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Effects of Somatostatin and Glucagon on the Utilization of [2-^{14}C]Propionate in Glucose Production *in vivo* in Sheep

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Abstract

This study examined the effects of hypoglucagonaemia and hyperglucagonaemia on the incorporation of ^{14}C from [2-^{14}C]propionate into plasma glucose of sheep *in vivo*. The sheep were adult ewes fed a maintenance diet of lucerne pellets delivered in equal aliquots hourly. The irreversible loss of glucose was determined by the continuous infusion of [6-^{3}H]glucose. During the control period (the hour immediately preceding infusion of hormones) $63\pm2\%$ of the propionate was converted to glucose, accounting for $30\pm2\%$ of glucose production. Glucagon deficiency, induced by infusion of somatostatin (100 μg/h), did not affect gluconeogenesis and the irreversible loss of glucose significantly. However, glucagon infusion at $11\cdot5\pm0\cdot6$ μg/h significantly increased the irreversible loss of glucose, with the greatest increase occurring in the first 15 min of infusion. The ^{14}C specific radioactivity of glucose and the fraction of glucose derived from propionate decreased significantly during glucagon infusion. The data are consistent with glucagon having a marked glycogenolytic effect initially, but little or no selective effect in promoting the utilization of propionate for glucose synthesis *in vivo* in sheep.

Introduction

Ordinarily ruminant animals on a roughage diet absorb very little dietary carbohydrate as hexose sugar (Schambye 1951; Bergman *et al.* 1970) because of the fermentation of the carbohydrate to short-chain fatty acids by ruminal microflora. Thus, their glucose requirements must be met by gluconeogenesis. One of the major glucose precursors is propionate, accounting for up to 50% of glucose synthesis (Bergman *et al.* 1966; Leng *et al.* 1967; Lindsay 1978). Despite the extensive investigations of the quantitative aspects of propionate metabolism in ruminants, relatively little is known about the hormonal influences on its regulation. The demonstration that propionate could stimulate the secretion of insulin (Manns *et al.* 1967; Bassett 1972) and glucagon (Bassett 1972) *in vivo* suggested that these hormones may be intimately involved in the regulation of propionate metabolism. Subsequent *in vitro* studies (Linzell *et al.*, cited by Lindsay 1971; Clark *et al.* 1976; Richardson and Livesey 1978) suggested that at high concentrations glucagon could enhance hepatic glucose production with propionate as the substrate. However, all *in vitro* studies do not agree. Savan *et al.* (1976) were unable to demonstrate that glucagon could enhance the utilization of propionate in glucose synthesis.

In vivo studies (Brockman and Bergman 1975; Brockman *et al.* 1975*a*) indicated that glucagon may facilitate gluconeogenesis by increasing the extraction of glucogenic

amino acids by the liver. The present study was undertaken to evaluate the effects of glucagon deficiency and glucagon excess on glucose production from propionate. Glucagon deficiency was induced by the infusion of somatostatin (SRIF) (Brockman and Johnson 1977; Brockman 1979*b*) and glucagon excess was obtained by the intravenous infusion of glucagon.

Materials and Methods

Animals and Diet

Mature, non-lactating, non-pregnant, mixed-breed (predominantly Columbia–Suffolk) ewes weighing 53±1 (mean±s.e., $n = 8$) kg were used in these experiments. The sheep were maintained in a temperature-controlled room with constant lighting. They were fed 800 g of lucerne pellets daily, delivered hourly in 24 equal aliquots by an automated feeder in order to facilitate steady-state digestion and absorption. Water and salt (blocks consisting of 99% (w/w) NaCl, 0·007% (w/w) iodine and 0·004% (w/w) cobalt) were provided *ad libitum*.

