1981. Development of a sensitive somatostatin radioimmunoassay and its application to plasma of stressed and non-stressed rats. Horm. Metab. Res. 13: 318-323.

Faulkner, A., Thompson, E.M., Bassett, J.M., and Thompson, G.E. 1980. Cold Exposure and Mammary Glucose Metabolism in the Lactating Goat. Br. J. Nutr. 43: 163-170.

Gray, D.E., Lickley, H.L.A., and Vranic, M. 1980. Physiologic Effects of Epinephrine on Glucose Turnover and Plasma Free Fatty Acid Concentrations Mediated Independently of Glucagon. Diabetes 29: 600-608.

Harris, V., Faloona, G.R., and Unger, R.H. 1979. Glucagon. *In* B.M. Jaffe and H.R. Behrman eds. Methods of Hormone Radioimmunoassay. 2nd ed. Academic Press Inc., New York, NY, p. 643.

Lucey, M.R. 1986. Endogenous Somatostatin and the Gut. Gut 27: 457-467.

Mears, G.J., Vesely, J.A., and Cheng, K.-J. 1988. Plasma Insulin and Growth Hormone in Growing Lambs Fed Monensin. Can. J. Anim. Sci. 68: 165-171.

Olsen, J.D. and Trenkle, A, 1973. Exposure of Cattle to Controlled Subzero Temperature: Growth Hormone, Glucose, and Free Fatty Acid Concentrations in Plasma. Am. J. Vet. Res. 34: 747-751.

Reid, A.M., Titchen, D.A., and Walker, D.M. 1984. Effects of Dietary Protein on Basal and Post-Prandial Plasma Gastrin and Somatostatin in the Suckled Lamb. Dig. Dis. Sci. 29(8): 69S (Abstract).

SAS User's Guide: Statistics. 1982. SAS Inst. Inc., Cary, NC.

Sasaki, Y. and Weekes TEC. 1986. Metabolic Responses to Cold. Pages 326-346. *In* L.P. Milligan, W.L. Grovum, and A. Dobson eds. Control of Digestion and Metabolism in Ruminants. Proceedings of the VI International Symposium on Ruminant Physiology. Prentice-Hall, Englewood Cliffs, NJ.

Sasaki, Y., Hiratsuka, H., and Ishida, M. 1984. Effect of Cold Exposure on Insulin Response to Feeding in Sheep. Can. J. Anim. Sci. 64(Suppl.): 269-270.

Schusdziarra, V. 1987. Evidence for the Endocrine Role of Somatostatin. Pages 209-217. *In* S. Reichlin ed. Somatosatin Basic and Clinical Status. Plenum Press, New York, NY.

Schusdziarra, V. and Schmid, R. 1986. Physiological and Pathophysiological Aspects of Somatostatin. Scand. J. Gastroenterol. 21(Suppl 119): 29-41.

Shipley, R.A. and Clark, R.E. 1972. Tracer Methods for in vivo Kinetics: Theory and Applications, Academic Press, New York, NY.

Steele, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics. A Biometrical Approach. McGraw-Hill Book Co., New York, NY. Pages 186-187.

Varner, M.A, Davis, S.L., and Reeves, J.J. 1980. Temporal Serum Concentrations of Growth Hormone, Thyrotropin, Insulin, and Glucagon in Sheep Immunized against Somatostatin. Endocrinol. 106(3): 1027-1032.

Vlaminck, K., Oyaert, W., Muylle, E., Van Den Hende, C., and Pipeleers, D. 1986. Blood Levels of Somatostatin, Pancreatic Polypeptide and Gastrin in Normal Cows and in Cows Suffering from Abomasal Dilatation. J. Vet. Med. A. 33: 241-246.

Weekes, T.E.C., Sasaki, Y., and Tsuda, T. 1983. Enhanced Responsiveness to Insulin in Sheep Exposed to Cold. Am. J. Physiol. 244 (Endocrinol. Metab. 7): E335-E345.

Table IV-1. Parameters of two-component exponential equations for clearance of plasma somatostatin after injection of somatostatin-14 (1.13 $\mu g\ kg^{-1}$) via the jugular vein in lambs adapted to either thermoneutral (20°C n=5) or chronic cold (0°C n=4) environments.

Parameters[1]	Environment		
	Thermoneutral	Cold	SEM
A_1, mL^{-1} [2]	0.365	0.240	0.053
g_1, min^{-1}	1.835[a]	0.848[b]	0.164
H_1	0.966	0.956	0.007
A_2, mL^{-1} [2]	0.011	0.011	0.002
g_2, min^{-1}	0.050	0.040	0.012
H_2	0.034	0.044	0.007

a,b Means with different subscripts were different ($P<0.05$).

1 The zero-time intercepts are A_1 and A_2, the time constants are g_1 and g_2, and the normalized intercepts are H_1 and H_2 for the first and second components, respectively, of disappearance curves for somatostatin concentration divided by dose.

2 Zero-time intercepts for the first and second components in this table were multiplied by 1,000: i.e., 0.000365 x 1,000 = 0.365 as reported for A_1 for the thermoneutral group.

Table IV-2. Basal concentration, pool size, distribution space, half-lives, turnover rate, metabolic clearance rate and secretion rate of somatostatin in lambs adapted to either TN (20°C n=5) or chronic cold (0°C n=4) environment.

Parameters	Environment		
	Thermoneutral	Cold	SEM
Basal Concentration, pg mL^{-1}	292.3	276.2	7.1
Pool Size, μg	0.88	1.12	0.04
Distribution space, L 50 kg^{-1}	3.42	4.63	0.61
Half-lives, min			
fast	0.40[a]	0.86[b]	0.08
slow	19.98	18.42	4.80
Turnover Rate, min^{-1}	1.79[a]	0.81[b]	0.17
Metabolic Clearance Rate, L/(h x 50 kg)	345.5	218.0	46.9
Secretion Rate, μg/(hx50 kg)	87.8[a]	53.1[b]	8.5

a,b Means within the same row with different subscripts are different ($P<0.05$).

Figure IV-1. Effect of meal feeding a concentrate diet on concentration of plasma somatostatin-like immunoreactivity in lambs adapted to either a thermoneutral (20°C) or chronic cold (0°C) environment. (Feeding was at time=0) (mean ± SEM).

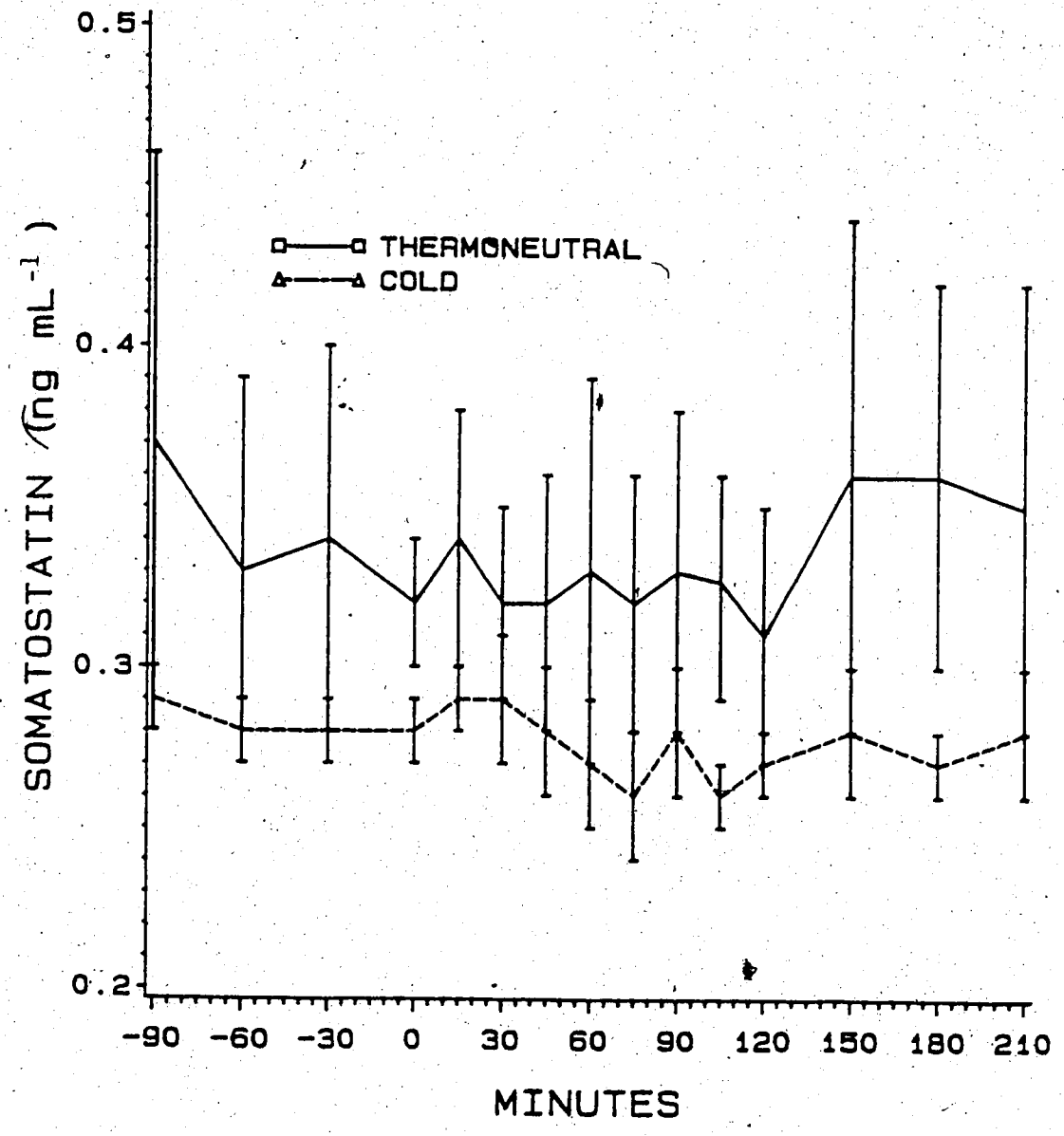


Figure IV-2. Effect of meal feeding a concentrate diet on plasma growth hormone concentrations in lambs adapted to either a thermoneutral (20°C) or chronic cold (0°C) environment. (Feeding was at time=0) (mean ± SEM).

