THE TRANSFER OF NITROGEN FROM THE BLOOD TO THE RUMEN IN SHEEP

By R. H. Weston* and J. P. Hogan*

[*Manuscript received March* 14, 1967]

Summary

Urea providing from 1 to 15 g nitrogen/day was continuously infused per rumen or per abomasum into sheep consuming a diet of chopped wheaten hay.

Blood urea was directly proportional to the quantity of urea nitrogen infused. The infusion of urea per abomasum produced an increase in the concentration of ammonia in rumen liquor due to an enhanced transfer of nitrogen from the blood to the rumen.

By comparing the increments in rumen ammonia concentration produced by urea infusions per abomasum and per rumen, it was shown that when 2·5 g or less of urea nitrogen were infused per abomasum, transfer of nitrogen to the rumen increased by an amount similar to that infused; increase in the quantity infused beyond approximately 3·5 g nitrogen/day, however, failed to increase nitrogen transfer further. This limit to nitrogen transfer was reached when the concentration of blood urea nitrogen was 16–18 mg/100 ml. It was calculated, after making certain assumptions, that the maximum amount of nitrogen transferred to the rumen was 4–5 g/day, the equivalent of 1·5–1·8 times the body urea nitrogen pool at blood urea nitrogen levels of 16–18 mg/100 ml.

I. Introduction

Several workers have shown that nitrogenous substances pass from the blood into the rumen via the salivary glands (McDonald 1948) and through the rumen wall (Simonnet, Le Bars, and Molle 1957; Houpt 1959). A large proportion of the salivary nitrogen is urea (Bailey and Balch 1961; Somers 1961*a*) while Ash and Dobson (1963) have shown that urea is one of the nitrogenous substances entering the rumen through the rumen wall.

There appear to be no satisfactory quantitative measures of the extent of the transfer of nitrogen from the blood to the rumen. Studies on diets with low nitrogen content (Gray, Pilgrim, and Weller 1958; Kay and Phillipson 1964; Hogan and Weston 1967) have shown that the amount of nitrogen leaving the pylorus exceeds the intake of nitrogen in the diet; this gain of nitrogen, however, represents the net result of the transfer of nitrogen into all compartments of the stomach and its absorption therefrom. The quantity of urea transferred to the alimentary tract as a whole has been studied using ^{14}C-labelled urea (Decker *et al.* 1960, 1961; Gartner, Decker, and Hill 1961; Engelhardt and Nickel 1965); it is not possible, however, to determine the proportion of this quantity that is transferred to the rumen. Gartner, Decker, and Hill (1961) observed in short-term experiments that rumen

* Division of Animal Physiology, CSIRO, Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W.

ammonia concentration remained steady when blood urea concentration was increased, and assumed that the transfer of urea into the rumen could reach an upper limit, due possibly to an active transport of urea across the rumen wall.

In recent studies in which a diet of wheaten hay was used we observed increases in both blood urea and rumen ammonia levels when protein was infused into the abomasum. This enhanced transfer of nitrogen to the rumen accompanying protein infusion had important nutritional implications in the studies concerned. Accordingly, the present studies were designed to investigate, in a more quantitative way, the fate of nitrogen infused into the abomasum; in particular, it was of interest to determine whether there was a limit to the transfer of nitrogen into the rumen, under these experimental conditions.

II. Materials and Methods

(a) *Animals and Experimental Procedures*

Adult Merino wethers with cannulae in the rumen and abomasum were used. The diet of chopped wheaten hay (wheaten chaff) contained 0·66% nitrogen, 27% cellulose, 12% soluble carbohydrate, and had an organic matter digestibility of 53%. The sheep were offered 640 g of the diet each day in eight equal portions at 3-hr intervals; this quantity was equal to 85–95% of *ad libitum* intake.

Experimental periods were of at least 6 days' duration; throughout each period the appropriate quantity of urea (analytical reagent grade) or casein, dissolved in 1·5 litres of water, was infused at a constant rate throughout the whole 24 hr of each day. Three samples of blood and six samples of rumen liquor were obtained at hourly intervals on each of the last 2 or 3 days of the period. Thirty-five experiments were conducted with six sheep; 27 of the experiments were conducted on three of the sheep. Urea was infused into the rumen (16 experiments), the abomasum (17 experiments), and the jugular vein (1 experiment); casein was infused into the abomasum in one experiment.

