Contribution of Propionate to Glucose Synthesis in Sheep

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(Received 19 December 1966)

1. The production rate of propionate in the rumen and the entry rate of glucose into the body pool of glucose in sheep were measured by isotope-dilution methods. Propionate production rates were measured by using a continuous infusion of specifically labelled [^{14}C]propionate. Glucose entry rates were estimated by using either a primed infusion or a continuous infusion of [$\text{U-}^{14}\text{C}$]glucose. 2. The specific radioactivity of plasma glucose was constant between 4 and 9 hr. after the commencement of intravenous infusion of [$\text{U-}^{14}\text{C}$]glucose and between 1 and 3 hr. when a primed infusion was used. 3. Infusion of [^{14}C]propionate intraruminally resulted in a fairly constant specific radioactivity of rumen propionate between about 4 and 9 hr. and of plasma glucose between 6 and 9 hr. after the commencement of the infusion. Comparison of the mean specific radioactivities of glucose and propionate during these periods allowed estimates to be made of the contribution of propionate to glucose synthesis. 4. Comparisons of the specific radioactivities of plasma glucose and rumen propionate during intraruminal infusions of one of [$1\text{-}^{14}\text{C}$]-, [$2\text{-}^{14}\text{C}$]-, [$3\text{-}^{14}\text{C}$]- and [$\text{U-}^{14}\text{C}$]-propionate indicated considerable exchange of C-1 of propionate on conversion into glucose. The incorporation of C-2 and C-3 of propionate into glucose and lactate indicated that 54% of both the glucose and lactate synthesized arose from propionate carbon. 5. No differences were found for glucose entry rates measured either by a primed infusion or by a continuous infusion. The mean entry rate ($\pm$ s.e.m.) of glucose estimated by using a continuous infusion into sheep was 0.33 ± 0.03 (4) m-mole/min. and by using a primed infusion was 0.32 ± 0.01 (4) m-mole/min. The mean propionate production rate was 1.24 ± 0.03 (8) m-moles/min. The conversion of propionate into glucose was 0.36 m-mole/min., indicating that 32% of the propionate produced in the rumen is used for glucose synthesis. 6. It was indicated that a considerable amount of the propionate converted into glucose was first converted into lactate.

Glucose entry rates in sheep are only slightly less than in non-ruminant animals in the post-absorptive state when compared on a body-weight basis (Annison & White, 1961; Kronfeld & Simesen, 1961; Bergman, 1963; Ford, 1963). Although glucose is an intermediate in the microbial breakdown of carbohydrates, it is not usually present in detectable quantities in the rumen of animals given roughage diets. Some glucose may be absorbed from the lower intestines of ruminants as a result of the enzymic degradation of microbial polysaccharides, but the quantity is probably of little significance (Heald, 1952; MacRae & Armstrong, 1966). Studies of the differences in the concentration of glucose in carotid and portal blood have also indicated that there is little or no net gain of glucose from the alimentary tract in sheep given roughage

rations (Schambye, 1951; Annison, Hill & Lewis, 1957). However, it is probable that the glucose entry rate and quantity of glucose absorbed from the alimentary tract of sheep is dependent to a large extent on the ration. Considerable quantities of glucose may be absorbed from the gastro-intestinal tract in sheep given rations high in concentrates, since MacRae & Armstrong (1966) have shown that up to 22 g. of α -linked polymers of glucose enters the duodenum in animals given diets of hay and grain, and Fries & Conner (1960) have shown, in cattle given high maize rations, that large amounts of starch escape rumen fermentation.

Although the quantity of glucose absorbed from the alimentary tract of sheep given rations containing a high proportion of grain is not known, on roughage diets most of the glucose entering the body pool in sheep is probably synthesized *in situ*. Gluconeogenesis in sheep is of considerable interest

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since, of the major volatile fatty acids absorbed from the rumen, only propionate could contribute significantly to the synthesis of glucose (Leng & Annison, 1963; Black, Kleiber & Brown, 1961; Annison, Leng, Lindsay & White, 1963). Recent measurements of production rates of volatile fatty acids in sheep fed at frequent intervals indicated that propionate production rates are probably higher than previously estimated (Leng & Leonard, 1965; Bergman, Reid, Murray, Brockway & Whitelaw, 1965), and, if all the propionate produced in the rumen was converted into glucose, it could account for 100% of the glucose synthesized in fed animals (Annison & White, 1961).