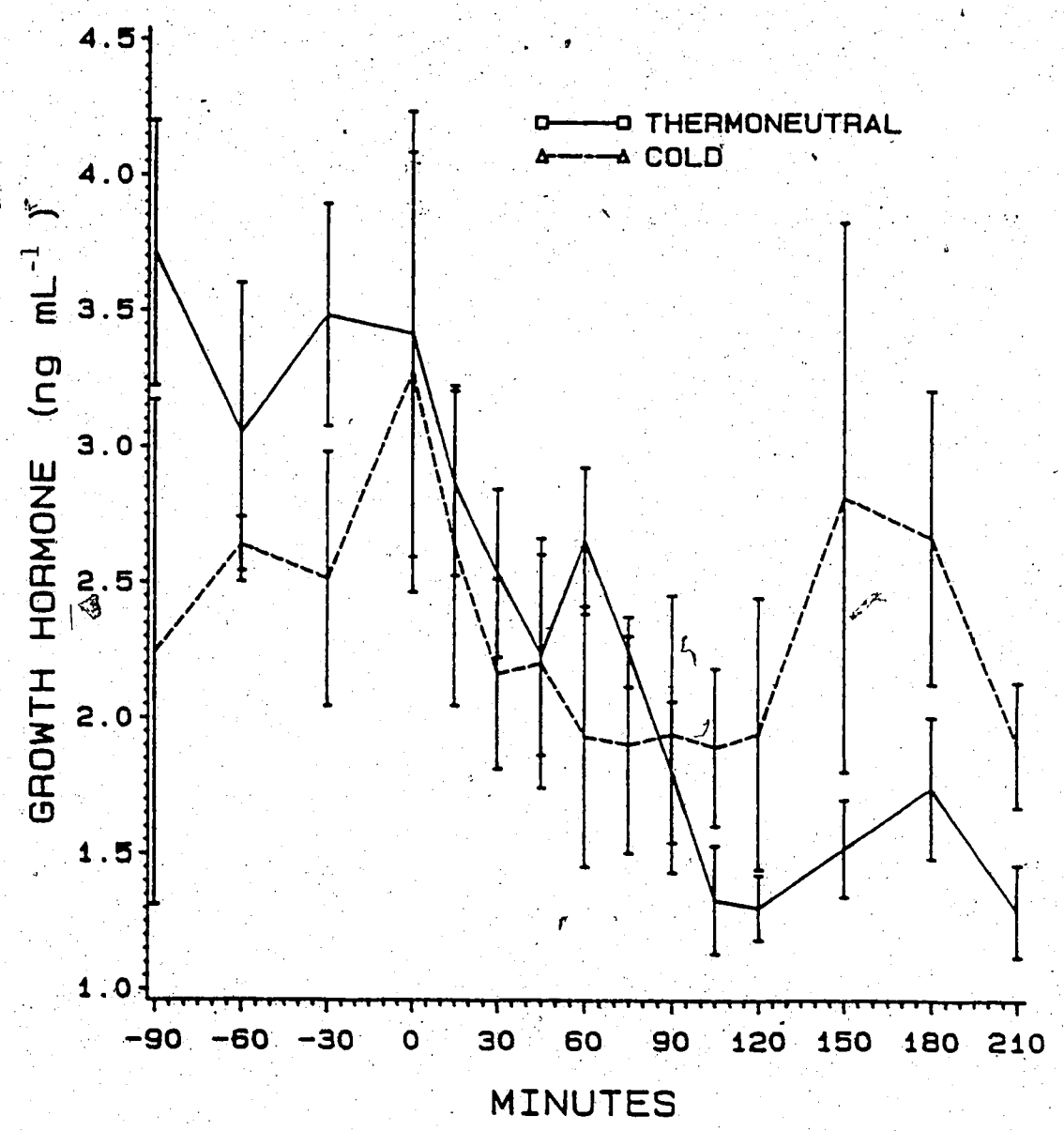


Figure IV-3. Effect of meal feeding a concentrate diet on plasma glucagon concentrations in lambs adapted to either a thermoneutral (20°C) or chronic cold (0°C) environment. (Feeding was at time=0) (mean ± SEM).

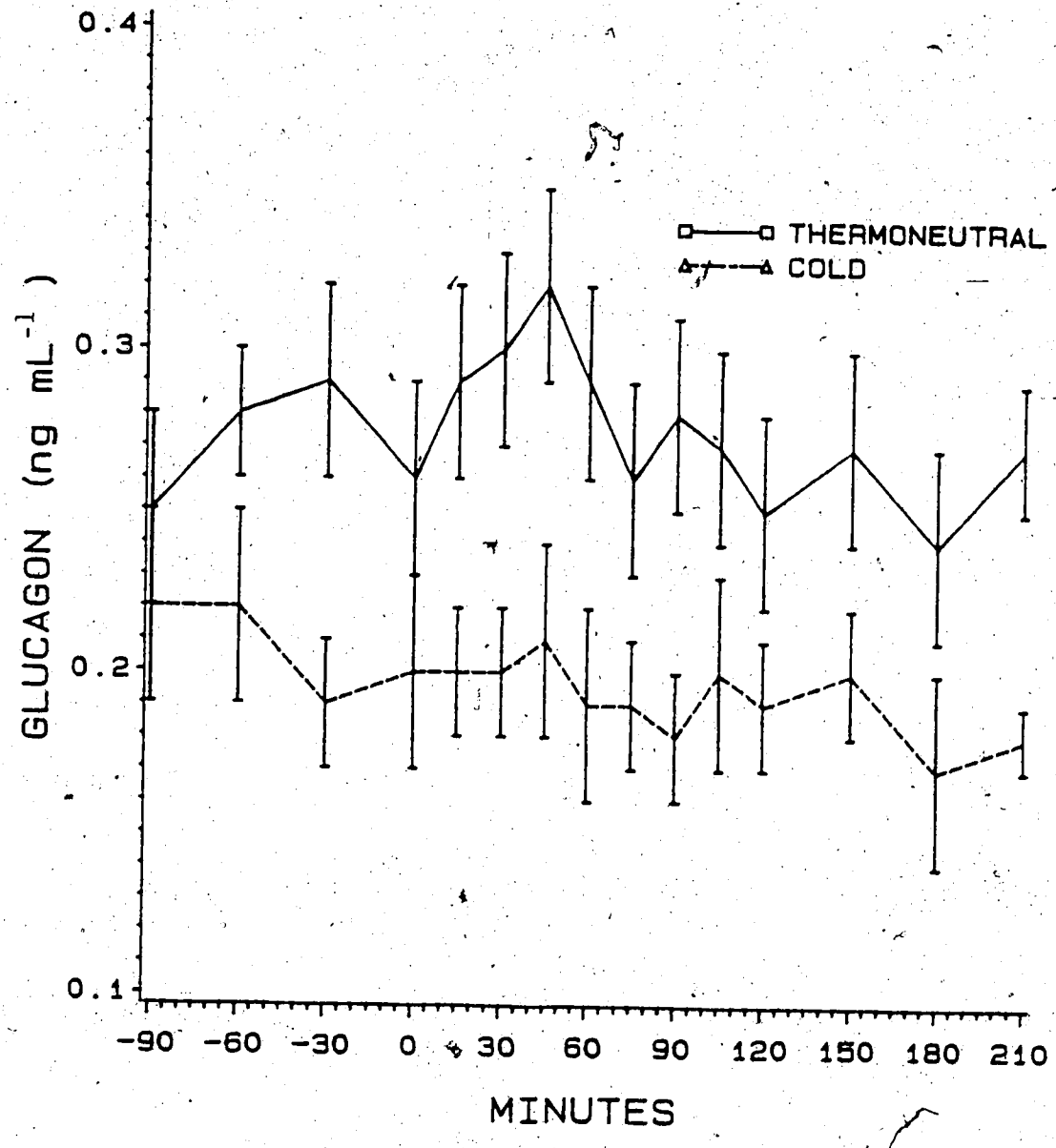


Figure IV-4. Effect of meal feeding a concentrate diet on plasma insulin concentrations in lambs adapted to either a thermoneutral (20°C) or chronic cold (0°C) environment. (Feeding was at time=0) (mean ± SEM).

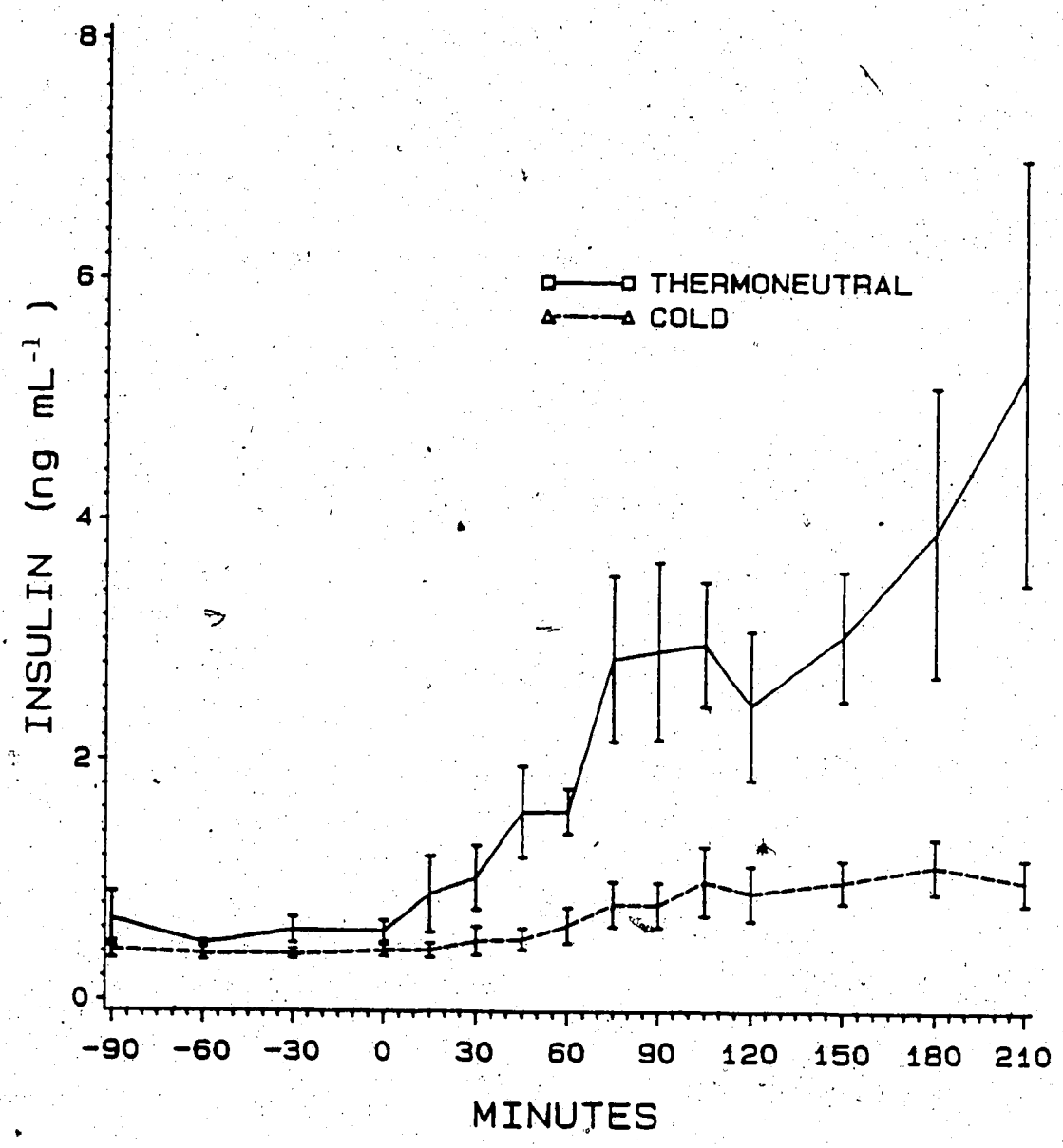


Figure IV-5. Effect of meal feeding a concentrate diet on plasma insulin:glucagon ratio in lambs adapted to either a thermoneutral (20°C) or chronic cold (0°C) environment. (Feeding was at time-0) (mean ± SEM).

