THE EFFECTS OF GLUCAGON ON PLASMA CONCENTRATIONS OF INSULIN, GROWTH HORMONE, GLUCOSE, AND FREE FATTY ACIDS IN SHEEP: COMPARISON WITH THE EFFECTS OF CATECHOLAMINES

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Abstract

Glucagon, adrenaline, and isoprenaline were administered to adult sheep by intravenous infusion at a rate of 5 μg/min for 2 hr.

Plasma glucose concentration increased more rapidly during glucagon infusion than during infusion of either adrenaline or isoprenaline.

Plasma insulin concentration increased rapidly during glucagon infusion, but the increase was less than that observed during infusion of isoprenaline. There was almost no increase in plasma insulin during infusion of adrenaline despite marked hyperglycaemia.

Plasma growth hormone concentration increased during glucagon infusion, but high levels were not maintained. Both adrenaline and isoprenaline decreased growth hormone concentration.

Plasma free fatty acid concentrations decreased during glucagon infusion. Plasma sodium and potassium concentrations also decreased but the plasma concentration of urea nitrogen was not altered during glucagon infusion.

Intravenous infusion of glucagon at rates of 0·5 and 0·1 μg/min for 2 hr also significantly increased plasma glucose, insulin, and growth hormone concentration and decreased plasma free fatty acid concentration. Infusion of glucagon at lower rates (0·05 and 0·01 μg/min) had no significant effects.

I. INTRODUCTION

Glucagon, the hormone secreted by the alpha cells of the islets of Langerhans in the pancreas, is now generally regarded as playing an essential role in glucose homeostasis through its stimulatory effects on hepatic glycogenolysis and gluconeogenesis (Sokal 1966*a*).

In ruminants, glucose homeostasis depends to a large extent on the regulation of gluconeogenesis and hepatic glucose output since little glucose is derived, as such, from dietary sources (Katz and Bergman 1969). It has been shown that glucagon in high doses can increase the blood glucose level of sheep (Ho and Reber 1957; Phillips *et al.* 1969) but the exact role of glucagon in ruminants remains virtually unknown.

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The present paper reports experiments undertaken to examine the effects of glucagon on metabolism in sheep, and to determine their sensitivity to glucagon. In addition the effects of glucagon have been compared with those of the catecholamines given under similar conditions.

II. Materials and Methods

(a) *Animals*

Ten Border Leicester–Merino crossbred wethers aged 1 year and weighing 48·0 kg (S.D. ±2·5 kg) were used. They were maintained individually in an animal house and fed 800 g daily of a ration consisting of chopped lucerne hay and oat grain (1:1). The animals remained free and unrestrained in their pens throughout the experiments.

(b) *Experimental*

Polyethylene catheters were implanted in one external jugular vein on the day prior to each infusion experiment. On the day of the experiment, food was withheld until observations were completed. Experiments were carried out at weekly intervals. All 10 sheep were infused simultaneously using two multichannel peristaltic infusion pumps and infusion catheters were supported from above the sheep. Each infusion lasted 2 hr.

During the experiments the following solutions were infused:

(1) Saline, 0·9% NaCl containing 0·1% bovine albumin.

(2) Glucagon (Eli Lilly beef pork glucagon Lot No. 258-234B-167-1) in 0·9% NaCl containing 0·1% bovine albumin. The final concentration and infusion rates were varied so that the sheep received either 5, 0·5, 0·1, 0·05, or 0·01 μg/min in the various experiments.

(3) Adrenaline, as the bitartrate salt in 0·9% NaCl containing 0·3% ascorbic acid, infused at a rate of 5 μg base/min.

(4) Isoprenaline, as the hydrochloride, in 0·9% NaCl containing 0·3% ascorbic acid, infused at a rate of 5 μg base/min.

(c) *Blood Sampling*

Blood samples were obtained with minimal disturbance by venipuncture of the jugular vein opposite to that used for infusion. The samples were transferred immediately to heparinized centrifuge tubes cooled in a bath of iced water. Plasma was separated by centrifugation at 4°C within 30 min and was stored at −20°C.