Bergman, Roe & Kon (1966), by using an infusion of [2-¹⁴C]propionate into a ruminal vein, with isolation of propionate and glucose from the portal vein, have demonstrated that only 27% of the glucose entry rate originated from propionate absorbed into the portal blood. However, probable conversion of propionate into lactate (Pennington & Sutherland, 1956) in the rumen wall was not taken into consideration. The studies now presented were designed to estimate the contribution of propionate to glucose synthesis during the period of continuous feeding in sheep given roughage rations by using an intraruminal infusion of [¹⁴C]propionate. It appears that approx. 54% of the glucose carbon was coming from propionate produced in the rumen.

MATERIALS AND METHODS

Experimental animals. Merino wethers and ewes aged between 3 and 5 years were housed in individual metabolism cages and given daily rations of 800g. of lucerne. Animals each had a rumen cannula that had been fitted for approx. 1 year. During experimental periods (5 days) the sheep were given their ration in 12 equal amounts at hourly intervals between 07.00 and 18.00 hr. This feeding regime was known to produce a fairly constant level of volatile fatty acids in the rumen between 14.00 and 21.00 hr. (Leng & Leonard, 1965). On the last day of each period the animals were infused with ¹⁴C-labelled substrates, either intravenously (with [¹⁴C]-glucose) (see Annison & White, 1961) or intraruminally (with [¹⁴C]propionate) (see Leng & Leonard, 1965). The animals were trained to consume their ration during the infusion periods.

Measurement of glucose entry rates. Catheters were inserted into both jugular veins of the sheep on the evening before an experiment. [U-¹⁴C]Glucose was infused at a rate of approx. 0.15 μ C (1 μ mole)/min. for 9 hr. into one jugular vein and blood samples were taken from the other at regular intervals. In some experiments samples of rumen fluid were taken at the end of the infusion period for estimation of the specific radioactivity of propionate.

Measurement of propionate production rates in the rumen. Propionate labelled with ¹⁴C was infused intraruminally at a rate of between 0.06 and 0.20 μ C/min. by using the methods described by Leng & Leonard (1965). Samples of ruminal fluid were taken at regular intervals over the 9 hr. infusion and the propionate was isolated and assayed for radio-

activity. At the same time blood samples were taken for measurements of the concentrations and specific radioactivities of plasma glucose and blood lactate.

Chemical methods. Plasma glucose was estimated by the method of Huggett & Nixon (1957). Radioactivity measurements were made with a Nuclear-Chicago Scintillation System 725 (Nuclear-Chicago Corp., Des Plaines, Ill., U.S.A.) after isolation of plasma glucose as the penta-acetate derivative (Jones, 1965).

Lactate was estimated in blood by the lactate-dehydrogenase method (Barker & Britton, 1957) and isolated from blood by using silicic acid column chromatography with CHCl₃-2-methylbutan-2-ol as eluting solvents (Leng & Annison, 1963). Blood (10 ml.) plus 8 mg. of zinc lactate was deproteinized by successive additions of 20 ml. of water, 10 ml. of 20% (w/v) NaWO₄ and 10 ml. of 0.6N-H₂SO₄. The solution was filtered and the protein-free filtrate was neutralized (pH 7), concentrated under reduced pressure on a rotary evaporator and finally dried in a beaker *in vacuo* over conc. H₂SO₄. The lactic acid was isolated by column chromatography and counted by liquid-scintillation techniques, as described for blood β -hydroxybutyrate (Leng, 1965). The channels-ratio method was used to correct for quenching (Hendler, 1964).

Propionate was isolated from rumen fluid as described by Leng & Leonard (1965). Total volatile fatty acid concentrations in ruminal fluid were estimated by titration after steam-distillation in a Markham still and the percentages of individual acids were determined by gas-liquid chromatography (Erwin, Marco & Emery, 1961; Leng & Leonard, 1965).