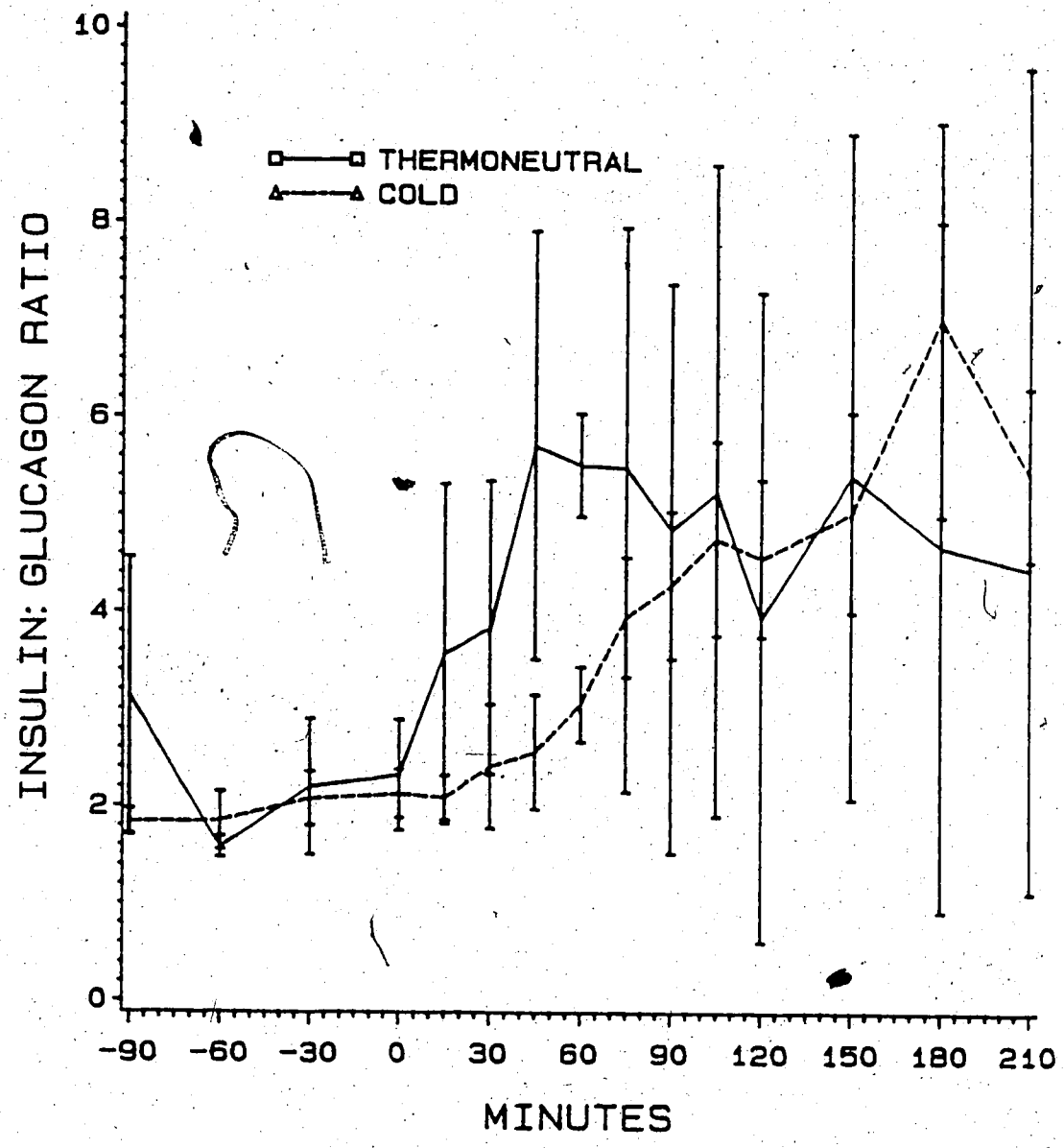


Figure IV-6. Effect of meal feeding a concentrate diet on plasma glucose concentrations in lambs adapted to either a thermoneutral (20°C) or chronic cold (0°C) environment. (Feeding was at time=0) (mean ± SEM).

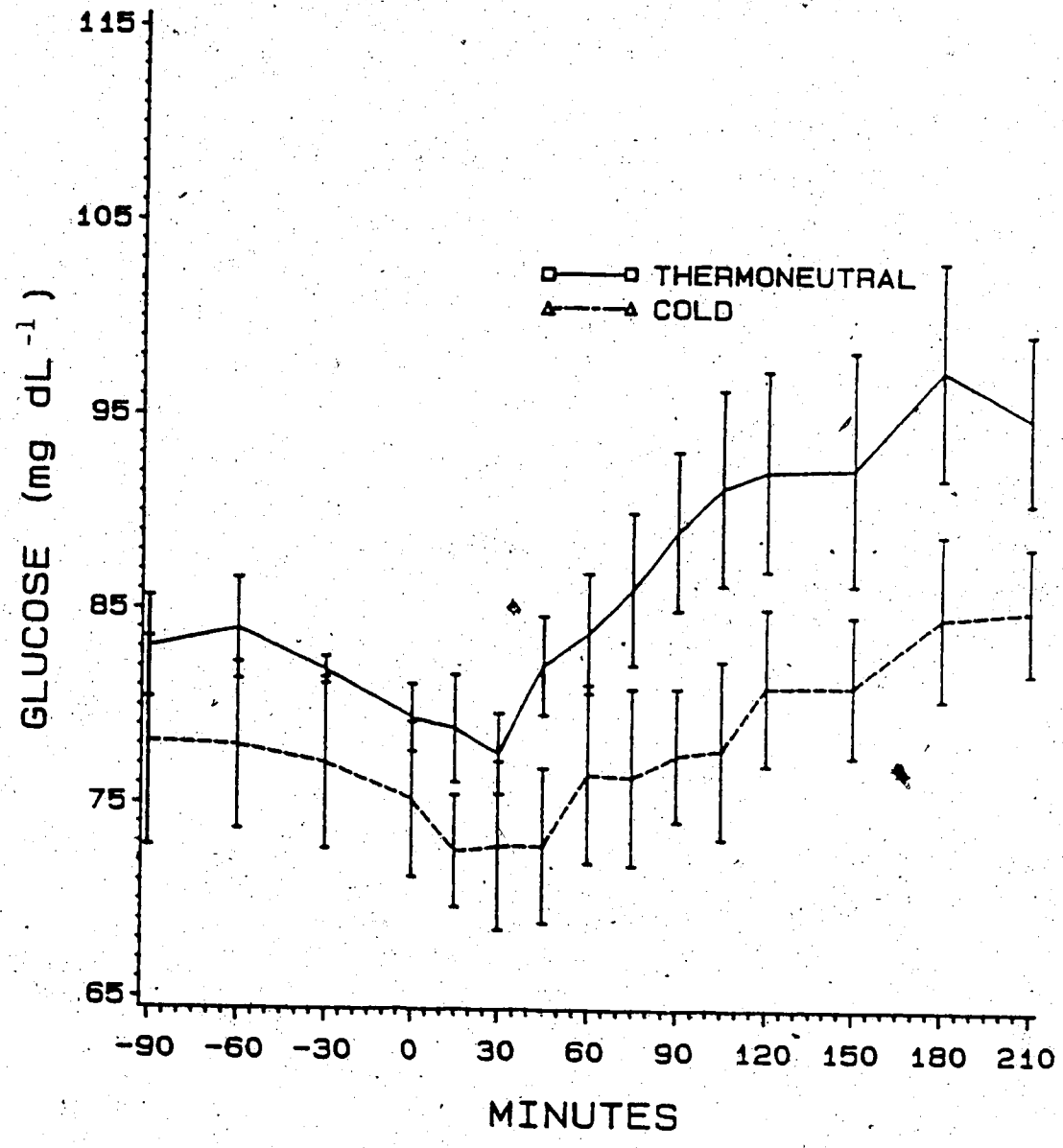


Figure IV-7. Effect of meal feeding a concentrate diet on plasma free fatty acid concentrations in lambs adapted to either a thermoneutral (20°C) or chronic cold (0°C) environment. (Feeding was at time=0) (mean ± SEM).