(d) *Analytical*

Plasma glucose was determined by the glucose oxidase method of Huggett and Nixon (1957); free fatty acids (FFA) by the method of Dole (1956), as modified by Annison (1960); and insulin and growth hormone by radio-immunoassay using talc to separate antibody-bound and free hormone (Rosselin *et al.* 1966; Wallace and Bassett 1970). Sodium and potassium were determined by flame-photometry with an autoanalyser (Technicon Instruments Corp. N.Y.) and urea was determined on an autoanalyser by the method of Wilson (1966).

(e) *Calculations*

Plasma concentrations of FFA, insulin, and growth hormone were transformed to logarithms (base 10) before calculation of means (Patterson *et al.* 1964; Welborn *et al.* 1966; Wallace and Bassett 1970). For clarity, antilogs of means are used in the presentation of results. Statistical significance was assessed by the *t*-test.

III. Results

(*a*) *Plasma Glucose and Insulin*

The infusion of glucagon at a rate of 5 μg/min (approximately 0·1 μg/kg/min) for 2 hr caused very large increases in the plasma levels of both glucose and insulin, with maximum concentrations being reached 60–90 min after the start of infusion, despite its continuation for 2 hr (Fig. 1). Infusion of adrenaline (5 μg/min) also caused a marked though somewhat slower increase in plasma glucose (Fig. 1). However, the increase continued throughout the 2 hr of the infusion and the maximum glucose concentration reached was similar to that seen during glucagon infusion. Isoprenaline caused a steady but much smaller increase in glucose concentration. During adrenaline infusion, despite hyperglycaemia, there was no increase in plasma insulin during the

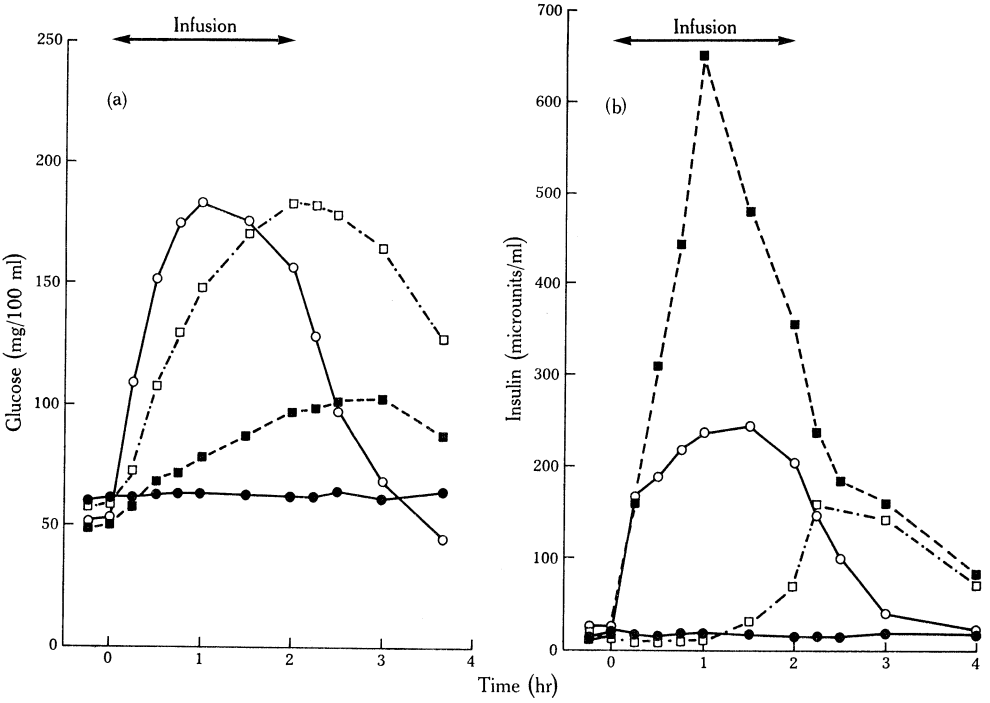

Fig. 1.—Changes in mean plasma concentrations of (*a*) glucose and (*b*) insulin in groups of five wether sheep during intravenous infusion of saline (●), 5 μg/min glucagon (○), 5 μg/min adrenaline (□), or 5 μg/min isoprenaline (■).

first hour of the infusion, and only a rather slow increase during the second hour. However, after the adrenaline infusion was stopped plasma insulin increased rapidly, but the maximum concentration reached was less than that obtained during glucagon infusion, despite the comparable hyperglycaemia. Isoprenaline infusion resulted in an increase in plasma insulin far greater than that seen during glucagon infusion. Infusion of saline alone did not significantly alter either glucose or insulin concentrations in plasma (Fig. 1).