Radioactive compounds. Sodium [1-¹⁴C]propionate and D-[U-¹⁴C]glucose were purchased from The Radiochemical Centre, Amersham, Bucks. Sodium [2-¹⁴C]propionate and sodium [3-¹⁴C]propionate were obtained from N. V. Phillips-Duphar, Amsterdam, The Netherlands. [U-¹⁴C]Propionate was prepared by mixing the three specifically labelled compounds. Infusion solutions containing propionate were assayed for radioactivity as described by Leng & Leonard (1965). Infusates containing [U-¹⁴C]glucose were assayed by counting 0.5 ml. of the solution in 10 ml. of a scintillation solution described by Bray (1960).

RESULTS

Measurements of glucose entry rates in sheep fed at hourly intervals. Intravenous infusions of [U-¹⁴C]-glucose into sheep over a 9 hr. period produced a constant specific radioactivity in plasma glucose between approx. 4 and 9 hr. of the infusion. Typical changes of specific radioactivity with time are shown in Fig. 1. Glucose entry rates (m-moles/min.) were calculated by comparing the mean specific radioactivity ($m\mu$ C/m-mole) of plasma glucose between 4 and 9 hr. with the rate of infusion of radioactivity ($m\mu$ C/min.). The mean plasma glucose concentration and glucose entry rate in four animals (mean wt. 36.6 kg.) were 68.2 ± 5.2 mg./100 ml. and 0.33 ± 0.03 m-mole/min. respectively. Primed infusions were made over a 3 hr. period and, as in the results of Annison & White (1961), a plateau specific radioactivity of plasma glucose was

Fig. 1. Specific radioactivity of plasma glucose during a continuous intravenous infusion of $[U-^{14}C]$ glucose. Experimental details are given in the text. Infusion rate was $0.15 \mu\text{Ci}$ ($1 \mu\text{mole}$)/min. ○, Specific radioactivity of plasma glucose; ●, plasma glucose concentration.

Fig. 2. Specific radioactivity and concentrations of ruminal fluid propionate, plasma glucose and blood lactate during a continuous intraruminal infusion of $[2-^{14}C]$ propionate. Infusion rate was $77.7 \text{ m}\mu\text{Ci}$ ($1 \mu\text{mole}$)/min. ○, Specific radioactivity of propionate; ●, specific radioactivity of plasma glucose; □, specific radioactivity of blood lactate; ■, plasma glucose concentration; △, rumen-fluid propionate concentration; ▲, blood lactate concentration.

obtained between 1 and 3 hr. Glucose entry rates were estimated over this period as described above. The mean plasma glucose concentration and the entry rate of glucose in four sheep (mean wt. 34.7 kg.) were $71.6 \pm 2.2 \text{ mg./100 ml.}$ and $0.32 \pm 0.01 \text{ m-mole/min.}$ respectively. There was no significant difference ($P > 0.1$) in entry rates measured by the two techniques, verifying other results from this Laboratory (J. R. Luick, R. G. White, J. W. Steel & R. A. Leng, unpublished work).

In two experiments, samples of ruminal fluid were taken at the end of the infusion and the specific radioactivity of propionate was determined. The radioactivity in propionate in comparison with that in circulating glucose was negligible.

Measurement of propionate production rates in the rumen and the contribution of propionate carbon to both lactate and glucose synthesis. Fig. 2 shows the results of an infusion of $[2-^{14}C]$ propionate into the rumen of a sheep. The specific radioactivity in ruminal-fluid propionate was fairly constant between approx. 4 and 9 hr. of the infusion and the specific-radioactivity-time curves were similar in all experiments and differed only in magnitude. The mean production rate of propionate in eight experiments was $1.24 \pm 0.03 \text{ m-moles/min.}$ (Table 1). Comparison of the specific radioactivities of both glucose and lactate with propionate over the plateau periods allowed estimates to be made of the proportion of the glucose and lactate synthesized that was arising from propionate carbon (Fig. 2). Table 1 shows results obtained with infusions of $[1-^{14}C]$ -, $[2-^{14}C]$ -, $[3-^{14}C]$ - and $[U-^{14}C]$ -propionate.