Ammonia in rumen liquor was determined by the method of McDonald (1952), plasma urea by the method of Wilson (1966), the quantity of water in the rumen by techniques described by Weston and Hogan (1967), and body water by the technique of Panaretto (1963). Urea space was calculated assuming that urea is distributed uniformly throughout the body water except in the digestive tract, at a level equal to that in blood plasma. On the basis of the data of Boyne *et al.* (1956), the quantity of water in the digestive tract was assumed to be 1·5 times the quantity in the rumen.

(b) *Calculation of the Increase in the Quantity of Nitrogen Entering the Rumen during Infusion of Nitrogen into the Abomasum*

It has been assumed that the additional nitrogen that enters the rumen in response to the infusion of urea into the abomasum is in a form such as urea that is readily degraded to ammonia. Accordingly, the proportion of a dose of urea nitrogen given per abomasum that enters the rumen has been calculated from the increase in rumen ammonia concentration that it produces; the quantity of nitrogen that is transferred is equal to the amount of urea nitrogen that must be infused intraruminally to produce the same increment in rumen ammonia concentration.

III. Results

(a) *Rumen Volume and Urea Space*

The three sheep used in most experiments had 3·2, 4·3, and 4·4 litres of water in their rumens; the urea spaces in these animals were 16·8, 18·7, and 18·6 litres respectively.

(b) *Effect of Infused Nitrogen on Blood Urea Concentration*

The infusion of urea, in amounts providing up to 15 g nitrogen/day, into the rumen or abomasum resulted in a linear increase in blood urea concentration (Fig. 1).

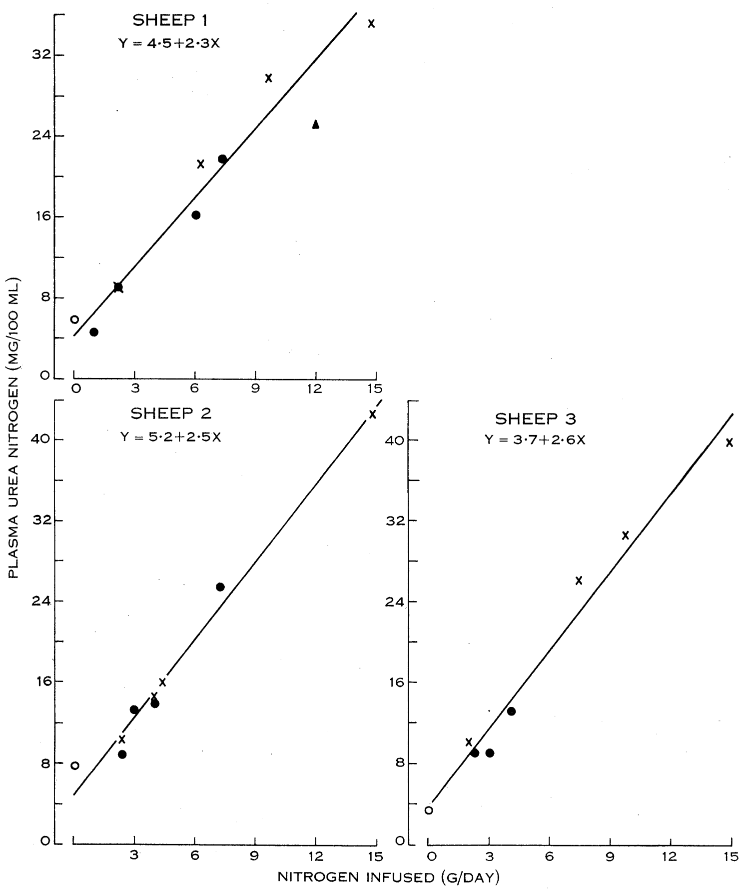

Fig. 1.—Relationship between plasma urea concentration and the amount of nitrogen infused as urea per rumen (●), per abomasum(×), intravenously (▲), and as casein per abomasum (△). Control values are shown by ○. Each plot for urea concentration is the mean of 6–9 blood samples.