To examine the minimum effective rate of glucagon infusion in sheep, the hormone was infused for 2 hr at rates of 0·5, 0·1, 0·05, and 0·01 μg/min. As the maximum glucose and insulin responses were obtained during the first 60 min of infusion, the increments during this period have been used in comparing the effects of these doses with those of saline and 5 μg glucagon/min (Table 1). Plasma concentrations of glucose and insulin at the end of the 2-hr infusion are also shown in Table 1.

TABLE 1

EFFECT OF INTRAVENOUS INFUSION OF GLUCAGON FOR 2 HR ON THE PLASMA CONCENTRATIONS OF GLUCOSE AND INSULIN

Glucagon Infusion Rate (μg/min)	Time of Sample (min) 0	60	120	Increment 0–60 min
	(*a*) Glucose (mg/100 ml)†			
5	53±1·3	183±7·2	157±13·8	130***
0·5	60±1·9	107±7·4	97± 9·7	47***
0·1	54±1·2	65±1·5	68± 1·2	11**
0·05	51±1·8	58±2·3	58± 1·8	7
0·01	61±1·7	60±2·1	61± 2·5	−1
0	62±2·5	63±3·9	62± 4·4	1
	(*b*) Insulin (microunits/ml)‡			
5	25·7	237·0	202·3	
	(1·41±0·06)	(2·37±0·08)	(2·31±0·05)	(0·96)***
0·5	13·4	66·4	44·1	
	(1·13±0·06)	(1·82±0·06)	(1·64±0·03)	(0·70)***
0·1	8·0	24·9	21·1	
	(0·91±0·07)	(1·40±0·06)	(1·32±0·07)	(0·49)***
0·05	13·5	9·4	13·0	
	(1·13±0·09)	(0·97±0·14)	(1·11±0·09)	(−0·16)
0·01	13·8	11·8	12·2	
	(1·14±0·04)	(1·07±0·04)	(1·08±0·03)	(−0·07)
0	20·1	16·0	13·7	
	(1·30±0·07)	(1·20±0·08)	(1·14±0·06)	(−0·10)

** Difference significant at $P < 0·01$.

*** Difference significant at $P < 0·001$.

† Values given are means ± standard error.

‡ Values in first lines are antilogarithms of mean values, whilst those in parentheses are $\log_{10}$ of mean values ± standard error.

Plasma concentrations of both glucose and insulin were increased during glucagon infusion at rates of 0·5 and 0·1 μg/min as well as at a rate of 5 μg/min (Table 1). Possibly there was also a very small increase in plasma glucose during the infusion of 0·05 μg glucagon/min, but there was no increase in insulin with either this or the smallest rate of infusion (0·01 μg/min) used. To compare the potency of glucagon and adrenaline as agents increasing the plasma glucose of sheep, the incre-

ments in plasma glucose during the first 30 min of glucagon and adrenaline infusion have been compared with those observed in another group of sheep given infusions of adrenaline at a variety of rates (Bassett 1970). The increment in glucose was clearly related to $\log_{10}$ of the glucagon infusion rate (Fig. 2), as has previously been observed with adrenaline, but when compared on a molar basis glucagon infusion at one-hundredth the rate of adrenaline produced equivalent increases in the plasma glucose concentration.