Rate of conversion of propionate into glucose. From a knowledge of the entry rate of glucose, the production rate of propionate and the percentage of the glucose arising from propionate produced in the

rumen, the rate of conversion of propionate into glucose was estimated as was the proportion of the propionate production rate being converted into glucose. An example of the calculation is as follows, with the results from sheep no. 147, which was infused with $[2-^{14}C]$ propionate.

(1) Entry rate of glucose = 0.33 m-mole/min.

(2) The specific radioactivity ($\mu\text{Ci/g. of C}$) of plasma glucose was 57.1% of the propionate in the rumen and thus $0.33 \times 57.1/100 \text{ m-mole of glucose/min.}$ is coming from propionate (i.e. 0.19 m-mole/min.).

(3) If it is assumed that 2 mol. of propionate is required for the synthesis of 1 mol. of glucose, then at least $0.38 \text{ m-mole of propionate/min.}$ is being converted into glucose.

(4) Propionate entry rate is 0.99 m-mole/min. and therefore 38.3% of the propionate being produced in the rumen is being converted into glucose.

In four experiments in which $[2-^{14}C]$ - or $[3-^{14}C]$ -propionate was infused between 25.0 and 38.3% of the propionate produced in the rumen was being converted into glucose.

Table 1. *Production rate of propionate in the rumen and the percentage contribution of propionate to glucose synthesis in sheep*

Sheep no.	Infusate	Infusion rate (mg./min.)	Sheep wt. (kg.)	Rumen-fluid concentrations* (m-moles/l.)			Specific radioactivity (μc/g. of C)			Production rate of rumen propionate (m-moles/min.)	Apparent percentage contribution of:		Rate of conversion of propionate into glucose (m-mole/min.)	Per-centage of propionate produced into glucose
				Total volatile fatty acids	Propionate	Plasma glucose concn.* (mg./100 ml.)	Blood lactate concn.† (mg./100 ml.)	Rumen propionate†	Blood lactate†		Propionate to lactate	Propionate to glucose		
68	[1- ¹⁴ C]Propionate	166.4	38.3	110.4	22.0	63.3	8.4	3.07	0.35	1.51	11.4	16.9	—	—
167	[1- ¹⁴ C]Propionate	204.8	37.6	80.9	15.9	64.4	9.2	4.88	0.49	1.17	10.0	18.2	—	—
147	[2- ¹⁴ C]Propionate	77.7	37.9	97.0	25.9	68.1	10.4	2.19	0.54	0.99	24.7	57.1	0.38	38.3
68	[2- ¹⁴ C]Propionate	70.5	36.2	111.2	22.5	83.2	7.2	2.26	1.88	0.87	83.2	50.0	0.33	37.9
37	[3- ¹⁴ C]Propionate	62.4	37.8	99.6	21.8	69.0	7.3	1.06	—	1.04	—	62.3	0.41	25.0
147	[3- ¹⁴ C]Propionate	131.6	37.4	94.1	18.5	60.2	4.2	3.16	1.70	1.16	53.8	45.3	0.30	25.9
167	[U- ¹⁴ C]Propionate	92.2	40.4	103.7	23.6	66.2	10.0	1.64	0.71	1.56	43.3	59.1	—	—
37	[U- ¹⁴ C]Propionate	115.4	35.0	74.6	18.5	63.3	6.8	3.20	1.90	1.00	59.4	42.5	—	—

* Means of ten results taken over the plateau specific-radioactivity period (4 to 9 hr. after commencement of the infusion).

† Means of seven results taken over the plateau specific-radioactivity period (4 to 9 hr.) of blood lactate.

‡ Means of seven results taken over the plateau specific-radioactivity period (6 to 9 hr.) of plasma glucose.

