CONTRIBUTIONS OF PROTEIN TO GLUCOSE SYNTHESIS IN PREGNANT AND NON-PREGNANT SHEEP

J. V. NOLAN* and R. A. LENG*

Summary

Urea entry rates measured with a radioisotope dilution technique were used as an index of possible gluconeogenesis from amino acids. Both pregnant and non-pregnant sheep given a ration of 1000g of equal parts of crushed oats and chaffed lucerne per day, had a maximum possible rate of glucose synthesis from deaminated amino acids of 64g/day. When the ration was reduced abruptly to 250g/day about 14 days from lambing, the pregnant ewes became hypoglycaemic and it was. calculated that even ewes carrying twins were synthesising, at the most, 52g of glucose per day from protein. This was only 17g/day more than the possible synthesis of glucose from protein in the non-pregnant ewes.

It appears that even previously well-nourished ewes subjected to short periods of reduced food intake in late pregnancy have a low absolute capacity to use protein in lieu of other dietary glucogenic precursors, and thus do not meet fully their high glucose requirements.

I. INTRODUCTION

Hypoglycaemia following undernutrition in late pregnancy, which is a critical factor in the pathogenesis of ovine pregnancy toxaemia, is more marked in twin-pregnancy than in single-pregnancy (McClymont and Setchell 1955). This is not surprising as glucose is believed to be the main energy substrate for the foetus (Barcroft 1946; Reid 1960) and recent evidence has indicated that the energy requirements of the foetus are higher than previously supposed (Huckabee et ***al.*** 1961; Graham 1964). The hypoglycaemia must result because these high glucose requirements of late pregnancy are not fully met by gluconeogenesis from propionate or from dietary and tissue amino acids. Numerous measurements of the total glucose entry rate in pregnant and non-pregnant ewes have been made (Annison and White 1961; Kronfeld and Simesen 1961; Bergman 1963; Ford 1963; Ford 1965a). The contribution of propionate to glucose synthesis has been determined for non-pregnant sheep (Bergman, Roe and Kon 1966; Leng, Steel and Luick 1967) but the contribution of protein has been determined only by difference (Ford 1965*b*), or by measuring total nitrogen excretion in urine (Bergman, Roe and Kon 1966).

In the study reported here, urea entry rate was used as a measure of gluconeogenesis from amino acids in sheep; this was part of a series of studies of the effects of undernutrition in late pregnancy (Graham 1968) made at the C.S.I.R.O. Division of Animal Physiology.

*Department of Biochemistry and Nutrition, The University of New England, Armidale, N.S.W.

II. MATERIAL AND METHODS

(a) Experimental methods

Adult Corriedale ewes were mated to a Dorset Horn ram and after pregnancy diagnoses made by X-ray about 80 days after mating were kept indoors in individual pens or cages. Here they were given 1000 g per day of a diet (16.1% crude protein) of equal parts of crushed oats and chaffed lucerne until about 14 days from lambing (period A) when this ration was reduced abruptly to 250 g per day for 6 days (period B). The measurements described below were made on two different days, the first on a day ***(a)*** at the end of period A, and the second on a day (b) after four days of period B. From seven days before the first measurements, the animals were given the daily ration in equal portions every 3 h and this feeding regime produced a nearly constant concentration of urea in the blood.

(b) Measurement of *urea entry rates*

Sheep were prepared with two jugular vein catheters the day before an experiment; [^{14}C] urea (6 mg, 70μc) was injected at 0600 h on days ***a*** and ***b.*** Blood samples were taken before the injection, after 2 and 4 h, and at 30 min intervals from 6 to 10.5 h after the injection. Entry rates were calculated from the specific radioactivity of blood urea as previously described (Cocimano and Leng 1966).

(c) Measurement of *excretion rates of urea*

On the days *a* and ***b,*** urine was collected quantitatively into 5 ml of concentrated sulphuric acid using the technique of Raabe (1967). Urea concentration in samples of urine which had been stored at 3°C was measured by the urease method of Wilson (1966).