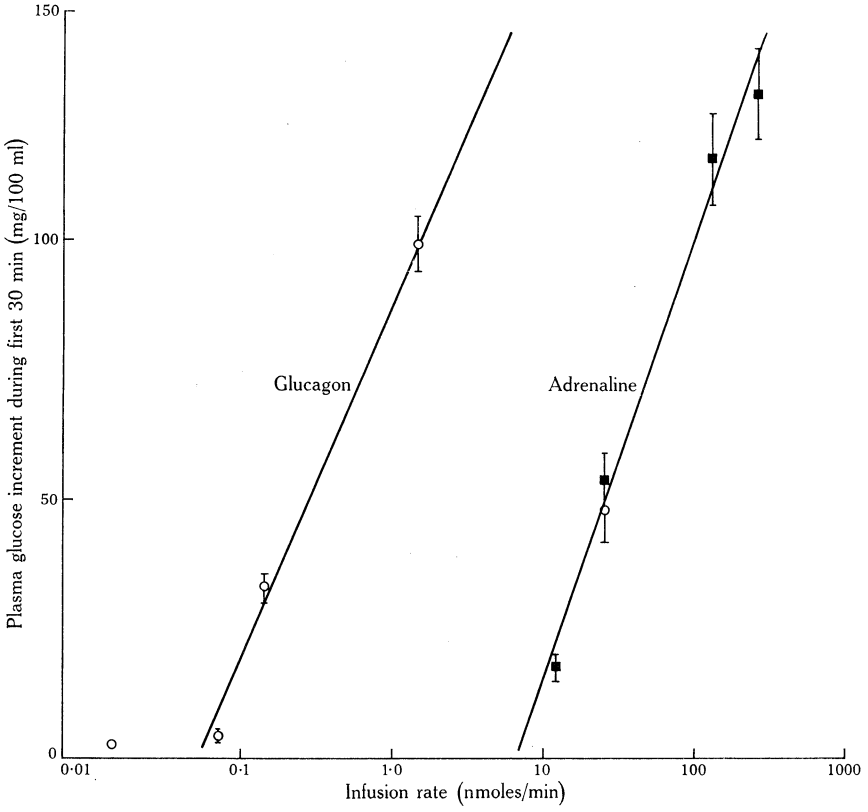

Fig. 2.—Comparison of the effects of glucagon and adrenaline on the increase in plasma glucose during the first 30 min of infusion in the present experiments (○) with the effects of adrenaline in earlier experiments (Bassett 1970) (■). Vertical bars indicate standard errors.

(*b*) *Plasma Growth Hormone*

Glucagon infusion at 5·0, 0·5, and 0·1 μg/min altered plasma growth hormone concentrations significantly [Fig. 3(*a*)]. During the first hour of infusion there were frequently large increases in growth hormone concentrations and the maximum values in each case were significantly higher ($P < 0·05$) than the pre-infusion values. However, the increases were neither prolonged nor consistent in their time of occurrence, and were not related clearly to the infusion rate. Smaller doses of glucagon

[Fig. 3(*a*)] and saline [Fig. 3(*b*)] had no significant effects on the plasma growth hormone level, but during the infusion of adrenaline or isoprenaline [Fig. 3(*b*)] the plasma growth hormone level declined significantly ($P < 0{\cdot}05$).