Experimental Procedure

At least 1 week prior to experimentation catheters were surgically implanted into the femoral artery and femoral and portal veins and a mesenteric vein tributary (Katz and Bergman 1969). Each experiment was begun by giving a priming dose of 4·0 μCi of [2-^{14}C]propionate (at −5 h) followed by continuous infusion at 7·0 μCi/h via the mesenteric venous catheter. Two hours later (at −3 h) a priming dose of 33 μCi of [6-^{3}H]glucose was administered followed by infusion at 22 μCi/h via the femoral venous catheter. After the zero hour samples were taken, 50 μg of SRIF (lot No. AY-24910, Ayerst Laboratories, Dorval, Quebec) were injected followed by infusion at 100 μg/h for 3·5 h via the femoral venous catheter. Porcine–bovine glucagon (lot No. GLF 599A, Lilly Research Laboratories, Indianapolis, Indiana) infusion was initiated at 1·5 h and continued for 2 h. It was infused into the mesenteric venous catheter at 11·5±0·6 (mean±s.e.) μg/h. Isotope infusions were terminated 1 h after termination of the hormone infusions.

All infusates were made up in pyrogen-free sterile saline (0·9% w/v NaCl). Carrier was added to the isotopes (obtained from Amersham Corporation, Oakville, Ontario) such that the infusion of unlabelled metabolites was less than 0·5% of the estimated turnover rates in the sheep. Lactose (25 μg per 100 μg of SRIF) was the carrier in the SRIF solutions. Glucagon was made up in saline containing 0·2% ovine serum albumin (fraction V powder, Sigma Chemical Co., St. Louis, Missouri). All infusates were delivered at 22 ml/h.

Blood samples for insulin, glucagon and propionate analyses were taken from the portal venous catheter. The samples were taken every half hour with the first sample at −1 h. Arterial samples were taken for glucose analysis. They were taken at half-hourly intervals throughout the infusion of [6-^{3}H]glucose, with the first sample taken 1 h after the priming dose of [6-^{3}H]glucose was administered (−2 h). Also, an additional sample was taken 15 min after the glucagon infusion was begun.

All blood samples were taken in heparinized syringes and immediately placed into tubes chilled on ice. The portal blood samples were centrifuged at 2°C and the plasma harvested. Samples for hormone analyses were stored in tubes containing 500 kallikrein inactivating units of aprotinin [Trasylol^{R}, Boehringer-Ingleheim (Canada) Ltd, Dorval, Quebec] and 3 mg of EDTA (dipotassium salt) per millilitre of plasma. All plasma samples were stored at −20°C until analysed. Neutral filtrates of arterial blood were prepared by immediately transferring 5 ml of blood into flasks containing 30 ml of 35 mM $Ba(OH)_2$. These were swirled and after at least 10 min, 15 ml of 70 mM $ZnSO_4$ were added. The supernatant was stored at −20°C until analysed.

Analytical Procedures

Glucagon and insulin in plasma were determined by radioimmunoassay as described previously (Brockman 1979*a*). Antibodies to insulin and glucagon were developed in guinea pigs. Antibody to guinea pig gammaglobulin was from sheep. Radio-iodinated [^{125}I]glucagon and [^{125}I]insulin were acquired from New England Nuclear Canada, Ltd (Lachine, Quebec) and Amersham Corporation (Oakville, Ontario), respectively. The sensitivities of the assays were 20 ng/l and 1 mU/l for glucagon

and insulin, respectively. Intra- and interassay repeatabilities were 9±2 and 16±3%, respectively, for glucagon and 6±1 and 7±1%, respectively, for insulin.

Propionate concentrations and specific radioactivities (SRA) were determined by gas–liquid chromatography (Baumgardt 1964; Corse and Elliot 1970). A Pye 104 gas chromatograph was equipped with a 100:1 stream splitter. A flame ionization detector was attached to the smaller stream. A glass side-arm in a methanol–dry ice bath was used to collect the volatile fatty acids from the larger stream. The side-arm was rinsed with 2 ml of water which was collected in a counting vial. 10 ml of a commercial phase combining solution (PCS, Amersham Corporation, Oakville, Ontario) were added. The samples were counted in a liquid scintillation counter set for ^{14}C.