(d) Measurement of *specific radioactivity* of *plasma urea*

Each plasma sample was deproteinized by Ba/Zn precipitation (Somogyi 1952) and 1 ml of the filtrate was counted in 10 ml of triton X-100 scintillation mixture (Patterson and Greene 1965). Urea concentration was measured on the same filtrate using an Auto Analyzer* and the diacetyl monoxime method of Marsh, Fingerhut and Kirsch (1957).

(e) Measurement of *plasma glucose concentration*

Blood was taken by catheter at 1530 h, midway between three-hourly feeds, without disturbing the animals. Plasma was separated immediately and stored at –20°C until analysis by the glucose-oxidase method of Huggett and Nixon (1957).

(f) *Calculation* of *possible glucose synthesis from amino acids*

The calculations were made on the assumptions that 35 g of urea would result from deamination of amino acids from 100 g of protein, and that (Krebs 1964) the deaminated residues could in turn yield 55 g of glucose.

III. RESULTS

Urea entry rate on day ***a*** (Table 1) was about 40 g/day and there was no significant difference between pregnant and non-pregnant ewes. On day ***b,*** urea entry rate had declined in all sheep and was 22 g/day in the non-pregnant ewes which was lower ($P<0.05$) than for the pregnant ewes. On day ***a,*** urea excretion rate was lower ($P<0.05$) in the pregnant than in the non-pregnant ewes despite the similar entry rate and this was reflected in a tendency towards a greater rate of degradation of urea in the digestive tract of the pregnant ewes. This higher

*Technicon, New York.

TABLE 1

Urea metabolism in pregnant and non-pregnant sheep (mean results ± S.D. for all animals in each group)

	Animals per group	Sheep Weight (kg)		Pool Size (g)		Entry Rate (g/day)		Excretion Rate (g/day)		Degradation rate (entry-excretion rate) (g/day)	
		a	*b*	*a*	*b*	*a*	*b*	*a*	*b*	*a*	*b*
twin foetuses	2	65 ± 5.4	61 ± 4.6	13 ± 1.4	12 ± 2.1	39 ± 1.7	33 ± 4.5	19 ± 3.1	28 ± 2.9	20 ± 3.9	5.7 ± 1.6
single foetus	7	56 ± 6.7	52 ± 4.6	12 ± 3.1	11 ± 1.1	40 ± 1.1	29 ± 1.1	24 ± 1.1	21 ± 2.1	16 ± 2.8	7.8 ± 2.0
non-pregnant	6	50 ± 6.5	46 ± 4.9	11 ± 2.6	9 ± 1.3	41 ± 1.7	22 ± 1.9	29 ± 1.2	18 ± 2.6	12 ± 1.2	4.8 ± 1.7

Sheep were given 1000 g per day of a diet of equal parts of crushed oats and chaffed lucerne until a fortnight before lambing when the measurements *a* were made. One or two days after *a*, the ration was reduced to 250 g per day, and four days later the measurements *b* were made.

TABLE 2

Effect **of** *reduction* **of** *the ration on urea entry rate, potential rate* **of** *glucose synthesis and plasma glucose concentration in pregnant and nun-pregnant ewes (mean results ± S.D. for all animals in each group)*

	Sheep Fed 1000 g/day (Day *a*)			Sheep Fed 250 g/day (Day *b*)		
	Urea Entry Rate	Possible Glucose Synthesis from Amino Acids	Plasma Glucose Concentration	Urea Entry Rate	Possible Glucose Synthesis from Amino Acids	Plasma Glucose Concentration
	(g/day)	(g/day)	(mg/100ml)	(g/day)	(g/day)	(mg/100ml)
twin foetuses	39 ± 1.7	61 ± 2.7	56 ± 1.4	33 ± 4.5	52 ± 7.1	30 ± 2.0
single foetuses	40 ± 1.1	63 ± 1.7	72 ± 6.5	29 ± 1.1	45 ± 1.8	36 ± 4.3
non-pregnant	41 ± 1.7	64 ± 2.7	77 ± 6.2	22 ± 1.9	35 ± 3.0	67 ± 9.8

degradation rate tended to be maintained on day ***b*** although excretion rate was also greater in the pregnant groups ($P<0.05$). The mean size of the urea pool was within the range of 9 to 13 g. Differences in body weight between pregnant and non-pregnant ewes were significant ($P<0.05$) but were attributable largely to the weights of conceptus which were estimated from the birth weight of lambs.