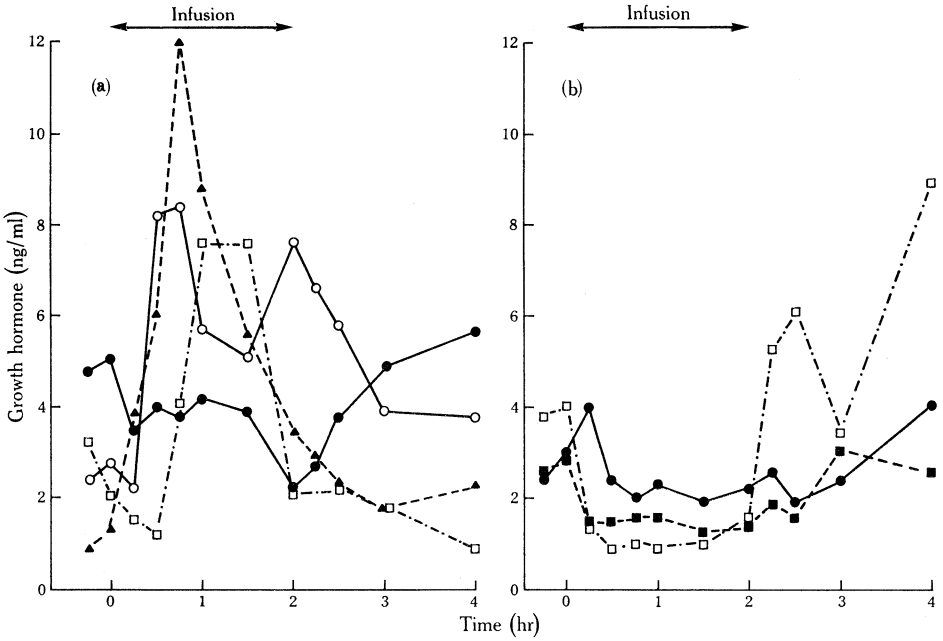

Fig. 3.—Changes in mean plasma growth hormone concentrations of groups of five wether sheep during intravenous infusion of (*a*) glucagon at 5 μg/min (○), 0·5 μg/min (▲), 0·1 μg/min (□), or 0·05 and 0·01 μg/min (10 sheep) (●), or (*b*) saline (●), 5 μg/min adrenaline (□), or 5 μg/min isoprenaline (■).

(*c*) *Plasma Free Fatty Acids*

Frequently during the first 15 min of glucagon infusion the plasma FFA concentration increased (Fig. 4), but the magnitude of this increase did not appear related to the infusion rate. After 15 min, however, there was a marked decrease in plasma FFA during infusion of glucagon at either 5 or 0·5 μg/min and a distinct, though smaller, decrease with 0·1 μg/min. With the latter infusion rate the plasma FFA level was never significantly below pre-infusion levels, but concentrations during the second hour of infusion were significantly lower than the values after 15 and 30 min of infusion. Plasma FFA increased in the 2 hr following cessation of glucagon infusion in all these experiments. Infusion of saline or of glucagon at lower rates had no significant effect on plasma FFA. The FFA concentrations in plasma during infusion of adrenaline and isoprenaline were not measured. The effects of these drugs on the plasma FFA concentration have already been reported (Bassett 1970).

(*d*) *Plasma Urea, Sodium, and Potassium Concentrations*

Infusion of glucagon at 5 μg/min had no statistically significant effect on the plasma concentration of urea, but sodium and potassium concentrations were significantly lower at the end of the infusion (Table 2).

IV. Discussion

The results show that intravenous infusion of as little as 0·1 μg glucagon/min (approx. 2 ng/kg/min) into adult wether sheep results in significant increases in plasma glucose and insulin levels. In dogs intraportal infusion of 3 ng glucagon/kg/min results in significant increases in blood glucose (Ezdinli and Sokal 1966), and in man intravenous infusion of 0·2 μg/min causes significant increases in blood glucose (Butterfield, Fry, and Whichelow 1960). The sheep is thus at least as sensitive as these