DISCUSSION

Glucose entry rates in sheep have been measured by isotope-dilution techniques, either by using a single injection of [¹⁴C]glucose (Kronfeld & Simesen, 1961; Annison & White, 1961), or by using a primed infusion of [¹⁴C]glucose (Annison & White, 1961; Bergman, 1963; Ford, 1963; Kronfeld & Simesen, 1961). The present investigations appear to be the first studies on glucose metabolism in sheep in which it has been found possible to use a continuous infusion of [U-¹⁴C]glucose without a priming dose for these measurements. The plasma glucose specific radioactivity became virtually constant between 4 and 9 hr. of the infusion and the variation about the plateau specific radioactivity was small (see Fig. 1, which is a typical result). The plateau specific radioactivity of plasma glucose between 4 and 9 hr. after the beginning of the experiment with no indication of a positive or negative slope suggests the absence of either a slowly mixing pool of glucose or extensive recycling. In these studies a primed infusion of glucose, as used by Annison & White (1961), gave essentially the same results as reported for the continuous infusion alone. Hetenyi, Ninomiya & Wrenshall (1966) have shown that similar results for glucose entry rates in dogs can be obtained with either a primed infusion or a single injection of [¹⁴C]glucose.

When [¹⁴C]propionate was infused intraruminally plateau specific radioactivity of ruminal-fluid propionate occurred between 4 and 9 hr. and of plasma glucose between 6 and 9 hr. Obtaining a plateau specific radioactivity in plasma glucose during infusion of either [¹⁴C]propionate or [¹⁴C]glucose is a necessary criterion for the accurate determination of interconversion rates of propionate to glucose. Direct comparisons of the mean specific radioactivities over these plateau periods allowed estimates to be made of the proportion of the glucose synthesized from propionate. From a knowledge of glucose entry rate, propionate production rates and the percentage of the glucose arising from propionate, the net conversion rate of propionate into glucose was calculated. It must be emphasized that these interrelationships have only been estimated over the period of maximum rate of propionate production in the rumen.

A knowledge of the pathways of propionate metabolism in sheep tissues suggests that conversion of propionate into glucose may undergo the following reactions, which are in outline only:

Reaction (1) occurs in the rumen epithelium (Pennington & Sutherland, 1956) and reactions (1), (2) and (3) occur in sheep liver (Leng & Annison, 1963). In reactions (1) and (3) the propionate molecule undergoes successive carboxylation and decarboxylation and, since a symmetrical C₄ compound is an intermediate, half the original carbon atoms in the C-1 position of propionate have been substituted on conversion into lactate or glucose respectively. Conversion of lactate into glucose (reaction 2) would also result in a further loss of radioactivity from the C-1 of the original propionate, since conversion of lactate into pyruvate into phosphoenolpyruvate and thence into glucose entails carboxylation of pyruvate to oxaloacetate, equilibration of this with succinate and loss of half the radioactivity on decarboxylation to form phosphoenolpyruvate. C-2 and C-3 of propionate are not exchanged in any of these pathways.

The net incorporation of C-1 of [1-¹⁴C]propionate into glucose depends on the overall rate of glucose synthesis and also on the rate of conversion of propionate into glucose via the direct pathway (reaction 3) relative to the indirect pathway in which lactate is an intermediate (reactions 1 and 2). C-1 of propionate in these studies was incorporated into glucose and lactate to a much less extent than C-2 and C-3. The incorporation of ¹⁴C from [1-¹⁴C]-propionate to that from [2-¹⁴C]- or [3-¹⁴C]-propionate was much less than 50% and indicates that there is probably extensive incorporation of propionate carbon into lactate before conversion into glucose.

In two out of three experiments the specific radioactivity of blood lactate was higher than that of plasma glucose during [2-¹⁴C]- or [3-¹⁴C]-propionate infusions into the rumen (Table 1), adding support to the suggestion of extensive conversion of propionate into lactate. The conversion of propionate into lactate may occur in the rumen epithelium during absorption of propionate (Pennington & Sutherland, 1956). It is therefore only permissible to use the conversion of C-2 or C-3 of propionate into glucose and lactate as an indication of glucose or lactate synthesis from propionate, and approx. 54% of both the glucose and lactate entry rates was apparently arising from propionate produced in the rumen (Table 1).