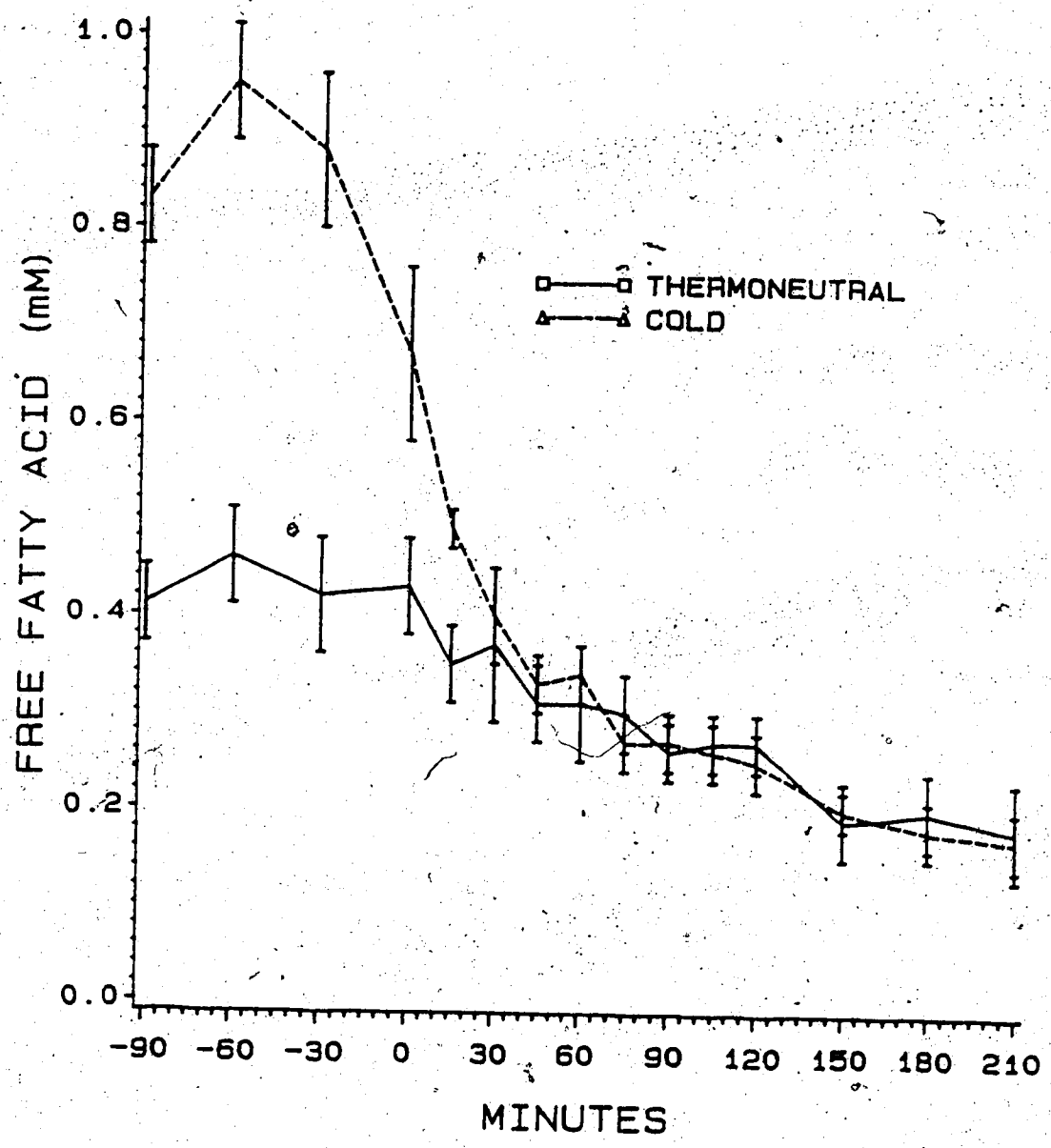


Figure IV-8. Mean plasma somatostatin disappearance curves after a pulse dose (1.13± 0.08 $\mu g\ kg^{-1}$) of somatostatin-14 for lambs in the thermoneutral (20°C) or chronic cold (0°C) environments. Data points represent observed values. The equation for the line of best fit (R^2= 0.999) for a two-component exponential model for the TN environment was $Y = 0.000365e^{-1.851t} + 0.0000115e^{-0.050t}$ and for the CC environment was $Y = 0.000240e^{-0.847t} + 0.0000112e^{-0.043t}$, where t is min after injection.

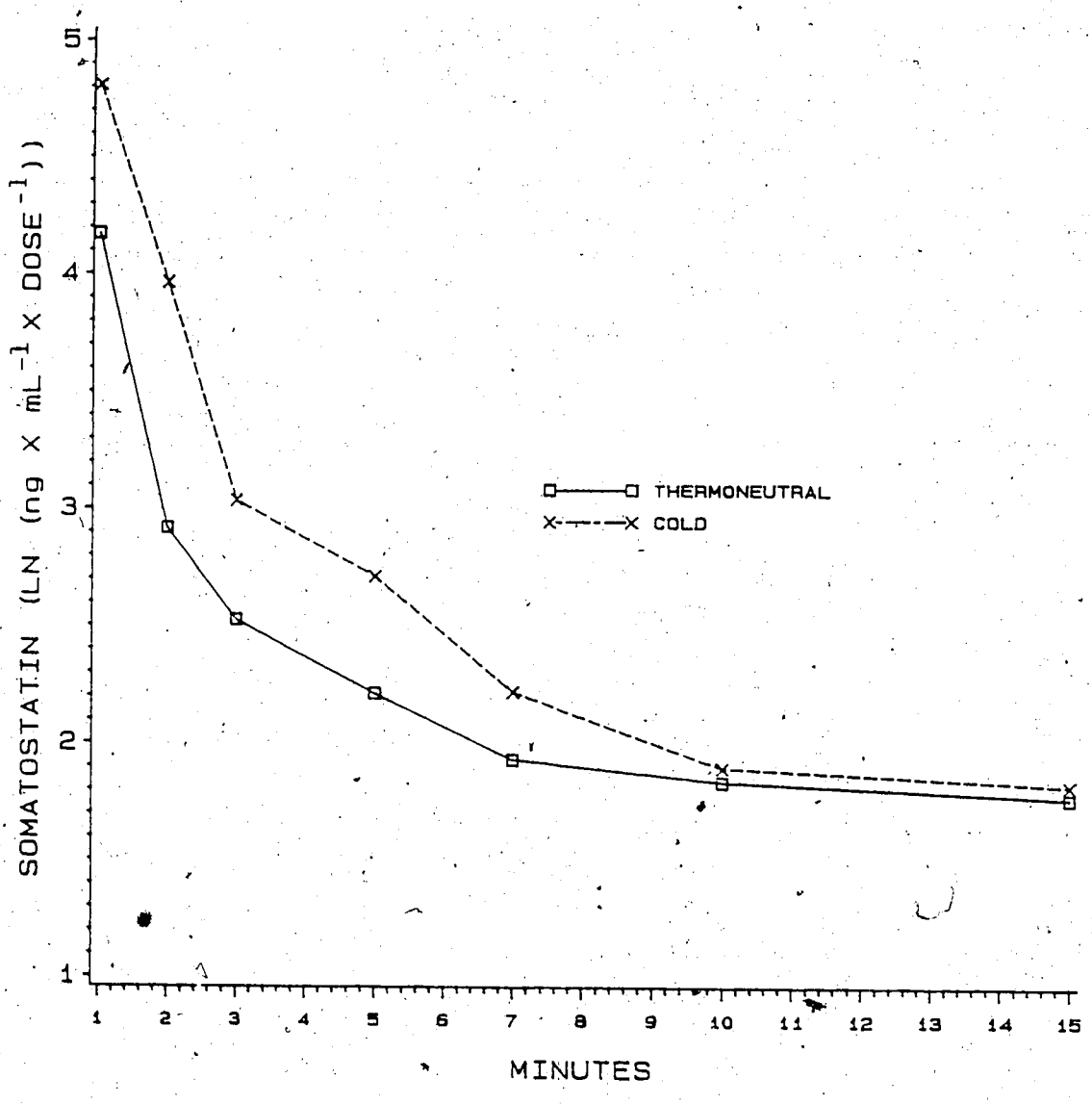


Figure IV-9. Effect of somatostatin-14 challenge (1.13 μg kg^{-1}) on concentration of plasma somatostatin-like immunoreactivity in 10 lambs adapted to either thermoneutral (20°C) or cold (0°C) environment. (Injection was at time = 0) (mean ± SEM).

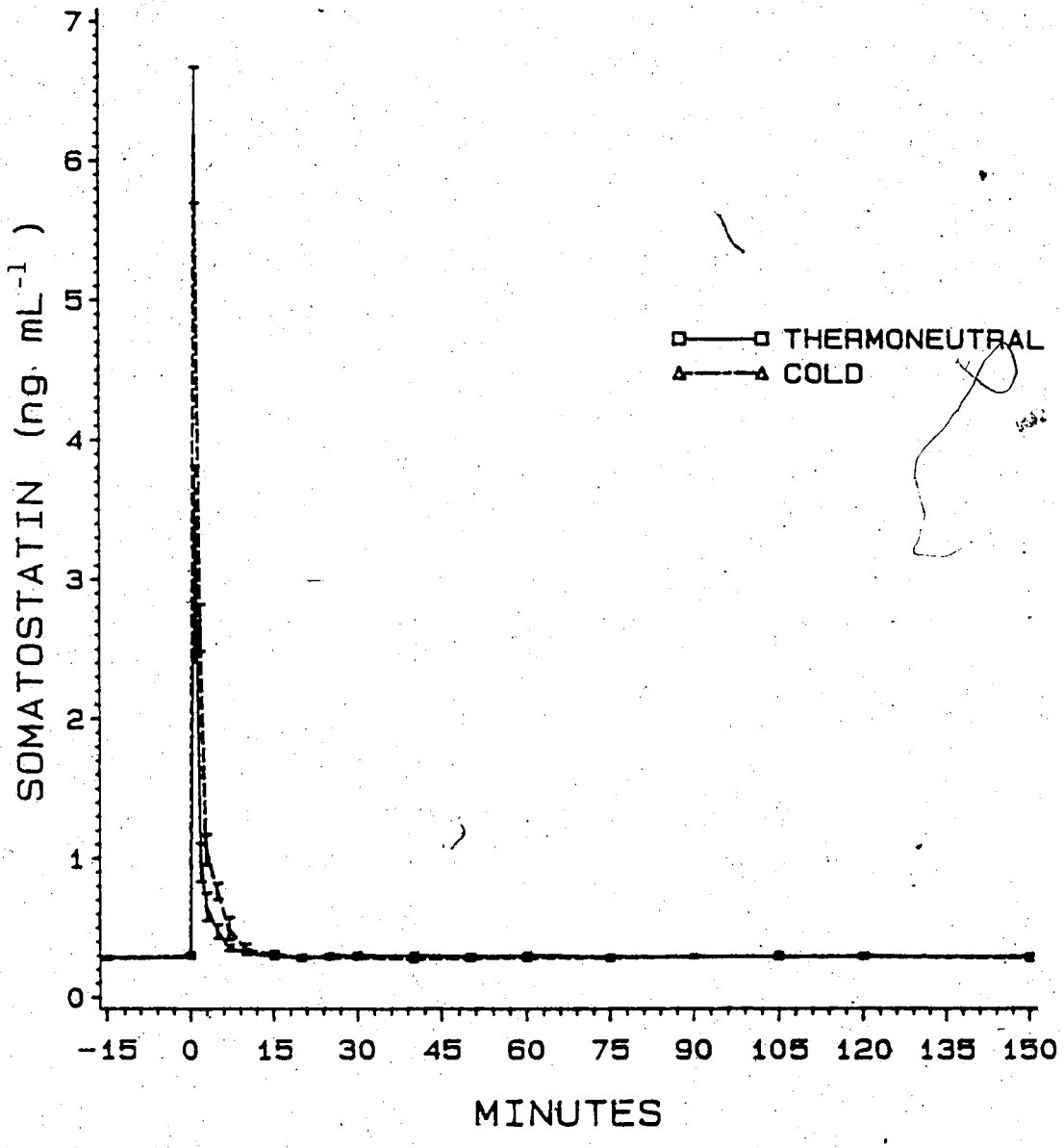


Figure IV-10. Effect of somatostatin-14 challenge (1.13 μg kg^{-1}) on plasma glucagon concentrations in 10 lambs adapted to either thermoneutral (20°C) or cold (0°C) environment. (Injection was at time = 0) (mean ± SEM).