The infusion of urea nitrogen into the jugular vein produced a response similar to that of rumen or abomasal infusion of urea (sheep 1, Fig. 1), whereas casein nitrogen infused per abomasum resulted in a proportionately smaller increase in blood urea (sheep 3, Fig. 1).

The increase in the body urea nitrogen pool produced by the infusion of urea per abomasum, as calculated from urea space and the increase in blood urea nitrogen concentration (Fig. 1), was 0·44 (S.E. $\pm$0·02)g nitrogen/g urea nitrogen infused.

(c) *Effect of Infused Nitrogen on Rumen Ammonia Concentration*

The infusion of urea into the rumen was accompanied by a linear increase in rumen ammonia concentration. The increase in the quantity of ammonia present in the rumen, accompanying intraruminal infusion, as calculated from rumen volume and the rate of increase of rumen ammonia (Fig. 2) was 0·09 (S.E. $\pm$0·003)g nitrogen/g urea nitrogen infused. When urea given per abomasum provided less than 2·5–3·5 g nitrogen/day, rumen ammonia levels increased by amounts comparable to those

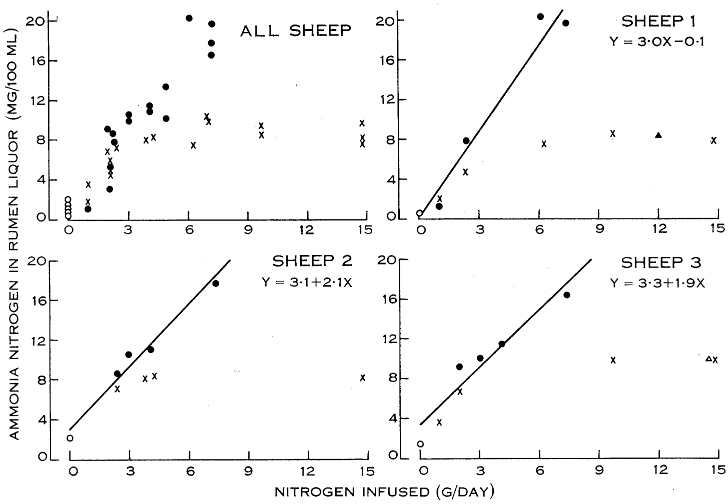

Fig. 2.—Relationship between ammonia concentration in rumen liquor and the amount of nitrogen infused as urea per rumen (●), per abomasum (×), intravenously (▲), and as casein per abomasum (△). Control values are shown by ○. Each plot for ammonia concentration is the mean of 12–18 samples.

obtained with corresponding doses into the rumen. At higher rates of infusion, however, the level of rumen ammonia was constant; the constant value was 8 mg ammonia nitrogen/100 ml for two of the sheep and 10 mg/100 ml for the third. The levels obtained with urea infused intravenously (sheep 1, Fig. 2) and casein infused per abomasum (sheep 3, Fig. 2) were comparable to those obtained with a corresponding dose of urea nitrogen infused per abomasum.

(d) *Relationship between Rumen Ammonia and Blood Urea Concentrations*

Figure 3 shows the relationship between the concentration of ammonia in rumen liquor and urea in blood in experiments other than those where urea was infused intraruminally. Rumen ammonia concentration was proportional to blood urea level when the latter was in the range of 4 to 16–18 mg urea nitrogen/100 ml; increase in blood urea level beyond this range was not accompanied by an increase in rumen ammonia.