Glucose concentrations were determined by the glucose oxidase method (Glucostat, Worthington Biochemical Corporation, Freehold, New Jersey). Radioactivity was determined by isolating glucose as potassium gluconate (Blair and Segal 1960). Recoveries were determined from the weight of potassium gluconate. The potassium gluconate was taken up in 3 ml water and transferred to a counting vial. 10 ml PCS were added. The samples were counted in a liquid scintillation counter set for two-channel dual isotope counting. Counting efficiencies for ^{3}H and ^{14}C and ^{14}C spillover into the ^{3}H channel were determined by external standard ratios method.

Calculations

The rate of production or irreversible loss of glucose was calculated using the equation for non-steady-state conditions (Cowan and Hetenyi 1971). The glucose pool size was calculated by the method of Steele *et al.* (1956). The fraction of glucose derived from propionate was estimated from the ratio of the SRA of arterial glucose to portal propionate (μCi/g-atom C). Glucose synthesized from propionate was calculated by multiplying the fraction of glucose derived from propionate by the irreversible loss of glucose.

The entry rate of propionate was determined from the ratio of infusion rate of [2-^{14}C]propionate (μCi/min) to the propionate SRA (μCi/mmol). The fraction of propionate used in glucose production was expressed as SRA of glucose (μCi/mmol) times its irreversible loss (mmol/min) divided by the infusion rate of [2-^{14}C]propionate (μCi/min).

Statistical significance of changes induced by the hormones within groups was evaluated by two-way analysis of variance. Each sample taken during hormone infusion was compared to the combined three-control (controls were samples taken at −1, −0·5 and 0 h) values by using the individual degree of freedom (Li 1964). Samples taken during glucagon plus SRIF infusion were similarly compared to combined SRIF values.

Results

The concentrations of glucagon and insulin in plasma are shown in Fig. 1. Glucagon and insulin concentrations decreased significantly ($P < 0{\cdot}05$) during infusion of SRIF, and increased during infusion of glucagon plus SRIF.

The concentration of propionate in the portal venous plasma was 235±16 (mean±s.e., $n = 8$) μM. Its specific radioactivity was 0·24±0·01 μCi ^{14}C/mmol. Both remained constant throughout the experiments. The entry rate of propionate was 29±2 mmol/h.

The concentration of glucose increased during glucagon infusion (Fig. 2). This was associated with an increase in the irreversible loss of glucose. SRIF infusion did not significantly affect plasma concentrations and rates of irreversible loss of glucose. The ^{14}C SRA of glucose and the fraction of glucose derived from propionate increased significantly during SRIF infusion (Fig. 3). They decreased significantly from SRIF values during the combined SRIF and glucagon infusions.

During the prehormone infusion period 63±2% (mean±s.e., $n = 8$) of infused isotope ([2-^{14}C]propionate) appeared in glucose (Fig. 4). This did not change during SRIF infusion but during the initial 15 min of glucagon infusion the rate of appearance of [^{14}C]glucose into plasma exceeded the rate of infusion of [2-^{14}C]propionate.

Thus, the rate of appearance in plasma of glucose derived from propionate exceeded the entry rate of propionate.