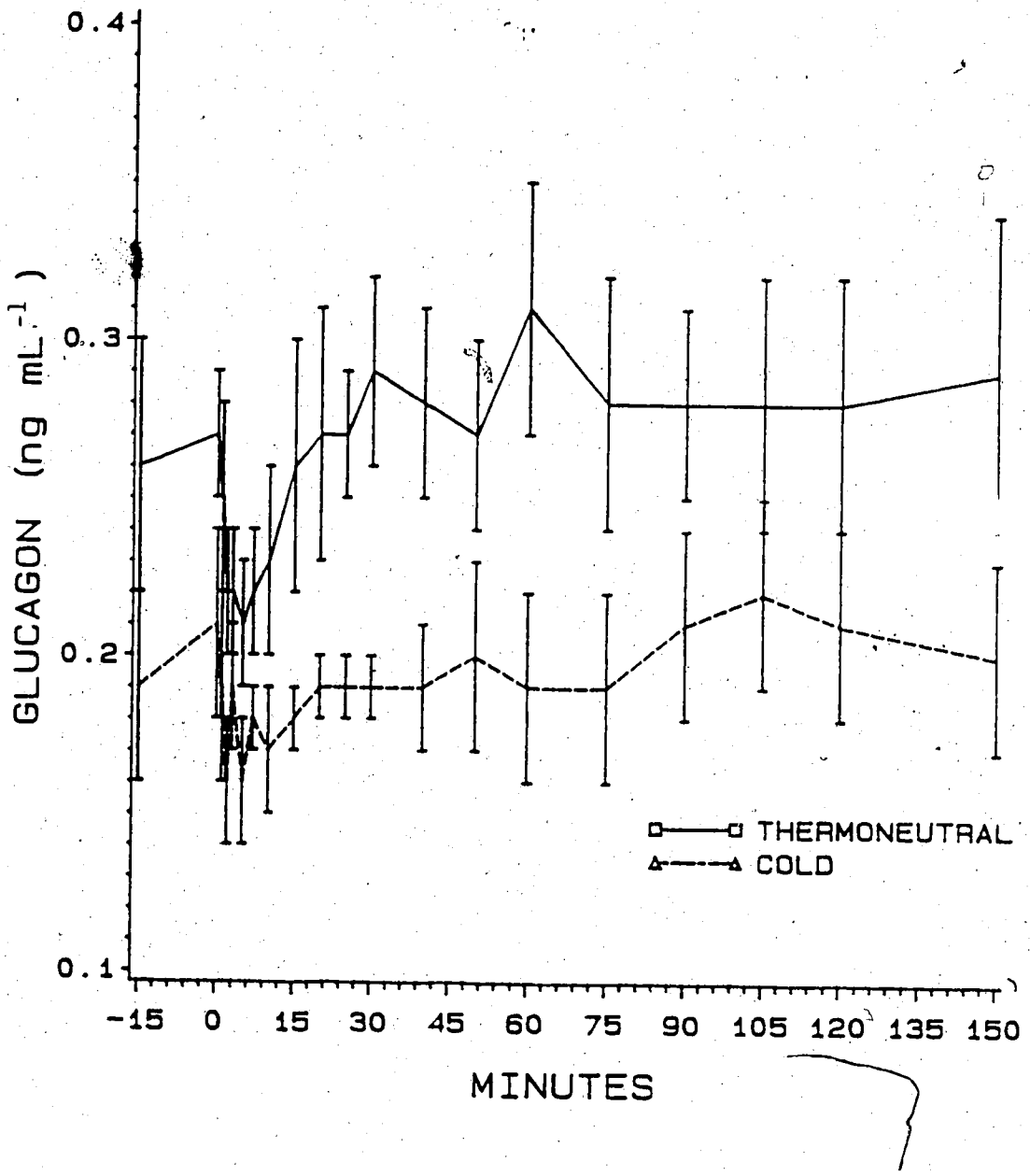


Figure IV-11. Effect of somatostatin-14 challenge (1.13 μg kg^{-1}) on plasma insulin concentrations in 10 lambs adapted to either thermoneutral (20°C) or cold (0°C) environment. (Injection was at time = 0) (mean ± SEM).

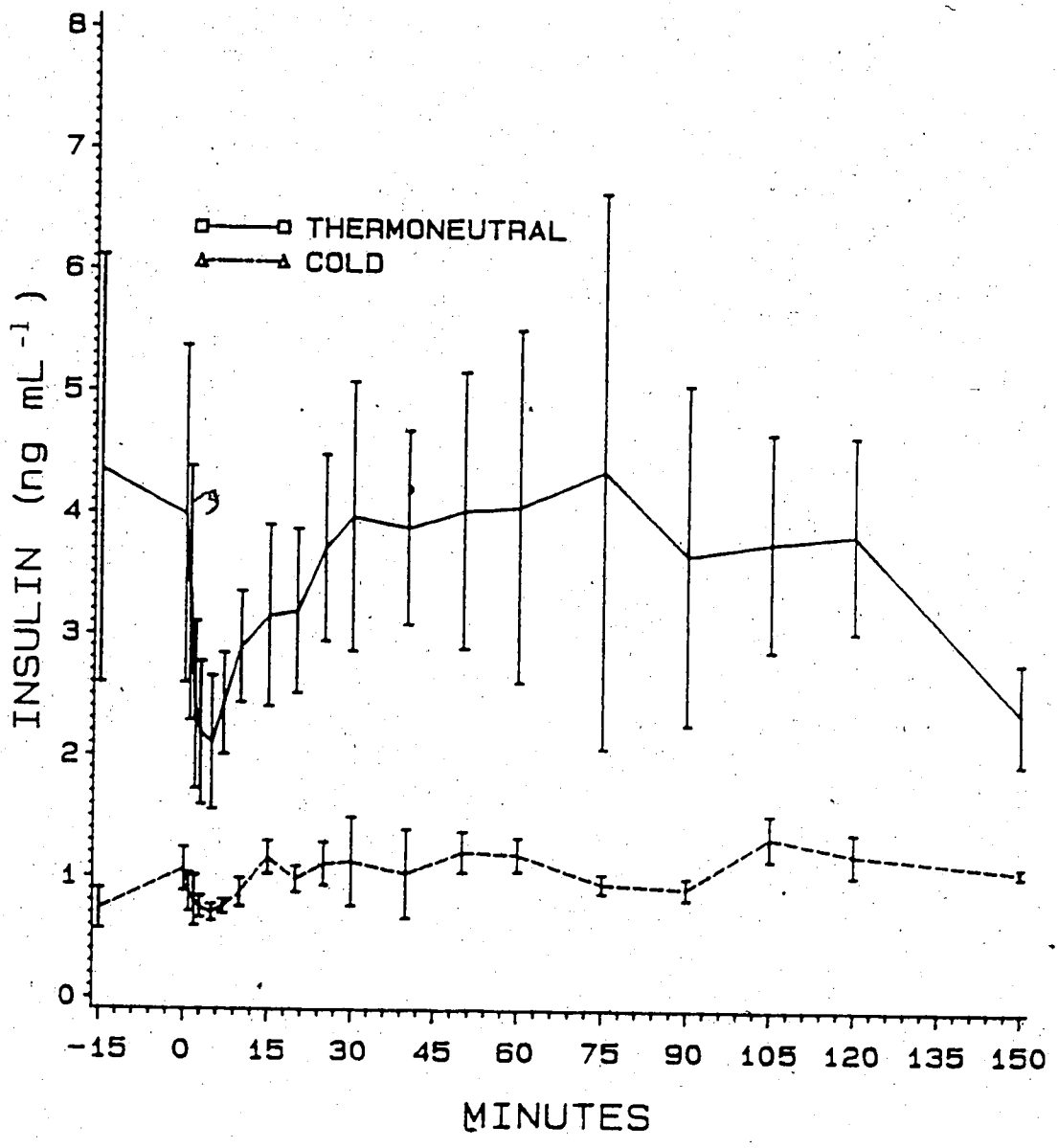


Figure IV-12. Effect of somatostatin-14 challenge (1.13 $\mu g\ kg^{-1}$) on plasma insulin:glucagon ratio in 10 lambs adapted to either thermoneutral (20°C) or cold (0°C) environment. (Injection was at time = 0) (mean ± SEM).