The calculated maximum rate of glucose synthesis from amino acids (Table 2) was similar in all animals on day ***a*** and was not more than 64 g/day. On day ***b,*** this glucose synthesis had declined in all sheep; it was 35 g/day in non-pregnant ewes, with the pregnant ewes 10-17 g/day higher than this. Despite hypoglycaemia in all pregnant animals, only one ewe showed mild clinical signs of pregnancy toxaemia but she recovered and lambed normally.

IV. DISCUSSION

As an aid to the discussion, the salient features of ammonia and urea metabolism are shown schematically in Figure 1. The urea entering the body pool of urea as measured by the isotope dilution technique (i.e. total urea synthesis) is derived from NH_4^+ carried from the gut in the portal system and from NH_4^+ released during deamination of amino acids in the tissues. (Any small net production or utilization of ammonia during purine or pyrimidine metabolism may

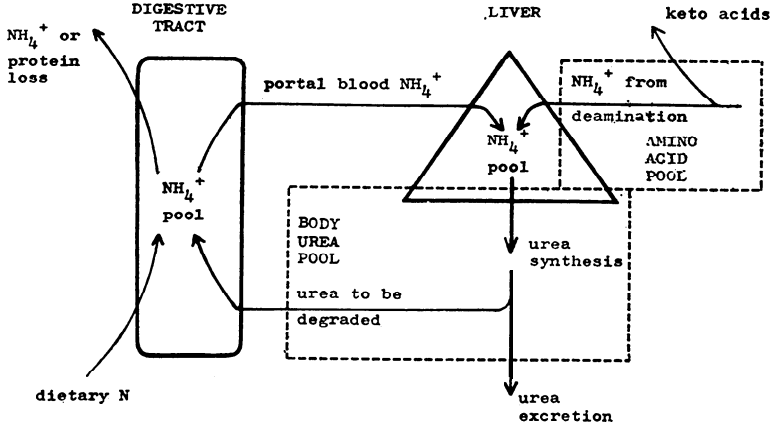

Fig. 1.—Schematic representation of ammonia and urea metabolism in sheep.

be neglected.) The urea synthesised can be stored in the body pool of urea, excreted, or recycled to the digestive tract to be degraded to NH_4^+. The latter can represent from 28 to 92% of the urea synthesised (Cocimano and Leng 1966).

The use of the rate of excretion of urea (or total nitrogen) in urine as an index of the rate of deamination of amino acids would be unsatisfactory. NH_4^+ excretion as urea will be equivalent to NH_4^+ from deamination in tissues only if the size of the urea pool does not change and if the contribution of NH_4^+ from the gut is balanced exactly by an equal contribution of NH_4^+ to the gut by urea being degraded (Figure 1). In the absence of such a balance, NH_4^+ excreted as urea may be greater or less than the NH_4^+ released by deamination of amino acids. The use of the rate of urinary total nitrogen excretion, a considerable proportion of which occurs as urea, would be subject to at least the same limitations as an index of deamination as urea itself.

Whereas estimates of deamination based on the rates of nitrogenous excretion may be high or low, estimates based on the rate of urea synthesis must be high by the extent of the contribution made by NH_4^+ carried from the gut to the liver in portal blood (Figure 1). Estimates of glucose synthesis based on any estimates of deamination rate will be accurate only if a known proportion of the deaminated residues is used for this synthesis; hence Krebs' (1964) conversion gives only the possible yield of glucose from a given quantity of protein. The results obtained in this study (Table 2) will therefore provide an upper limit to the possible synthesis of glucose.

On the 1000 g ration, neither pregnant nor non-pregnant ewes produced more than 64 g of their total daily synthesis of glucose from dietary or tissue amino acids. This could represent from 40 to 60 percent of the likely glucose entry rate (Steel and Leng 1968) which agrees with conclusions of Bergman, Roe and Kon (1966) and Ford (1965b).