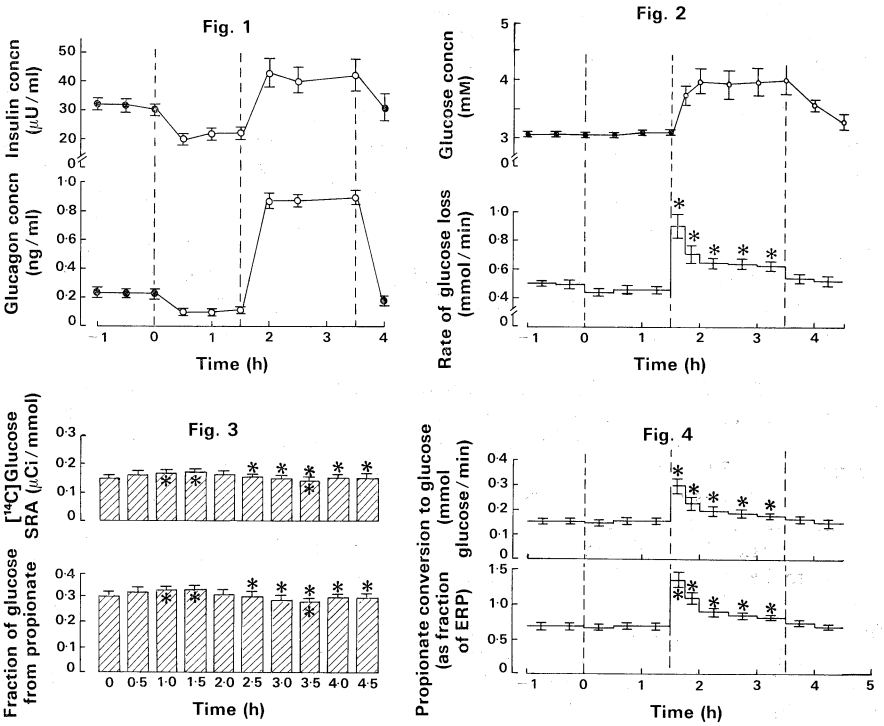

Fig. 1. Effect of SRIF and SRIF plus glucagon infusions on insulin and glucagon concentrations in plasma. Values are means±s.e. of eight animals. SRIF was infused for 3·5 h (over the interval 0–3·5 h) and glucagon over the interval 1·5–2·5 h. Open symbols denote significant differences ($P<0·05$, paired analyses) from values for −1, −0·5 and 0 h.

Fig. 2. Glucose concentration and rate of irreversible loss of glucose, before, during and after infusion of SRIF (from 0 to 1·5 h) and SRIF plus glucagon (from 1·5 to 3·5 h). Values are means ±s.e., $n = 8$. Open symbols and asterisks denote significant differences ($P<0·05$, paired analyses) from the samples for −1, 0·5 and 0 h.

Fig. 3. SRA of [^{14}C]glucose and fraction of glucose derived from propionate before, during and after infusion of SRIF (interval 0–1·5 h) and SRIF plus glucagon (interval 1·5–3·5 h). Values are means ±s.e., $n = 8$. Asterisks outside histograms denote significant differences from the three SRIF infusion values ($P<0·05$, paired analyses). Asterisks within histograms denote significant differences ($P<0·05$) from the control values.

Fig. 4. Propionate conversion to glucose as millimoles of glucose per minute and as a fraction of the entry rate of propionate (ERP). Values are means±s.e., $n = 8$. SRIF was infused from 0 to 3·5 h and glucagon over the interval 1·5–3·5 h. Asterisks indicate values are significantly different from control and SRIF infusion values.

Discussion

The feed was delivered in aliquots hourly to facilitate steady-state digestion. Therefore, the ruminal production and rate of absorption of the products of digestion, including propionate, should be constant throughout each experiment. Since the infusion rate of labelled propionate also was constant and into the portal vein, the

equilibrium SRA of propionate would be attained quickly and remain constant during the experiments. In addition, since ruminal propionate is the only source of extracellular propionate, SRA of arterial propionate would be the same as the portal SRA. Therefore, in the calculations, the mean ^{14}C SRA of portal venous propionate was used.

The calculation of irreversible loss of metabolites during non-steady state has limitations. Norwich *et al.* (1974) using inulin estimated that this approach has an error of $11 \pm 3\%$. This error may increase with the glucose system as a result of changes in insulin release during the non-steady state. However, the close correlation between net hepatic output and irreversible loss of glucose during glucagon infusion in another study (Brockman and Bergman 1975) suggests that the error is not of sufficient magnitude to alter the interpretation of the data.