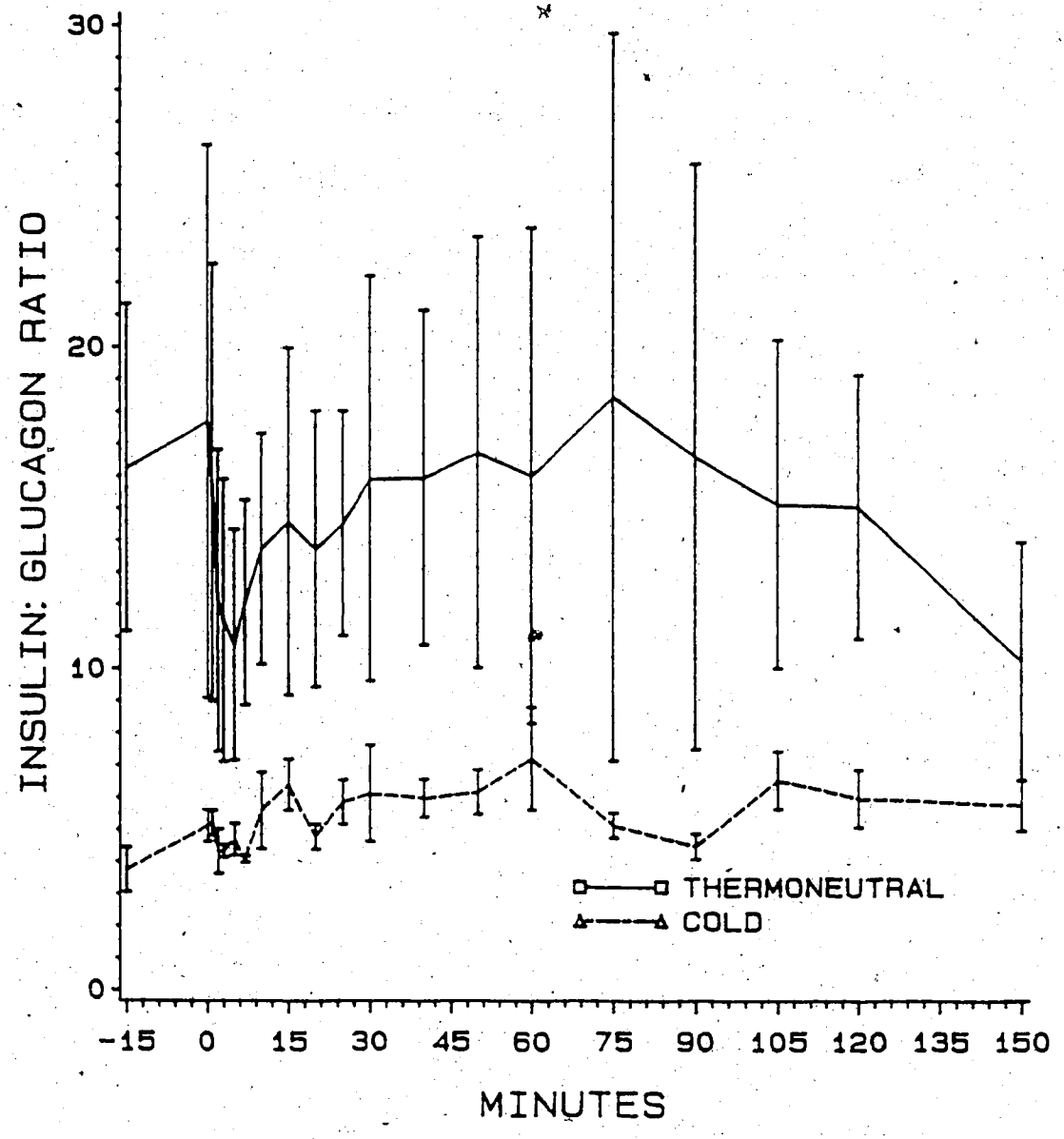


Figure IV-13. Effect of somatostatin-14 challenge (1.13 $\mu g\ kg^{-1}$) on plasma growth hormone concentrations in 10 lambs adapted to either thermoneutral (20°C) or cold (0°C) environment. (Injection was at time = 0) (mean ± SEM).

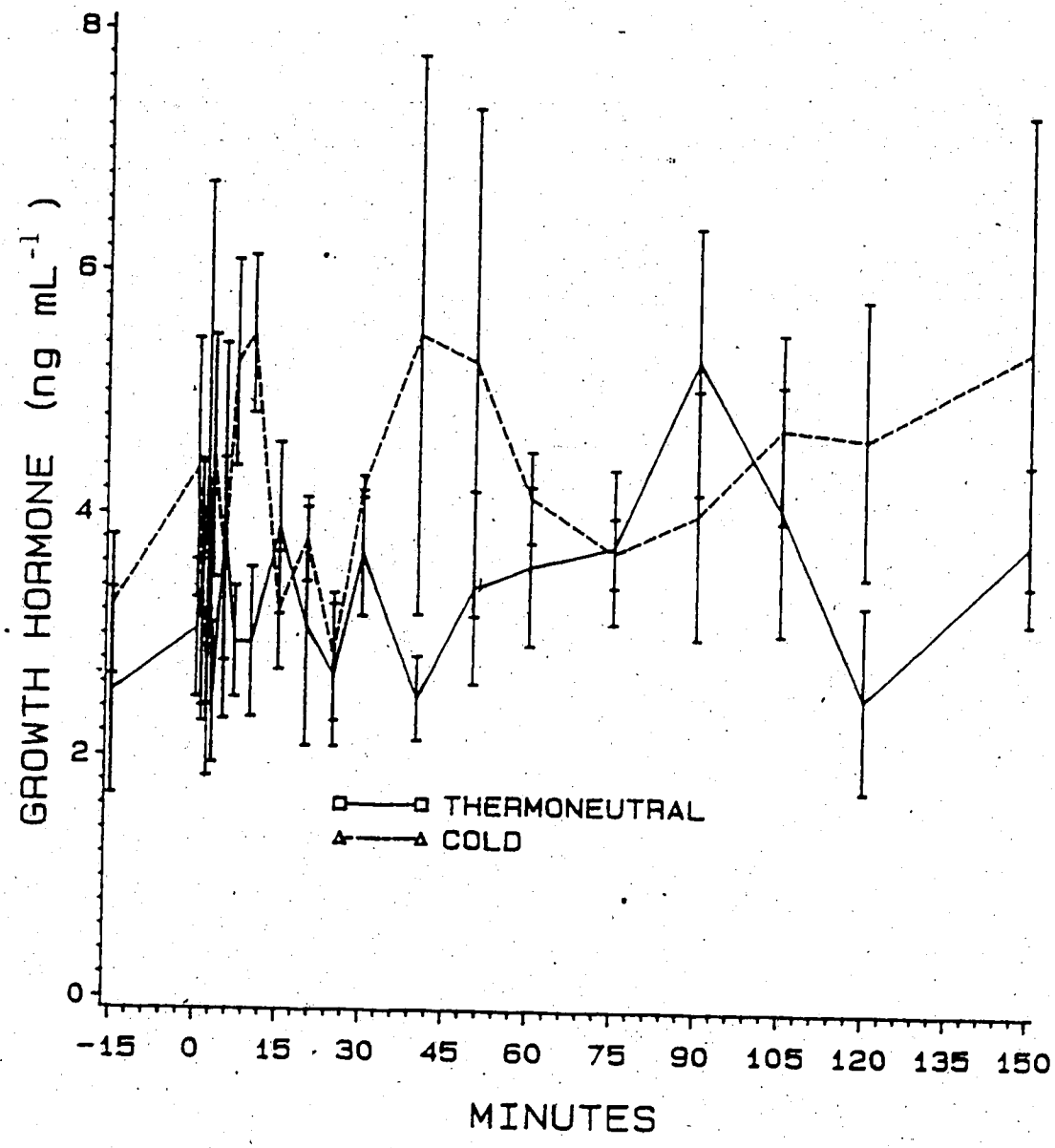


Figure IV-14. Effect of somatostatin-14 challenge (1.13 μg kg^{-1}) on plasma glucose concentrations in 10 lambs adapted to either thermoneutral (20°C) or cold (0°C) environment. (Injection was at time = 0) (mean ± SEM).

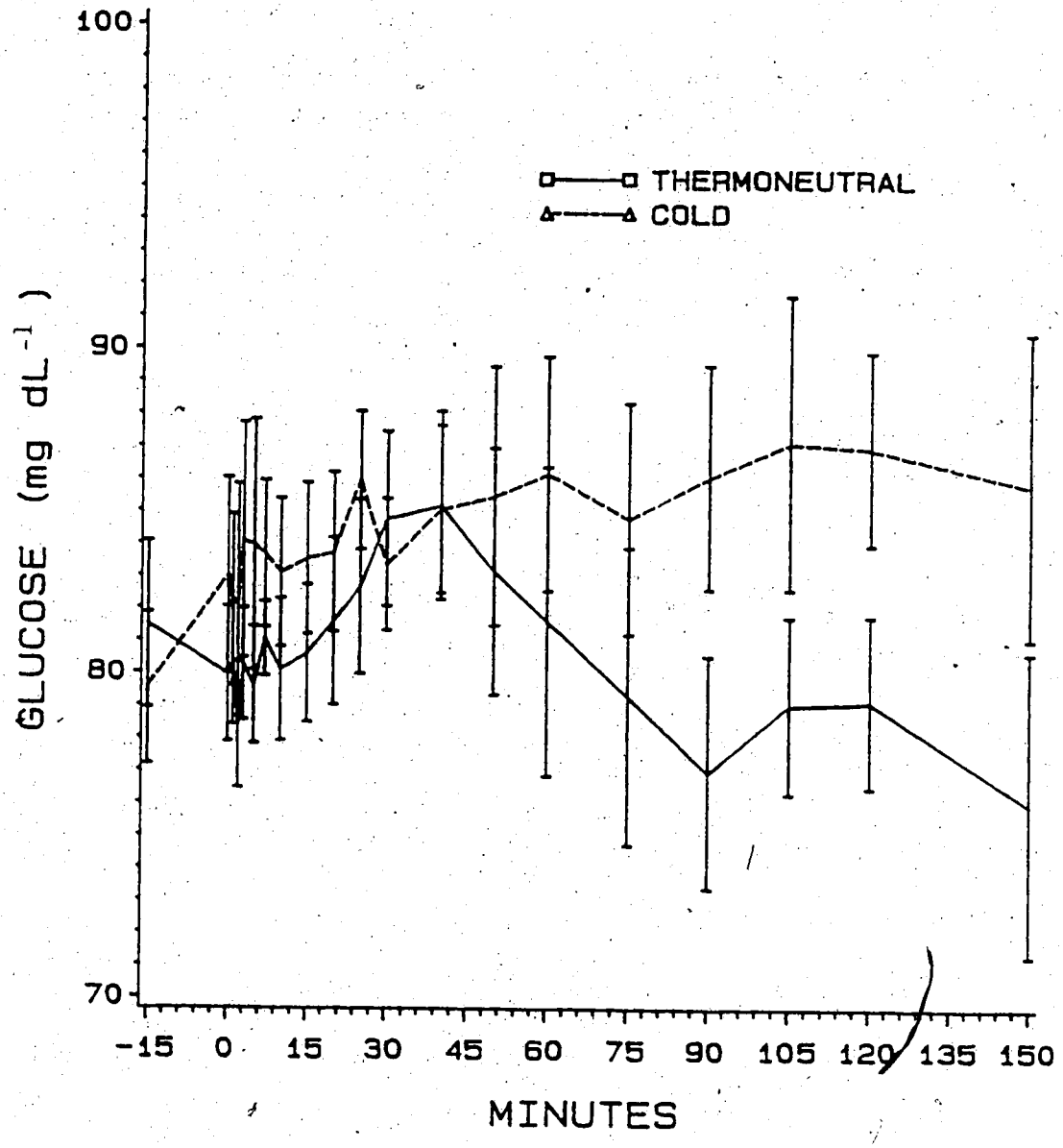
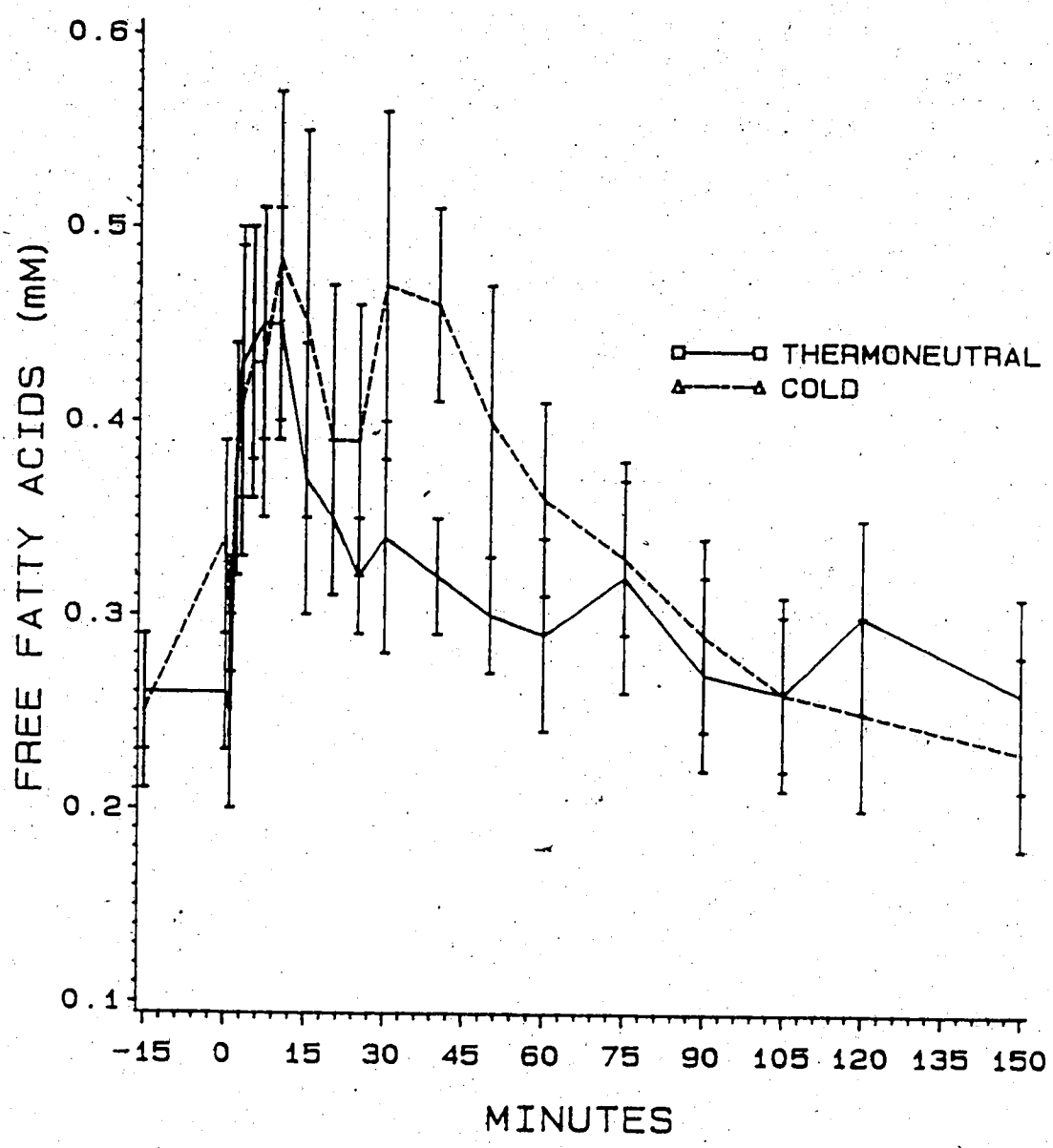