(e) *Transfer to the Rumen of Nitrogen Infused per Abomasum*

The relationships between rumen ammonia concentration and level of nitrogen infused (Fig. 2) show that when less than 2·5–3·5 g urea nitrogen were infused per day, the increment in rumen ammonia was similar for both rumen and abomasum infusions. Hence, in this range of urea nitrogen infusions, a large proportion, if not all, of the urea nitrogen infused per abomasum was transferred to the rumen. However, rumen ammonia remained steady when more than 2·5–3·5 g urea nitrogen were infused per abomasum and presumably a constant amount of nitrogen was returned

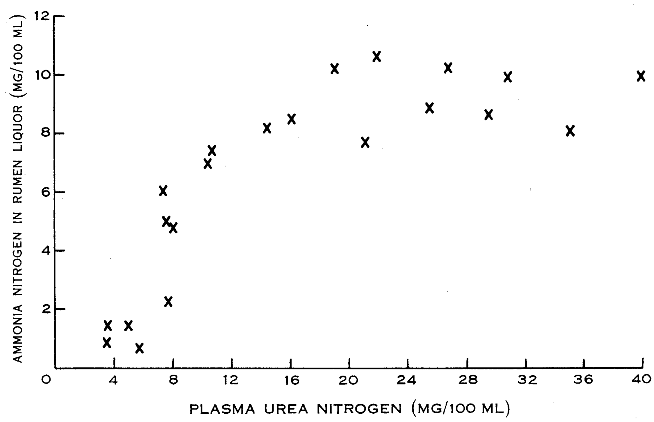

Fig. 3.—Relationship between ammonia concentration in rumen liquor and urea concentration in plasma. Each plot is the mean of 6–9 samples.

to the rumen in this range. Thus the maximum increment in nitrogen transfer to the rumen resulting from the nitrogen infused was 2·5–3·5 g/day, the equivalent of 0·06–0·08 g nitrogen/kg body weight. This maximum recycling of infused nitrogen was achieved when blood urea nitrogen levels reached 16–18 mg/100 ml (Fig. 3) or when the body urea pool was approximately 2·9–3·2 g urea nitrogen.

IV. Discussion

Under the experimental conditions that prevailed, there was a limit to the extent of transfer of nitrogen from the blood to the rumen. This limit appeared to be reached when the concentration of urea nitrogen in blood was 16–18 mg/100 ml; at lower concentrations nitrogen transfer appeared to be proportional to concentration. The limit to nitrogen transfer was not restricted to situations where blood urea concentrations were elevated by the infusion of urea per abomasum; it also existed when blood urea concentrations were elevated by the intravenous infusion of urea or the infusion of casein per abomasum. Gartner, Decker, and Hill (1961) observed the existence of an upper limit to nitrogen recycling to the rumen in an experiment of approximately 3 hr duration; the present experiments conducted over a much longer period confirm this observation. The data of these workers show further that a limit existed to the transfer of urea to the alimentary tract as a whole, although subsequent work from the same institute (Engelhardt and Nickel 1965) did not show this limit; in the latter studies the rate of entry of urea into the alimentary tract was proportional to blood urea concentration.

The nitrogen transferred to the rumen is probably derived both from saliva and by direct passage through the rumen wall. The studies of Somers (1961*b*) indicate that secretion of nitrogen in saliva has an upper limit; this work showed that following a single intracarotid infusion of urea, the secretion of total nitrogen or urea in parotid saliva reached an upper limit when blood urea nitrogen level was approximately 30 mg/100 ml and did not increase with further increase in blood urea concentration. It is possible that a similar limitation to salivary nitrogen secretion existed in the present studies but at a lower blood urea concentration. The existence of an upper limit to urea transfer through the rumen wall was demonstrated by the use of an *in vitro* technique by Gartner (1962, 1963); in the earlier studies maximum transfer of urea was found when the concentration of urea nitrogen in the external solution was 10–20 mg/100 ml while in the latter urea transfer increased with increasing urea nitrogen concentration up to 10 mg/100 ml and then remained constant. In more recent studies with an *in vitro* system, Engelhardt and Nickel (1965) could not demonstrate a limitation to urea transfer through the rumen wall, the transfer being in accordance with concentration gradient over a wide range of urea concentrations. The foregoing discussion indicates the need for further work on mechanisms regulating urea transport into the rumen via both the salivary glands and the rumen wall.