Dual isotopes have been used previously to study gluconeogenesis in sheep (Brockman and Bergman 1975). One of the limitations is that the plasma SRA of the precursor is used in the calculations rather than the SRA of the substrate inside the cell where gluconeogenesis occurs. If the intracellular SRA is lower than the plasma SRA the rate of gluconeogenesis is underestimated. The major reduction of intracellular SRA of propionate would occur from 'crossing over' with the citric acid cycle intermediates (Krebs *et al.* 1966; Young 1977). It appears, however, that this underestimation is no more than 10–20% (Lindsay 1978). In addition, recycling of label through lactate may contribute to an overestimation of the utilization of propionate for glucogenesis. However, error associated with recycling of ^{14}C from glucose through products of glucose metabolism appears to be small in sheep (Brockman *et al.* 1975*b*). The recycling is not altered by hormone administration.

In ruminant animals the activity of hepatic glucokinase is low (Ballard *et al.* 1969). Therefore, estimation of glucose production with [6-^{3}H]glucose does not include the recycling of glucose through glycogen. However, ^{14}C from propionate can appear in liver glycogen. *In vitro* studies indicate that the incorporation of ^{14}C from labelled propionate into liver glycogen occurs at 24% of the rate of incorporation into glucose (Ballard and Oliver 1965). At steady state, [^{14}C]glycogen would equal about 24% of the glucose pool, and for the sheep in this study this amounts to 11 mmoles (pool size = 46 ± 2 mmoles). The total amount of glucose released by the liver during the first 15 min of glycogen administration was $13 \cdot 6 \pm 1 \cdot 2$ mmoles. The mean$\pm$s.e. irreversible loss of glucose during SRIF infusion was $0 \cdot 46 \pm 0 \cdot 02$ mmol/min. Subtracting the basal release for 15 min leaves 6·7 mmoles released by administration of glucagon. Thus, the amount of labelled glycogen would have been sufficient to account for the extra [^{14}C]glucose released by the liver during the initial 15 min of glucagon infusion. The release of [^{14}C]glucose as a result of glycogenolysis associated with infusion of glucagon would not be distinguishable from gluconeogenesis. Because the last glycogen synthesized is the first to be broken down, the SRA of glucose derived from glycogenolysis would be the same as that of glucose synthesized prior to hormone treatment. A reduction in SRA, therefore, would occur when 'cold' glycogen is broken down. On the other hand, if glucagon stimulates the utilization of propionate to a greater extent than other precursors in the synthesis of glucose, i.e. in gluconeogenesis, the ^{14}C SRA of the newly synthesized glucose and plasma glucose should increase. If all processes are increased equally, the SRA would be unchanged.

There are a limited number of ways to provide additional propionate for glucogenesis. Sparing of propionate metabolism for use in glucose synthesis is one (Blair

et al. 1973). However, since 63% of propionate is already used in synthesis of glucose, and since it is unlikely that all the propionate would be used in glucogenesis, sparing of propionate could not provide a substantial increase in the amount available for glucogenesis. Increased hepatic extraction of propionate, at best, could account only for a minor contribution. Ordinarily the liver extracts 90% of the propionate presented to it (Bergman and Wolff 1971).

The infusion of somatostatin was associated with a slight (about 7%), but significant, increase in [^{14}C]glucose SRA (Fig. 3). At the same time there was a slight (about 7%) depression of the irreversible loss of glucose (Fig. 2) such that there was no change in rate of incorporation of propionate into glucose (Fig. 4). These data suggest that basal levels of glucagon in plasma have little or no effect on propionate metabolism. However, it is possible that the simultaneous decrease in plasma insulin concentrations (Fig. 1) may offset any effect resulting from depression of glucagon concentrations. It is possible that SRIF may influence carbohydrate metabolism by depressing growth hormone concentrations. However, SRIF does not appear to reduce basal growth hormone concentrations, although it may depress the response to arginine or propionate (Bryce *et al.* 1975; Davis 1975), in sheep.

