THE METABOLISM OF GLUCOSE AND BUTYRATE BY THE OMASUM OF THE SHEEP

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SUMMARY

The metabolism of glucose and butyrate by omasum tissue from the sheep has been investigated. Butyrate, but not glucose, is a potent precursor of acetoacetate. The addition of glucose to the system metabolising butyrate causes a marked stimulation in the oxygen consumption by the tissue. This is accompanied by a marked stimulation in the metabolism of butyrate to ketone bodies and to a much less extent to carbon dioxide. In the presence of glucose there is a large increase in the proportion of β-hydroxybutyrate.

Assuming random recombination of acetyl CoA, derived from butyrate, it has been calculated that roughly 75 % of intact carbon skeletons of butyrate are converted directly to ketone bodies.

The significance of these observations and of ketone body formation is discussed.

INTRODUCTION

PENNINGTON and his coworkers[1-6] have done a considerable amount of work on the respiratory and ketogenic activity of several tissues of the sheep. They have given particular attention to the metabolism of acetate, propionate and butyrate, especially in the epithelium of the rumen. The strong ketogenic property of butyrate found by PENNINGTON stimulated us to investigate in more detail the metabolism of labelled butyrate and glucose separately and together. The results described below are an account of such an investigation using tissue from the leaves of the third stomach—the omasum. Previous expts. done by PENNINGTON[1] had shown that as far as had been investigated this tissue was metabolically similar to the epithelial tissue of the rumen.

MATERIALS AND METHODS

Materials

Sodium β hydroxybutyrate – L. Light & Co. Ltd. Dried in a desiccator over P_2O_5 before use.

Acetone – A.R. acetone was distilled twice from and stored over anhydrous K_2CO_3.

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Carbon tetrachloride – Analytical reagents as supplied by B.D.H. Ltd. (Eng.) and May & Baker Ltd. (Eng.) were found to give suitable low blank values.

2.4 Dinitrophenylhydrazine A.R. – 0.1 % (w/v) solution in 2 *N* HCl. This soln. was extracted at least twice by vigorous shaking with carbon tetrachloride in order to reduce the blank value to a low figure.

Generally labelled [^{14}C]glucose and sodium [1-^{14}C]butyrate were obtained from the Radiochemical Centre, Amersham, England.

Experimental procedure

Leaves of tissue of the omasum were cut out at the abattoirs from freshly killed sheep, washed free of solid material with normal saline and taken to the laboratory in ice-cold Krebs-Ringer-phosphate buffer. The leaves were trimmed and the thicker part was discarded. The tissue was then cut into pieces approx. 4 mm^2 and washed again in ice-cold Krebs-Ringer-phosphate buffer. During this final washing oxygen was bubbled through the soln. The pieces were then blotted with filter paper and approx. 300 mg tissue added to each Warburg vessel which contained 3.0 ml of incubating medium. This consisted of Krebs-Ringer-phosphate buffer at pH 7.4 containing the appropriate concn. of substrate(s). NaOH (0.1 ml 2 *N*) was added to the centre well.

The vessels were incubated at 38° with constant shaking at 150 oscillations/min in a conventional Warburg apparatus. The gas phase was 100 % O_2 unless otherwise stated. Readings were taken at intervals for 1.5 to 3 h.

At the end of the incubation period, the incubating fluid was removed and frozen at —15° and the tissue dried at 105° for 16 h. When radioactive substrates were used, the alkali in the centre well was washed out and the washings plus the paper were used to prepare barium carbonate for the estimation of $^{14}CO_2$ produced. No correction has been made for $^{14}CO_2$ retained in the medium and the tissue. This has been found to be about 10 to 15 % of the $^{14}CO_2$ collected in the centre well.

Normally 40 to 80 min elapsed from the time the omasum was removed from the sheep to the time the tissue went into the bath. Bubbling oxygen through the buffer during transport from the abattoirs had no effect on the activity of the tissue. Transporting the tissue in the presence of glucose plus butyrate gave a subsequent activity with these substrates of about 20 % more than tissue transported in buffer alone. Because prior treatment with substrates would have made interpretation difficult, tissue not so treated was thought to be satisfactory especially since in both cases respiration was linear over the exptl. period.

Tissue from mature sheep was used in most of the expts. but occasionally tissue from lambs about 9 months old was taken. The results were similar in both cases. All the results obtained have been calculated to 50 mg dry wt. of tissue.

Estimation of ketone bodies

The estimation of ketone bodies (acetone, acetoacetate and β-hydroxybutyrate) in mixtures presents difficulties that are not easily apparent in the published methods. These difficulties are worsened when applied to biological fluids containing oxidisable substances. One difficulty is met in the quantitative conversion of β-hydroxybutyrate to acetone and a further difficulty is caused by destruction of acetoacetate by the oxidising agent used to oxidise β-hydroxybutyrate.

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Conversion of β-hydroxybutyrate to acetone

The methods investigated depend upon the oxidation and decarboxylation of β-hydroxybutyric acid to acetone by dichromate in an acid medium.