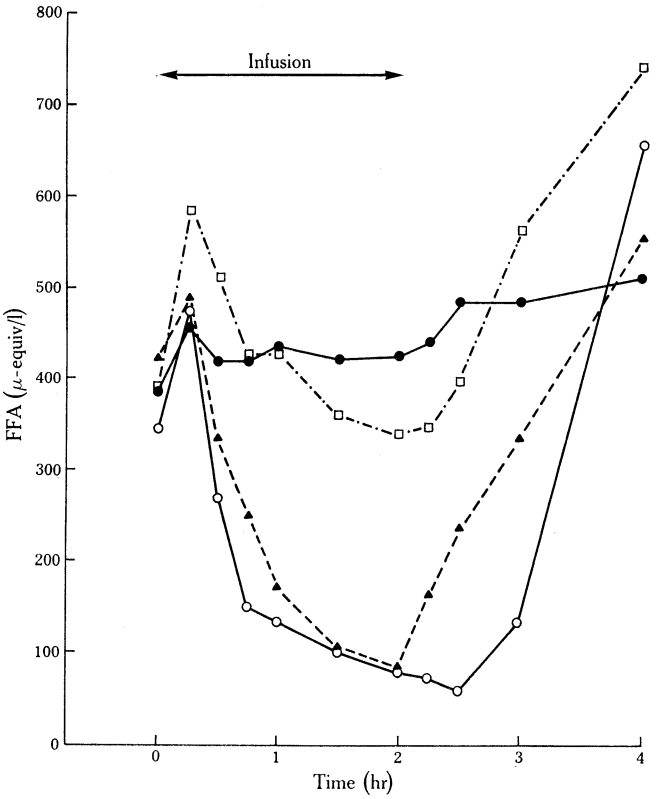

Fig. 4.—Changes in mean plasma FFA levels of groups of five wether sheep during intravenous infusion of glucagon at 5 μg/min (○), 0·5 μg/min (▲), 0·1 μg/min (□), or 0·05 and 0·01 μg/min (10 sheep) (●).

other species to the glycogenolytic action of glucagon. The similar magnitude of the changes during intravenous infusion of 5 μg glucagon/min into sheep and of those observed during infusion of 5 μg/min into human subjects (Crockford *et al.* 1966) is further confirmation of the similarity of these species in their sensitivity to the action of glucagon.

The experiments on sheep also show clearly, as do those on dogs (Ezdinli and Sokal 1966), that glucagon is a more potent glycogenolytic agent than adrenaline, especially when the effectiveness of the two is compared on a molar basis (Fig. 2).

This is so even though the hyperglycaemia observed during glucagon infusion is accompanied by a marked increase in the plasma insulin concentration, whereas that occurring during adrenaline infusion is not accompanied by any immediate increase in the plasma insulin concentration (Bassett 1970).

The present experiments do not demonstrate unequivocally that the increase in plasma insulin is a direct effect of glucagon on insulin secretion rather than a result of the hyperglycaemia caused by the hormone, as the insulin secretory response to glucose infusion was not studied. However, the increase in plasma insulin which followed the adrenaline infusion [Fig. 1(*b*)] was smaller than that occurring during glucagon infusion, even though the degree of hyperglycaemia was comparable [Fig. 1(*a*)]. It seems likely, therefore, that insulin secretion is directly stimulated by glucagon in sheep as it is in man (Samols, Marri, and Marks 1965; Crockford *et al.* 1966; Karam *et al.* 1966) and the occurrence of significant increases in plasma insulin during infusion of glucagon at 0·1 μg/min suggests that the insulin secretory mechanism is probably as sensitive to glucagon as is hepatic glycogenolysis.

TABLE 2

EFFECT OF GLUCAGON (5 μG/MIN) INTRAVENOUSLY FOR 2 HR OR SALINE INFUSION (CONTROL) ON MEAN PLASMA CONCENTRATIONS OF SODIUM, POTASSIUM, AND UREA IN A GROUP OF FIVE SHEEP

Values given are means ± standard error

Time of Sample (min)	Sodium Concn. (m-equiv/l)		Potassium Concn. (m-equiv/l)		Urea Concn. (mg/100 ml)	
	Glucagon	Control	Glucagon	Control	Glucagon	Control
0	147·9±0·5	145·7±0·7	4·61±0·13	4·86±0·03	13·7±1·1	14·6±1·4
30	146·9±0·7	146·1±0·4	4·09±0·11*	4·76±0·18	13·8±1·0	14·5±1·3
60	147·0±0	145·9±0·7	3·77±0·09***	4·56±0·09	13·9±0·9	14·4±1·1
90	146·3±0·1*	145·5±0·8	3·59±0·14***	4·54±0·07	13·8±0·9	14·2±1·1
120	145·4±0·3**	145·6±0·8	3·46±0·16***	4·57±0·09	13·9±0·9	14·4±1·1
180	145·1±0·6**	145·5±0·8	4·10±0·23	4·85±0·08	14·2±0·9	14·0±1·1
240	147·1±0·2	145·0±0·6	4·31±0·21	4·80±0·04	14·4±0·8	13·8±0·9

* Difference from time zero value significant at $P < 0·02$.