Figure IV-15. Effect of somatostatin-14 challenge (1.13 $\mu g\ kg^{-1}$) on plasma free fatty acid concentrations in 10 lambs adapted to either thermoneutral (20°C) or cold (0°C) environment. (Injection was at time = 0) (mean ± SEM).



GENERAL DISCUSSION

In the past 15 years much of the knowledge regarding the role of somatostatin in metabolism in nonruminants has been delineated (Brockman and Laarveld, 1986). In the few studies where the role of somatostatin has also been studied in ruminants the results are often similar to those for nonruminants. The results presented here provide some basic information on the role of somatostatin in response to cold exposure in ruminants.

Basal plasma somatostatin concentration was not affected by cold exposure, which was in contrast to the findings of Faichney and Barry, (1986) where mild heat exposure elevated somatostatin concentrations. In a steady state, plasma concentrations are a reflection of the balance between the secretion and metabolic clearance rates of a hormone and these were altered by cold exposure. Cold exposure prolonged the half-life of somatostatin and decreased overall secretion rate. Increasing the half-life of somatostatin could be the result of a decrease in degradation rate by plasma peptidases, in the removal by the liver and kidney, or uptake by tissues and organs. The rate of degradation of somatostatin by hypothalamic peptidases was decreased by administration of thyroxine in animals suffering from hypothyroidism (Dupont et al., 1978), although there is no information regarding the effects of thyroid hormones above the normal range. We found that chronic cold exposure significantly increased plasma tri-iodothyronine, but not thyroxine concentrations. Alteration of peptidase enzyme activity by thyroid hormones could be important in altering the degradation rate of many of the plasma hormones.

To maintain similar basal concentrations of somatostatin, while decreasing metabolic clearance rate, a decrease in secretion rate must also occur. Since somatostatin is released from a multitude of sites in response to a number of different stimuli, it is unclear whether secretion rate of somatostatin is altered equally at different sites by cold exposure.

We were unable to show any effect of somatostatin on reticular motility in either the thermoneutral or cold environment. This is consistant with the finding that prolonged infusion of somatostatin in a thermoneutral environment had no significant effect on the passage rate of digesta from the reticulorumen (Barry et al., 1985) and suggests that other mechanisms, such as cholinergic innervation from the medulla oblongata (Kennedy et al., 1986), are more important in the control of motility in the forestomach.

In this study, small increases in plasma somatostatin concentration produced inconsistent alteration of the migrating myoelectric complex. Both the direction and amplitude of this change in duration was not always the same. It is unclear why the duration of the migrating myoelectric complex was suppressed at the two higher doses of somatostatin in the acute cold experiment, while pharmacological doses of somatostatin have produced increases in the duration of the migrating myoelectric complex (Yamada, 1987). A direct correlation between the rate of passage of digesta and muscle contractions can not be made because intestinal transit is the result of several factors, including type and frequency of contraction waves, viscoelastic properties of chyme, and volume fluxes of the digesta (Meyer, 1987).

Ruckebusch, (1987) has suggested that in sheep and cattle the rate of passage of digesta along the intestine parallels the migrating myoelectric complex. If this is the case, our findings of a reduced duration of the MMC due to somatostatin would indicate an opportunity for a greater number of MMCs per day, and hence an increased rate of passage of digesta. Barry et al., (1985) found an increased retention time in the post-ruminal portion of the digestive tract. However, it is possible that the accumulation of digesta in the antral region, which they found, was due to somatostatin acting on the duodenal bulb to slow the rate of passage from the antrum into the duodenum (Ruckebusch and Merritt, 1985) and once in the duodenum digesta may have been rapidly propelled along the small intestine to the caecum.

In nonruminants, one of the major stimuli for somatostatin release within the digestive tract is feeding (Schusdziarra, 1980) or, more precisely, intragastric increases in protein, triglyceride, or carbohydrate concentrations. In our study we did not detect any change in peripheral plasma somatostatin concentration in response to feeding, although the liver plays an important role in the removal of somatostatin from the blood, and thus might have masked any small changes in somatostatin concentration within the hepatic portal blood. One of the functions of somatostatin release in the gut is thought to be control of the rate of nutrient entry into the body (Schusdziarra, 1980). This function may be less important in the ruminant where the reticulorumen acts as a buffer with a continuous outflow of nutrients entering the lower digestive tract (Bassett, 1975), which may affect the release of somatostatin from the digestive tract and from the pancreas.

Somatostatin was effective in increasing plasma free fatty acid concentations in a dose-dependent manner and potentiated the cold-induced rise in plasma free fatty acid levels during acute cold stress. It is unclear from this study whether this was due to direct or indirect effects on lipolysis or due to a decrease in utilization by tissues. Both types of response have been proposed (Gray et al., 1980; Minaire et al., 1981). The effect of somatostatin on plasma glucose concentration was much smaller than for free fatty acids and could indicate that glucose concentration is more tightly regulated. It may also be that somatostatin has a smaller effect on the liver than on adipose tissue. This is supported by in vitro work, where somatostatin inhibited both glucagon-stimulated glycogenolysis and gluconeogenesis, whereas it had no effect on epinephrine-stimulated glycogenolysis (Oliver and Wagle, 1975). Since at the doses of somatostatin used in these experiments somatostatin had no effect on plasma glucagon concentration, the effect on the liver might be substantially smaller.

Over the short-term basis of these experiments, exogenous somatostatin had little effect on either rectal or peripheral skin temperature, although somatostatin did decrease heat production in sheep acclimated to thermoneutral and cold environments. Heat production may have been reduced as a result of decreased uptake of substrates by tissues and thus would support the concept that glucose and free fatty acid utilization were decreased. We can not rule out the possibility that these increases in plasma glucose and free fatty acids resulted from increased absorption from the gut, release from endogenous stores, or from increased de novo synthesis. Intravenous

infusion of somatostatin seems to affect only the rate of heat production, whereas injection of somatostatin into the CNS (intraventricular) decreases heat production and peripheral vasodilation (Chandra et al., 1986), possibly by altering epinephrine release from the adrenal gland (Brown and Fisher, 1984). Thus it seems that the actions of somatostatin may be different depending upon the site of injection.

In these experiments, pancreatic hormone secretions were not affected by the continuous infusion of somatostatin, although the pancreas tended to be more responsive to the high dose used in the metabolic clearance rate trial. This is similar to the findings of other studies with sheep (Barry et al., 1985; Brockman and Laarveld, 1986). The effects of cold exposure on plasma insulin and glucagon concentrations were variable. Both insulin and glucagon concentrations were lower in the cold during the feeding and metabolic clearance rate trial, but were not affected by temperature in any of the other experiments. It is unclear as to whether this was due to a difference in sex, length of cold exposure, or period of fasting, or to some other type of stimuli. However, similar variations in insulin and glucagon responses to cold have been previously found (Sasaki and Weekes, 1986).

Plasma growth hormone was not affected by cold exposure in these trials, in agreement with the findings of Faulkner et al., (1980). On the other hand, more severe cold exposure has been shown to increase growth hormone concentrations in both sheep (Blom et al., 1976) and cattle (Olsen and Trenkle, 1973). Cold exposure seems to alter the growth hormone response after feeding. Growth hormone concentrations

were only marginally decreased after feeding in the cold environment, whereas the decline was more substantial in the thermoneutral environment suggesting that temperature somehow modifies the release and/or metabolic clearance of growth hormone following feeding. Somatostatin inhibited the post-cold increase in growth hormone during the acute temperature experiment. Thus, environmental temperature may affect the responsiveness of the hypothalamus and reduce growth hormone secretory response.