After four days on the 250 g ration, the pregnant ewes apparently could could not compensate fully for the reduced availability of dietary glucogenic precursors by increasing the contribution of amino acids to glucose synthesis. This contribution was apparently greatest for the twin-pregnant ewes (which had the highest expected glucose demand) but was, at the most, 52 g/day; these ewes were hypoglycaemic (30 mg/ 100 ml of plasma) indicating that the total glucose synthesis was not sufficient to meet the requirements of glucose by the dam and foetuses. Thus, if protein is the main glucogenic substrate available to undernourished ewes, the reason for the sub-optimal synthesis of glucose must be a limited capacity to deaminate protein for the synthesis. This conclusion is supported by the finding (Steel and Leng 1968) that pregnant ewes after four days' starvation exhibited similar glucose entry rates of 63 g/day when measured at 80, 100, and 140 days of pregnancy although they became progressively more hypoglycaemic.

The low absolute capacity of previously well-nourished ewes to catabolize protein, at least during short periods of reduced feed intake, predisposes them to hypoglycaemia. The consequences may be a restriction in growth of the foetus and a proclivity of the ewe to develop pregnancy toxaemia.

V. ACKNOWLEDGMENTS

We are indebted to the Australian Wool Board, the Rural Developments Fund and the University of New England for financial support of this work, to Dr. N.

McC. Graham and the Division of Animal Physiology, C.S.I.R.O., Prospect, *N.S.W.*, for use of facilities, to Mr. R. Raabe for technical assistance and to Dr. R. F. Passey for much helpful discussion.

VI. REFERENCES

Annison, E. F., and **White,** R. R. (1961). ***Biochem. J. 80:*** 162.
Barcroft, J. (1946). "Researches on Pre-natal Life". (Blackwell: Oxford.).
Bergman, E. N. (1963). ***Am. J. Physiol.* 204:** 147.
Bergman, E. N. **Roe,** W. E., and **Kon, K.** (1966). ***Am. J. Physiol.*** 211: 793.
Cocimano, M. R., and **Leng,** R. A. (1966). ***Proc. Aust. Soc. Anim. Prod. 6: 378.***
Ford, E. J. H. (1963). ***Biochem. J. 88: 427.***
Ford, E. J. H. (1965*a*). ***J. agric. Sci., Camb. 65:*** 41.
Ford, E. J. H. (19653). In: "Energy Metabolism" (K. L. Blaxter, Ed.). London: Academic Press.
Graham, N. McC. (1964). ***Aust. J. agric. Res.*** 15: 127.
Graham, *N.* McC. (1968). ***Aust. J. agric. Res.*** (in press).
Huckabee, W. E., **Metcalfe,** J., **Prystowsky,** H., and **Barron,** D. H. (1961). ***Am. J. Physiol.*** 200: 274.
Huggett, A. St G., and **Nixon,** D. A. (1957). ***Biochem. J. 66:*** 12P.
Krebs, H. A. (1964). In: "Mammalian Protein Metabolism", Vol. I. (H. N. Munro and J. B. Allison, Eds.) London: Academic Press.
Kronfeld, D. S., and **Simesen,** M. ***G.*** (1961). ***Am. J. Physiol.*** 201: 639.
Leng, R. A., **Steel,** J. W., and **Luick,** J. R. (1967). ***Biochem. J.*** 103: 785.
Marsh, W. H., **Fingerhut,** B. and **Kirsch,** E. (1957). ***Am J. clin. Path. 28:*** 681.
McClymont, G. L., and **Setchell,** B. P. (1955). ***Aust. vet. J.*** 31: 53.
Patterson, M. S., and **Greene,** R. ***C.*** (1965). ***Analyt. Chem. 37: 854.***
Raabe, R. (1968). ***Lab. Pract.*** 17: 217.
Reid, R. L. (1960). ***Aust. J. agric. Res.*** 11: 364.
Somogyi, M. (1952). ***J. biol. Chem.*** 195: 19.
Steel, J. W., and **Leng,** R. A. (1968). ***Proc. Aust. Soc. Anim. Prod. 7: 342.***
Wilson, B. W. (1966). ***Clin. Chem.*** 12: 230.