The yields of acetone from β-hydroxybutyrate can very widely and the variation of these yields with different acid and dichromate concns. has been well established (SHAFFER[7], VAN SLYKE[8], WEICHSELBAUM AND SOMOGYI[9]). GREENBERG AND LESTER[10] obtained an 86 % yield but under the conditions reported by MICHAELS, MARGEN, LIEBERT AND KINSELL[11] 100 % yields are obtained. In our hands, however, this latter technique consistently gives yields of 96 %.

It was found that the presence of such reducing substances as glucose and lactate in the medium can lower the concn. of dichromate and hence cause an appreciable drop in the yield of acetone from β-hydroxybutyrate. In estimations on incubation media, which may vary widely in their content of reducing substances, it was found impossible to get accurate estimates of β-hydroxybutyrate from one sample to another. On any one sample, however, consistent yields could be obtained.

The procedure was adopted of adding a known amount of sodium β-hydroxybutyrate to 1 of 2 samples of the exptl. soln. to determine its recovery. The difference in acetone yield of these 2 samples then gave the yield of acetone from the added β-hydroxybutyrate. The yield from the β-hydroxybutyrate already present was assumed to be the same and the correction applied.

Conversion of acetoacetate to acetone

This method depends on the decarboxylation of acetoacetate to acetone by heating in acid. Dichromate was found to decrease markedly the yield of acetone from acetoacetate in both pure solution media in agreement with MICHAELS *et al.*[11] but in contrast to GREENBERG AND LESTER[10]. In our experience therefore acetoacetate must be converted to acetone before any dichromate is added.

Method used for estimation of ketone bodies

After experience with the above factors using the methods of GREENBERG AND LESTER[10], and of MICHAELS *et al.*[11] it was concluded that the latter method with some modification was preferable both from the point of view of accuracy and convenience. The method and modifications finally used are given.

Precipitation of protein

The small amount of tissue material in the incubation samples was removed by $Ba(OH)_2/ZnSO_4$ precipitation (WEICHSELBAUM AND SOMOGYI[9]).

Conversion of ketone bodies to acetone

Samples of 4.0 ml containing up to 0.9 μmoles of ketone bodies and no more than about 5 μmoles of glucose were added to Clin-Britic 1-oz vaccine bottles containing 1.0 ml 10 *N* H_2SO_4. 3 bottles were needed for the estimation of every sample. To 1 of these 1.0 ml of a soln. of Na-β-hydroxybutyrate containing 0.15–0.25 μmole was added and 1.0 ml of water was added to the other 2. The rubber and bakelite caps were put on and all the bottles autoclaved at 17–18 lbs pressure for 10 min.

When the bottles were cool, 1.0 ml 0.4 % (w/v) potassium dichromate was added from a syringe with a fine needle pushed down beside the rubber cap. 2 of the 3

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bottles from each sample, 1 of which contained the added Na-β-hydroxybutyrate, were now autoclaved for a further 30 min.

Appropriate acetone standards were treated in the same way. It is necessary to have standards for both periods of heating as the rubber caps contribute to a small but significant extent to the blank and this contribution varies with the time of heating.

Estimation of acetone

The method adopted was similar to that used by GREENBERG AND LESTER[10]. MICHAELS *et al.*[11] estimated the acetone in the same bottles in which the autoclaving was carried out. The rubber caps of the Clin-Britic bottles however were rapidly affected by carbon tetrachloride and it was not possible to do this. Samples (5.0 ml) were taken from the autoclaved bottles and transferred to glass stoppered test tubes (Quickfit B 19 6″·1″ Pyrex tubes) containing 1 ml 15 % anhydrous sodium sulphite (w/v) to reduce the dichromate. 2.4 Dinitrophenylhydrazine soln. (4 ml) and 4.0 ml carbon tetrachloride were added, the tubes were stoppered and shaken mechanically at a rate of about 270/min for 20 min.

When the carbon tetrachloride had settled, the upper aqueous layer was drawn off, 26 ml water added and the tubes shaken by hand about 30 times. The aqueous layer was drawn off again and the washing procedure repeated. After the addition of 6 ml 0.5 *N* NaOH the tubes were shaken for 5 min. The carbon tetrachloride fraction was then read at 420 mμ in a spectrophotometer. At low concns. of acetone a wavelength of 350 mμ was used; in this region the O.D. is 4 times that at 420 mμ.

The bottles autoclaved for 10 min give the concn. of the acetone plus acetoacetate. Those autoclaved for an extra 30 min give the concn. of total ketone bodies. The difference is taken as β-hydroxybutyrate and this figure is corrected for the percentage recovery of acetone obtained from the added Na-β-hydroxybutyrate.

^{14}C Content of ketone bodies

Samples of the carbon tetrachloride soln. of total ketone bodies were dried on stainless steel planchets and counted at infinite thinness in a gas flow counter. (Tracerlab. Inc. S.C. 16 Windowless Flow Counter and S.C. 51 Autoscaler). All samples were counted for sufficient time to give a standard error of less than 4 %.