** Difference from time zero value significant at $P < 0·01$.

*** Difference from time zero value significant at $P < 0·001$.

The decrease in the plasma FFA level of sheep during glucagon infusion is also consistent with the findings in man (Crockford *et al.* 1966) and indicates either that glucagon has little lipolytic activity in sheep or that any lipolytic action of glucagon [comparable to that observed in dogs (Lefebvre 1966) or during *in vitro* studies on rat adipose tissue (Hagen 1961)] is obscured by the antilipolytic effect of the insulin secreted during glucagon infusion. The initial increase in FFA during infusion of glucagon may indicate a lipolytic effect of the hormone obscured later by the antilipolytic effect of insulin; a similar biphasic effect has been observed in dogs after glucagon injection (Whitty *et al.* 1969). However, isoprenaline is a very powerful lipolytic agent in sheep (Bassett 1970) even though it is a powerful stimulator of insulin secretion, so it seems unlikely that glucagon can be regarded as an important lipolytic hormone in the sheep.

Glucagon given by subcutaneous injection has been reported to cause significant increases in the serum growth hormone concentration of human subjects within 2–3 hr of its administration (Mitchell, Byrne, and Silver 1969), and a continuous infusion into two human subjects at a rate of 0·1 mg/kg/min has also been shown to increase the plasma growth hormone concentration (Kipnis, Hertelendy, and Machlin 1969). The experiments reported here show that glucagon infusion into sheep also increases plasma growth hormone (Fig. 3), but reasons for variability in the increase and in its timing are not apparent. The failure of saline or the lower glucagon infusion rates to cause similar fluctuations in growth hormone indicates a specific response to glucagon. This response was also clearly distinct from that seen during adrenaline and isoprenaline infusion when, consistent with earlier observations (Wallace and Bassett 1970), growth hormone concentration decreased. It seems unlikely that the action of glucagon in stimulating secretion of insulin and growth hormone involves adrenergic receptor mechanisms, even though it probably involves activation of systems generating cyclic-3′5′-AMP. Beta receptors have a stimulatory effect and alpha receptors an inhibitory effect on insulin secretion in sheep (Bassett 1970), yet the effects of isoprenaline indicate an inhibitory effect of beta receptors on growth hormone secretion in this species, whereas glucagon has a stimulatory effect on the secretion of both hormones.

In addition to its stimulatory effects on hepatic glycogenolysis, glucagon also increases gluconeogenesis and urea production by rat liver (Miller 1960; Sokal 1966*b*). However, in the present experiments there was no evidence for an increase in hepatic urea production during glucagon infusion, since there was no significant increase in the plasma urea concentration. On the other hand, an increase in renal excretion of urea could have obscured any effect of an increase in urea production.

The decreases in plasma sodium and potassium concentrations during glucagon infusion are consistent with earlier observations on cats (Wolfson and Ellis 1956) and may be partly due to increased renal excretion of sodium and potassium seen after glucagon administration (Staub *et al.* 1957).

The observations presented in this paper indicate that the effects of glucagon in sheep are similar to the effects of the hormone in other species which have been studied. However, an understanding of the role of glucagon in sheep and in other ruminants must await information about the way the blood glucagon level is regulated. In man and dog, glucagon secretion is stimulated by fasting, hypoglycaemia, hyperaminoacidaemia, and by the gastrointestinal hormone pancreozymin (Unger and Eisentraut 1964; Ohneda *et al.* 1968; Buchanan *et al.* 1969; Ohneda *et al.* 1969; Unger *et al.* 1969) and clearly plays an important role in glucose homeostasis. There have, however, been no comparable studies on ruminants. In these species there is often no dietary source of glucose, and blood glucose homeostasis is largely dependent on the regulation of hepatic glucose production and gluconeogenesis. Despite this lack of a dietary source of glucose, there is good evidence (Katz and Bergman 1969) that the rate of hepatic glucose output is highest 2–4 hr after feeding. Since glucagon can increase hepatic glycogenolysis, glucagon secretion (triggered by processes in the gastrointestinal tract) might be involved in regulating hepatic glucose output at this time. The finding that the hyperglycaemic effect of propionic and butyric acids is dependent on the presence of an intact pancreas in

sheep (Phillips *et al.* 1969) suggests that these acids stimulate the secretion of glucagon as well as that of insulin (Manns and Boda 1967). This raises the possibility that increases in the concentration in plasma of these short-chain fatty acids may play a part in stimulating both glucagon and insulin secretion.