The infusion of glucagon caused a significant increase in irreversible loss of glucose. After 15 min of infusion [^{14}C]glucose SRA decreased significantly compared to values obtained during the infusion of SRIF alone. Furthermore, during the initial 15 min of glucagon infusion, the glucose derived from propionate exceeded the entry rate or rate of absorption of propionate. These findings clearly show that the increase in hepatic glucose output cannot be due solely to increased gluconeogenesis. Glycogenolysis must make a substantial contribution to the glucose output, at least in the early stages. The decrease in [^{14}C]glucose SRA, when considered with the observation that [^{14}C]glucose SRA tended to increase after termination of the hormone infusions, does not support the hypothesis that glucagon has a selective stimulatory effect on the utilization of propionate in glucogenesis. But these are consistent with an enhanced rate of utilization of other precursors in glucogenesis. This, however, does not rule out the possibility of increased conversion of propionate to glucose as the result of a general increase in the rate of gluconeogenesis.

The selectivity of the stimulatory effect of gluconeogenesis from other metabolites is supported by a study with [U-^{14}C]alanine (Brockman and Bergman 1975). In that study the fraction of glucose derived from alanine increased during the 30–120-min interval of glucagon infusion. Furthermore, the absolute amount of glucose derived from alanine progressively increased during glucagon infusion whereas in the present study it decreased from propionate. It appears, therefore, that although glucagon may enhance the utilization of alanine in gluconeogenesis it does not play a major role in the utilization of propionate in glucose production *in vivo*. The latter appears to be regulated by the amount absorbed and that which reaches the liver (Bergman *et al.* 1966).

The apparent discrepancy between *in vivo* and *in vitro* studies (Clark *et al.* 1976; Richardson and Livesey 1978) may be accounted for by two factors. Firstly, very high concentrations of glucagon were necessary to demonstrate gluconeogenesis effects *in vitro*. Secondly, it has been suggested that the stimulatory effect may occur as a result of low rates of basal glucose production in the *in vitro* preparations (Linzell *et al.*, cited by Lindsay 1971).

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References

Ballard, F. J., and Oliver, I. T. (1965). Carbohydrate metabolism in liver from foetal and neon ata sheep. *Biochem. J.* **95**, 191–200.

Ballard, F. J., Hanson, R. W., and Kronfeld, D. S. (1969). Gluconeogenesis and lipogenesis in tissue from ruminant and nonruminant animals. *Fed. Proc.* **28**, 218–31.

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**, 1277–87.

Baumgardt, B. R. (1964). Practical observations on the quantitative analysis of free volatile fatty acids (VFA) in aqueous solutions by GLC. Univ. Wis. Dep. Dairy Sci. Bull. No. 1.

Bergman, E. N., and Wolff, J. E. (1971). Metabolism of volatile fatty acids by liver and portal-drained viscera in sheep. *Am. J. Physiol.* **221**, 586–92.

Bergman, E. N., Katz, M. L., and Kaufman, C. F. (1970). Quantitative aspects of hepatic and portal glucose metabolism and turnover in sheep. *Am. J. Physiol.* **219**, 785–93.

Bergman, E. N., Roe, W. E., and Kon, K. (1966). Quantitative aspects of propionate metabolism and gluconeogenesis in sheep. *Am. J. Physiol.* **211**, 793–9.

Blair, A., and Segal, B. (1960). The isolation of blood glucose as potassium gluconate. *J. Lab. Clin. Med.* **55**, 959–64.

Blair, J. B., Cook, D. E., and Lardy, H. A. (1973). Interaction of propionate and lactate in the perfused rat liver. Effects of glucagon and oleate. *J. Biol. Chem.* **248**, 3608–14.

Brockman, R. P. (1979*a*). Glucagon response to exercise in trained and untrained sheep. *Aust. J. Biol. Sci.* **32**, 215–20.

Brockman, R. P. (1979*b*). Effect of somatostatin suppression of glucagon secretion on glucose production in sheep. *Can. J. Physiol. Pharmacol.* **57**, 848–52.

Brockman, R. P., and Bergman, E. N. (1975). Effect of glucagon on plasma alanine and glutamine metabolism and hepatic gluconeogenesis in sheep. *Am. J. Physiol.* **228**, 1627–33.

Brockman, R. P., and Johnson, M. R. (1977). Evidence of a role for glucagon in regulating glucose and β-hydroxy-butyrate metabolism in sheep. *Can. J. Anim. Sci.* **57**, 177–80.