In conclusion, environmental temperature does not seem to affect basal concentrations of somatostatin, although it does affect somatostatin kinetics. Somatostatin, at physiological doses, can modify plasma metabolite concentrations even though changes in several of the hormones which regulate these metabolites are not affected, including insulin, glucagon, and growth hormone. This might result from a decrease in utilization of the metabolites, since somatostatin decreases oxygen consumption in both the thermoneutral and cold environments, or it may be due to an effect on the rate of absorption from the lower gut.

BIBLIOGRAPHY

Barry, T.N., Faichney, G.J., and Redekopp, C. 1985. Gastro-intestinal Tract Function in Sheep Infused with Somatostatin. Aust. J. Biol. Sci. 38: 393-403.

Bassett, J.M. 1975. Dietary and Gastro-intestinal Control of Hormones Regulating Carbohydrate Metabolism in Ruminants. Pages 383-398. *In* I.W. McDonald and A.C.I. Warner eds. Proceeding of the IV International Symposium on Ruminant Physiology. Univ. New Eng. Publ. Unit, Armidale, NSW, Australia.

Blom, A.K., Hove, K., and Nedkvitne, J.J. 1976. Plasma Insulin and Growth Hormone Concentrations in Pregnant Sheep II: Post-absorptive Levels in Mid- and Late Pregnancy. Acta Endocrinologica 82: 553-560.

Brockman, R.P. and Laarveld, B. 1986. Hormonal Regulation of Metabolism in Ruminants; A Review. Livest. Prod. Sci. 14: 313-334.

Brown, M.R. and Fisher, L.A. 1984. Brain Peptide Regulation of Adrenal Epinephrine Secretion. Am. J. Physiol. 247 (Endocrinol. Metab. 10): E41-E46.

Chandra, A., Chou, H.C., Chang, C., and Lin, M.T. 1981. Effects of Intraventricular Administration of Neurotensin and Somatostatin on Thermoregulation in the Rat. Neuropharmacol. 20(7): 715-718.

Dupont, A., Merand, Y., and Barden, N. 1978. Effect of Propylthiouracil and Thyroxine on the Inactivation of Somatostatin by Rat Hypothalamus. Life Sci. 23: 2007-2012.

Faichney, G.J. and Barry, T.N. 1986. Effects of Mild Heat Exposure and Suppression of Prolactin Secretion on Gastro-intestinal Tract Function and Temperature Regulation in Sheep. Aust. J. Biol. Sci. 39: 85-97.

Faulkner, A., Thomson, E.M., Bassett, J.M., and Thomson, G.E. 1980. Cold Exposure and Mammary Glucose Metabolism in the Lactating Goat. Br. J. Nutr. 43: 163-170.

Gray, D.E., Lickley, H.L.A., and Vranic, M. 1980. Physiologic Effects of Epinephrine on Glucose Turnover and Plasma Free Fatty Acid Concentrations Mediated Independently of Glucagon. Diabetes 29: 600-608.

Kennedy, P.M., Christopherson, R.J., and Milligan, L.P. 1986. Digestive Responses to Cold. Pages 285-306. *In* L.P. Milligan, W.L. Grovum, and A. Dobson eds. Control of Digestion and Metabolism in Ruminants. Prentice-Hall, Englewood Cliffs, NJ.

Meyer, J. 1987. Relationship Between Small Intestinal Transit and Small Intestinal Motility. Pages 327-338. *In* J.H. Szurszewski ed. Cellular Physiology and Clinical Studies of Gastrointestinal Smooth Muscle. Excerpta Medica, Elsevier Science Publ., New York, NY.

Minaire, Y., Forichon, J., Dallevet, G., and Jomain, M.J. 1981. Combined Effects of Cold and Somatostatin on Glcuose Kinetics in Dogs. Eur. J. Appl. Physiol. 46: 249-259.

Oliver, J.R. and Wagle, S.R. 1975. Studies on the Inhibition of Insulin Release, Glycogenolysis and Gluconeogenesis by Somatostatin in the Rat Islets of Langerhans and Isolated Hepatocytes. Biochem. Biophys Res. Commun. 62(3): 772-777.

Olsen, J.D. and Trenkle, A. 1973. Exposure of Cattle to Controlled Subzero Temperature: Growth Hormone, Glucose, and Free Fatty Acid Concentrations in Plasma. Am. J. Vet. Res. 34(6): 747-751.

Ruckebusch, Y. 1987. Comparative Ontogenesis of Gastrointestinal Motor Functions. Pages 71-101. *In* J.H. Szurszewski ed. Cellular physiology and Clinical Studies of Gastrointestinal Smooth Muscle. Excerpta Medica, Elsevier Science Publ., New York, NY.

Ruckebusch, Y. and Merritt, A.M. 1985. Pharmacology of the Ruminant Gastroduodenal Junction. J. Vet. Pharmacol. Therap. 8: 339-351.

Sasaki, Y. and Weekes, T.E.C. 1986. Pages 326-343. *In* L.P. Milligan, W.L. Grovum, and A. Dobson eds. Control of Digestion and Metabolism in Ruminants. Prentice-Hall, Englewood Cliffs, NJ.

Schusdziarra, V. 1980. Somatostatin - A Regulatory Modulator Connecting Nutrient Entry and Metabolism. Horm. Metab. Res. 12: 563-577.

Yamada, T. 1987. Gut Somatostatin. Pages 221-228. *In* S. Reichlin ed. Somatostatin Basic and Clinical Status. Plenum Press. New York, NY.

APPENDIX

Table 1. Feed composition for 20.4% crude protein diet used for the acute cold experiment in chapter II and was fed at 28.8 g kg^{-1} body weight.

Barley	79.0
Soybean	18.0
Limestone	1.0
Permapel™	2.0
Vitamin A,D,E	0.2
	100.0%

expressed as fed basis, 85.2% DM.

Table 2. Feed composition for 18.8% crude protein diet used for the chronic cold experiment outlined in chapter III and was fed at 28.8 g kg^{-1} body weight.

Barley	79.0
Soybean	18.0
Limestone	1.0
Permapel™	2.0
Vitamin A,D,E	0.2
	100.0%

expressed as fed basis, 87.3% DM.

Table 3. Feed composition for 19.6% crude protein diet used in the effect of feeding and metabolic clearance rate experiments and was fed at 28.8 g kg^{-1} body weight.

Barley	79.0
Soybean	18.0
Limestone	1.0
Permapel™	2.0
Vitamin A,D,E	0.2
	100.0%

expressed as fed basis, 86.1% DM.

Figure 1. Demonstration of parallelism with increasing quantities of pooled sheep plasma (5, 10, 25, 50, 75, and 100 μL).

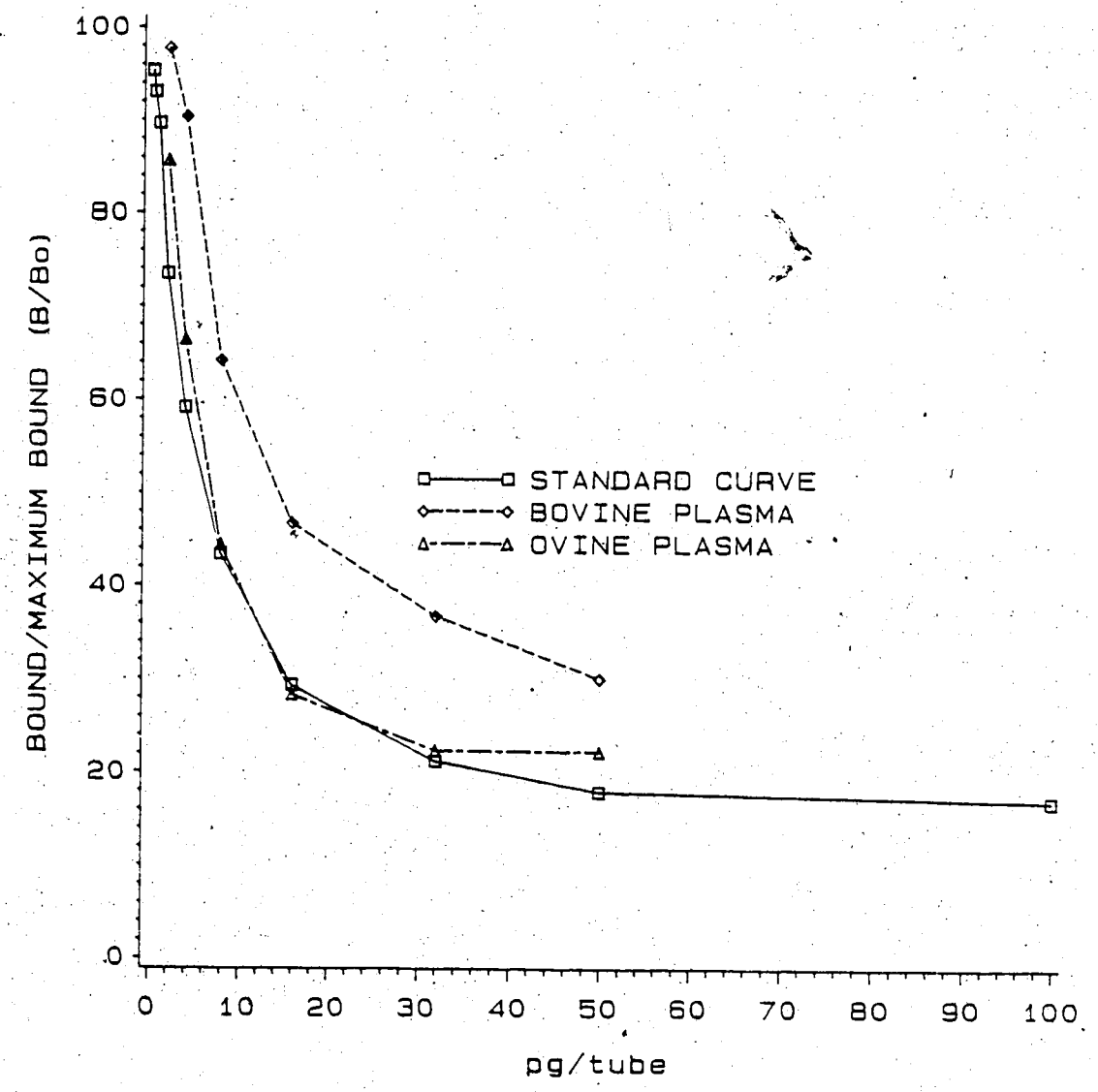


Figure 2. Parallel displacement of standard curve by addition of a constant amount (10 μL) of pooled sheep plasma.