In the present studies, the total amount of nitrogen transferred to the rumen, the sum of transfer on the basal diet and that accruing from the infused urea, cannot be accurately assessed because recycling on the basal diet has not been determined. In previous studies of the digestion of this diet (Hogan and Weston 1967) the quantity of nitrogen leaving the abomasum was $2 \cdot 8$ g/day greater than the quantity of nitrogen provided by 640 g of the diet. This gain is equal to the sum of nitrogen recycled to the rumen, and nitrogen entering the omasum and abomasum. The components of greatest quantitative significance are probably recycling of nitrogen to the rumen and the secretion of nitrogen into the abomasum in gastric juice. The other components are probably small and tend to counterbalance. Thus the quantity of urea entering the omasum and abomasum, except in gastric juice, was probably small as blood urea concentrations were low; similarly, little absorption of nitrogen from the forestomachs would be expected since the concentration of ammonia in digesta was low. Ash and Hogan (unpublished data) found the mean nitrogen content of 24 samples of gastric juice to be $20 \cdot 8$ (S.E. $\pm 2 \cdot 7$) mg/100 ml and Masson and Phillipson (1952) calculated the rate of gastric juice secretion to be 5 litres /day. Applying these values to the present experiments, it may be calculated that approximately 1 g nitrogen/day is secreted in gastric juice and hence nitrogen transfer to the rumen on the basal diet was $1 \cdot 8$ g/day. Accordingly, the total transfer of nitrogen to the rumen during urea infusions resulting in blood urea nitrogen levels of 16–18 mg/100 ml or above may be estimated to be $4 \cdot 3$–$5 \cdot 3$ g/day. At these blood urea levels the body urea nitrogen pool was in the range of $2 \cdot 9$–$3 \cdot 2$ g, hence the total transfer of nitrogen per day was equal to $1 \cdot 5$–$1 \cdot 8$ times the urea nitrogen pool.

V. Acknowledgments

The authors' thanks are due to Miss C. Spargo, Mr. P. J. Connell, and Mr. D. E. Margan for their skilled technical assistance.

VI. References

Ash, R. W., and Dobson, A. (1963).—*J. Physiol.* **169**, 39.

Bailey, C. B., and Balch, C. C. (1961).—*Br. J. Nutr.* **15**, 383.

Boyne, A. W., Campbell, R. M., Davidson, J., and Cuthbertson, D. P. (1956).—*Br. J. Nutr.* **10**, 325.

Decker, P., Gartner, K., Hornicke, H., and Hill, H. (1961).—*Pflügers Arch. ges. Physiol.* **274**, 289.

Decker, P., Hill, H., Gartner, K., and Hornicke, H. (1960).—*Dt. tierärztl. Wschr.* **67**, 539.

Engelhardt, W. V., and Nickel, W. (1965).—*Pflügers Arch. ges. Physiol.* **286**, 57.

Gartner, K. (1962).—*Pflügers Arch. ges. Physiol.* **276**, 292.

Gartner, K. (1963).—*Dt. tierärztl. Wschr.* **70**, 16.

Gartner, K., Decker, P., and Hill, H. (1961).—*Pflügers Arch. ges. Physiol.* **274**, 281.

Gray, F. V., Pilgrim, A. F., and Weller, R. A. (1958).—*Br. J. Nutr.* **12**, 413.

Hogan, J. P., and Weston, R. H., (1967).—*Aust. J. agric. Res.* **18**, 803.

Houpt, T. R. (1959).—*Am. J. Physiol.* **197**, 115.

Kay, R. N. B., and Phillipson, A. T. (1964).—*Proc. Nutr. Soc.* **23**, 46.

Masson, M. J., and Phillipson, A. T. (1952).—*J. Physiol.* **116**, 98.

McDonald, I. W. (1948).—*Biochem. J.* **42**, 584.

McDonald, I. W. (1952).—*Biochem. J.* **51**, 86.

Panaretto, B. A. (1963).—*Aust. J. agric. Res.* **14**, 944.

Simonnet, H., Le Bars, H., and Molle, J. (1957).—*C. hebd. Séanc. Acad. Sci., Paris* **244**, 943.

Somers, M. (1961*a*).—*Aust. J. exp. Biol. med. Sci.* **39**, 111.

Somers, M. (1961*b*).—*Aust. J. exp. Biol. med. Sci.* **39**, 145.

Weston, R. H., and Hogan, J. P. (1967).—*Aust. J. agric. Res.* **18**, 789.

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