Brockman, R. P., Bergman, E. N., Joo, P. K., and Manns, J. G. (1975*a*). Effects of glucagon and insulin on net hepatic metabolism of glucose precursors in sheep. *Am. J. Physiol.* **229**, 1344–50.

Brockman, R. P., Bergman, E. N., Pollak, W. L., and Brondum, J. (1975*b*). Studies of glucose production in sheep using [6-^{3}H]glucose and [U-^{14}C]glucose. *Can. J. Physiol. Pharmacol.* **53**, 1186–9.

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**, 842–50.

Clark, M. E., Filsell, O. H., and Jarrett, I. G. (1976). Gluconeogenesis in isolated intact lamb liver cells. Effects of glucagon and butyrate. *Biochem. J.* **156**, 671–80.

Corse, D. A., and Elliot, J. M. (1970). Propionate utilization by pregnant, lactating, and spontaneously ketotic dairy cows. *J. Dairy Sci.* **53**, 740–6.

Cowan, J. S., and Hetenyi, G., Jr. (1971). Glucoregulatory responses in normal and diabetic dogs recorded by a new tracer method. *Metab. Clin. Exp.* **20**, 360–72.

Davis, S. L. (1975). Somatostatin: its influence on plasma levels of growth hormone, prolactin and thyrotropin in sheep. *J. Anim. Sci.* **40**, 911–16.

Katz, M. L., and Bergman, E. N. (1969). A method for simultaneous cannulation of the major splanchnic blood vessels of the sheep. *Am. J. Vet. Res.* **30**, 655–61.

Krebs, H. A., Hems, R., Weidemann, M. J., and Speake, R. N. (1966). The fate of isotopic carbon in kidney cortex synthesizing glucose from lactate. *Biochem. J.* **101**, 242–9.

Leng, R. A., Steel, J. W., and Luick, J. R. (1967). Contribution of propionate to glucose synthesis in sheep. *Biochem. J.* **103**, 785–90.

Li, J. C. R. (1964). 'Statistical Inference.' Vol. I. pp. 252–65. (J. W. Edwards Inc.: Ann Arbor, Michigan.)

Lindsay, D. B. (1971). Changes in the pattern of glucose metabolism in growth, pregnancy and lactation in ruminants. *Proc. Nutr. Soc.* **30**, 272–7.

Lindsay, D. B. (1978). Gluconeogenesis in ruminants. *Biochem. Soc. Trans.* **6**, 1152–6.

Manns, J. G., Boda, J. M., and Willes, R. F. (1967). Probable role of propionate and butyrate in control of insulin secretion in sheep. *Am. J. Physiol.* **212**, 756–64.

Morgan, C. R., and Lazarow, A. (1963). Immunoassay of insulin: two antibody system. Plasma levels of normal, subdiabetic and diabetic rats. *Diabetes* **12**, 115–26.

Norwich, K. H., Radziuk, J., Lau, D., and Vranic, M. (1974). Experimental validation of nonsteady rate measurements using a tracer infusion method and inulin as tracer and tracee. *Can. J. Physiol. Pharmacol.* **52**, 508–21.

Richardson, R. I., and Livesey, A. F. (1978). Gluconeogenesis in isolated sheep hepatocytes. *Proc. Nutr. Soc.* **38**, 2A.

Savan, P. M. J., Jeacock, M. K., and Shepherd, D. A. L. (1976). Hepatic gluconeogenesis in foetal and suckling lambs. *Proc. Nutr. Soc.* **35**, 30A.

Schambye, P. (1951). Volatile acids and glucose in portal blood of sheep. *Nord. Veterinarmed.* **3**, 555–74.

Steele, R., Wall, J. S., Bodo, R. C. de, and Altszuler, N. (1956). Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am. J. Physiol.* **187**, 15–24.

Young, J. W. (1977). Gluconeogenesis in cattle: significance and methodology. *J. Dairy Sci.* **60**, 1–15.

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