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VI. References

Annison, E. F. (1960).—*Aust. J. agric. Res.* **11**, 58.

Bassett, J. M. (1970).—*Aust. J. biol. Sci.* **23**, 903.

Buchanan, K. D., Vance, J. E., Dinstl, K., and Williams, R. H. (1969).—*Diabetes* **18**, 11.

Butterfield, W. J. H., Fry, I. K., and Whichelow, M. J. (1960).—*Guy's Hosp. Rep.* **109**, 95.

Crockford, P. M., Porte, D., Wood, F. C., and Williams, R. H. (1966).—*Metabolism* **15**, 114.

Dole, V. P. (1956).—*J. clin. Invest.* **35**, 150.

Ezdinli, E. Z., and Sokal, J. E. (1966).—*Endocrinology* **78**, 47.

Hagen, J. H. (1961).—*J. biol. Chem.* **236**, 1023.

Ho, P., and Reber, E. F. (1957).—*Am. J. vet. Res.* **18**, 342.

Huggett, A. St. G., and Nixon, D. A. (1957).—*Lancet* **ii**, 368.

Karam, J. H., Grasso, S. G., Wegienka, L. C., Grodsky, G. M., and Forsham, P. H. (1966).—*Diabetes* **15**, 571.

Katz, M. L., and Bergman, E. N. (1969).—*Am. J. Physiol.* **216**, 953.

Kipnis, D. M., Hertelendy, F., and Machlin, L. J. (1969).—In "Progress in Endocrinology." Proc. 3rd Int. Congr. Endocr. Mexico 1968 (Ed. C. Gual.) p. 601. (Excerpta Medica Int. Congr. Ser. No. 184.)

Lefebvre, P. (1966).—*Diabetologia* **2**, 130.

Manns, J. G., and Boda, J. M. (1967).—*Am. J. Physiol.* **212**, 747.

Miller, L. L. (1960).—*Nature, Lond.* **185**, 248.

Mitchell, M. L., Byrne, M. J., and Silver, J. (1969).—*Lancet* **i**, 289.

Ohneda, A., Aguilar-Parada, E., Eisentraut, A. M., and Unger, R. H. (1969).—*Diabetes* **18**, 1.

Ohneda, A., Parada, E., Eisentraut, A. M., and Unger, R. H. (1968).—*J. clin. Invest.* **47**, 2305.

Patterson, D. S. P., Burns, K. N., Cunningham, N. F., Hebert, C. N., and Saba, N. (1964).—*J. agric. Sci., Camb.* **62**, 253.

Phillips, R. W., House, W. A., Miller, R. A., Mott, J. L., and Sooby, D. L. (1969).—*Am. J. Physiol.* **217**, 1265.

Rosselin, G., Assan, R., Yalow, R. S., and Berson, S. A. (1966).—*Nature, Lond.* **212**, 355.

Samols, E., Marri, G., and Marks, V. (1965).—*Lancet* **ii**, 415.

Sokal, J. E. (1966*a*).—*Am. J. Med.* **41**, 331.

Sokal, J. E. (1966*b*).—*Endocrinology* **78**, 538.

Staub, A., Springs, V., Stoll, F., and Elrick, H. (1957).—*Proc. Soc. exp. Biol. Med.* **94**, 57.

Unger, R. H., and Eisentraut, A. M. (1964).—*Diabetes* **13**, 563.

Unger, R. H., Ohneda, A., Aguilar-Parada, E., and Eisentraut, A. M. (1969).—*J. clin. Invest.* **48**, 810.

Wallace, A. L. C., and Bassett, J. M. (1970).—*J. Endocr.* **47**, 21.

Welborn, T. A., Rubenstein, A. H., Haslam, R., and Fraser, R. (1966).—*Lancet* **i**, 280.

Whitty, A. J., Shima, K., Trubow, M., and Foa, P. P. (1969).—*Proc. Soc. exp. Biol. Med.* **130**, 55.

Wilson, B. W. (1966).—*Clin. Chem.* **12**, 360.

Wolfson, S. K., and Ellis, S. (1956).—*Proc. Soc. exp. Biol. Med.* **91**, 226.