The results of Bergman *et al.* (1966) with sheep given a similar ration to that used in these studies show that only 27% of the glucose was arising from absorbed propionate and that of this 50% was converted into glucose. In the present investigations the ration was fed over 12 hr. whereas the feeding regime used by Bergman *et al.* (1966) extended over

24 hr. However, if the two investigations are taken as comparable, 27% of the glucose produced in sheep tissues may have been produced from compounds arising from propionate either before or on absorption into the blood draining the rumen. Since lactate is extensively labelled in these studies this adds support to the suggestion of extensive conversion of propionate into lactate by the rumen epithelium on absorption. The glucose entry rates reported by Bergman *et al.* (1966) were very similar to those obtained in the present investigations.

In addition to the pathways of glucose synthesis from propionate listed above other pathways are present for propionate metabolism in the liver. Propionate may be oxidized in the tricarboxylic acid cycle and propionate carbon may enter any of the synthetic pathways from both tricarboxylic acid-cycle and glycolytic intermediates. For instance, although the origin of glycerol for fatty acid synthesis is not known in sheep, it is possible that propionate may be converted into glycerol without prior conversion of propionate carbon into glucose.

The various pathways available for metabolism of propionate complicate estimations of the contribution of pathways (1), (2) and (3) to glucose synthesis from propionate. If propionate carbon is converted into glucose only via these reactions then the extent of conversion of propionate through lactate into glucose can be estimated from the ratio of incorporation of C-1 to that of C-2 or C-3 of propionate into glucose. That is, if only pathway (3) is operative then 50% as much C-1 as C-2 or C-3 of propionate would appear in glucose. If glucose is synthesized from propionate via reactions (1) and (2) then the incorporation of C-1 of propionate into glucose would be 25% of that incorporated from C-2 or C-3. The incorporation of C-1 relative to that of C-2 and C-3 in relation to the operation of the direct or indirect pathways can be illustrated graphically (Fig. 3). In the present studies the incorporation of C-1 was 0.33 of that of C-2 and C-3, indicating that approx. 70% of the propionate converted into glucose was first converted into lactate. This percentage is a maximum since metabolism by other pathways before conversion of propionate carbon into glucose would tend to exchange the C-1 of propionate. For instance, where [1-¹⁴C]propionate is oxidized partially in the tricarboxylic acid cycle all the radioactivity is lost as ¹⁴CO₂ in one turn of the cycle (i.e. succinate → succinate). In contrast, there is no loss of radioactivity from [2-¹⁴C]propionate during the first turn, but for each subsequent turn of the cycle 50% of the residual activity is lost. The extent of equilibration of propionate in the tricarboxylic acid cycle, however, is probably not great since infusion of [2-¹⁴C]propionate intraportally in sheep has shown only small incorpora-

Fig. 3. Incorporation into glucose of C-1 relative to C-2 and C-3 of propionate in relation to the 'direct' (A) or 'indirect' (B) pathways of synthesis. (Calculations are shown in the text.) The arrow indicates the ratio of mean incorporation into glucose of C-1 to that of C-2 and C-3 of propionate.

tion of ^{14}C into C-3 and C-4 of glucose relative to C-1, C-2, C-5 and C-6 (Annison *et al.* 1963).

Although between 45 and 62% of the glucose carbon arose from propionate carbon only 32% of the propionate produced in the rumen was actually converted into glucose. In the past it has been held that insufficient propionate was produced in the rumen to meet the demands for gluconeogenesis (see review by Lindsay, 1959). This now appears to be untrue, at least in sheep given rations of 800 g. of lucerne/day. On the contrary, there appears to be

considerably more propionate produced in the rumen than would be required to synthesize all the glucose entering the body pool.

We are indebted to the Australian Wool Board and the Rural Credits Development Fund for financial support for this project. J. R. L. was a recipient of a Fulbright Research Fellowship. The skilled technical assistance of Mr J. Wilcher, Mr. P. Russell and Mrs A. Luick is gratefully acknowledged.

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