^{14}C Content of carbon dioxide

The washings and paper from the centre well of the Warburg flask and a suitable quantity of sodium carbonate as carrier were acidified under vacuum and the $^{14}CO_2$ absorbed into saturated barium hydroxide. The precipitate was washed twice with water and once with acetone and plated out in ethanol on to stainless steel planchets. These were counted and corrected for self absorption to infinite thinness. The corrections used were those given by CALVIN *et al.*[12].

The method of plating directly in planchets was tedious and it is difficult to obtain precise surface areas. Because of these difficulties the method was changed to that given in ARONOFF[13]. The barium carbonate, formed from the washings from the centre well of the Warburg vessels and a suitable quantity of carrier sodium carbonate as before, is precipitated on to circles of Whatman No. 3 filter paper and washed with water, ethanol and acetone. The filter paper is transferred to a stain-

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less steel planchet and held in place with a stainless steel ring. The counts/min obtained were corrected to infinite thinness from an absorption curve prepared under the same conditions. All samples were counted for sufficient time to give a standard error of less than 2 %.

Specific activities of substrates

The specific activities of the [^{14}C]glucose and [1-^{14}C]butyrate solns. were determined by plating, in ethanol, a suitable series of dilutions and counting these at infinite thinness. In the case of [1-^{14}C]butyrate, alkaline solns. were used and the planchets were dried at room temperature. The glucose solutions were tested and shown to be pure by radioautography.

RESULTS

Influence of glucose and butyrate on the oxygen uptake by pieces of omasum tissue

When incubated in either 20 m*M* glucose or butyrate, pieces of omasum tissue generally have an oxygen uptake above the level with butyrate (Table I). When glucose and butyrate are metabolised together, however, there is a marked stimulation in oxygen uptake with increases of from 15 % to sometimes over 100 % above the level with butyrate (Table I). Results of 1 expt. are given in Fig. 1. It has been found that concns. of glucose as low as 1 m*M* can have a stimulatory effect on the oxygen consumption in the presence of both 10 m*M* and 20 m*M* butyrate.

TABLE I

OXYGEN UPTAKE BY OMASUM TISSUE IN THE PRESENCE OF VARIOUS SUBSTRATES

Tissue (200–300 mg wet wt.) incubated at 38° in 3.0 ml of Krebs-Ringer-phosphate buffer at pH 7.4 containing each substrate at a final concn. of 20 m*M*. Gas phase 100 % O_2. Results calculated to 50 mg dry wt.

Expt. No.	*Duration h*	*Oxygen uptake ($\mu l \times 10^{-2}$)*				*Stimulation of glucose plus butyrate above butyrate (%)*
		No. Substrate	*Glucose*	*Butyrate*	*Glucose plus butyrate*	
1	2.0	3.5	5.1	3.8	6.3	66
2	2.0	1.8	1.6	2.1	2.7	29
3	1.5	2.6	3.2	2.7	4.0	48
4	1.5	1.9	2.1	2.3	2.7	17
5	2.0	2.6	3.3	3.1	4.9	58
6	2.0	3.0	3.2	2.7	4.3	59
7	3.0	2.9	4.4	4.7	7.2	53
8	3.0	2.7	4.6	4.0	6.6	65
9	3.0	3.5	5.5	3.5	9.3	165
10	3.0	3.0	2.7	2.6	6.1	135
11	3.0	4.5	4.9	5.7	9.2	60
12	3.0	2.9	5.0	3.2	7.0	120

Tipping in either glucose or butyrate from the side arm of the Warburg flasks into a medium containing either butyrate or glucose respectively shows that this effect is actually a stimulatory one and is not related to preservation of the tissue in the presence of glucose and butyrate together. The rapid change in rate of oxygen

uptake is shown in Figs. 1 and 2. Control flasks in which glucose was added to glucose and butyrate to butyrate showed no change in the rate of oxygen uptake.

The results on oxygen uptake have therefore shown a stimulatory effect when butyrate is metabolised in the presence of glucose and it appears that this joint metabolism has resulted in the abolition of a rate-limiting step. However, it is impossible to consider properly any of the possible factors involved without additional information on the metabolism of the added substrates.

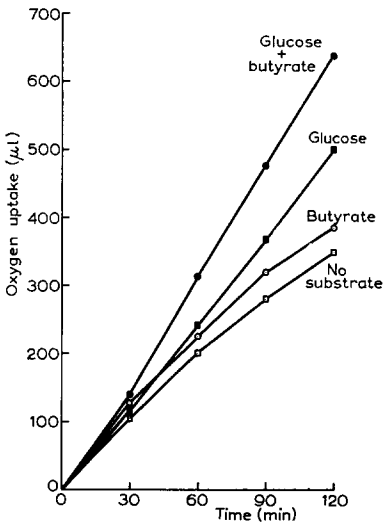

Fig. 1. Oxygen uptake by omasum tissue in the presence of various substrates. Tissue (300 mg wet wt.) incubated at 38° in 3.0 ml Krebs-Ringer-phosphate buffer at pH 7.4 containing each substrate at a final concn. of 20 m*M*. Gas phase 100% O_2. Results calculated to 50 mg dry wt.

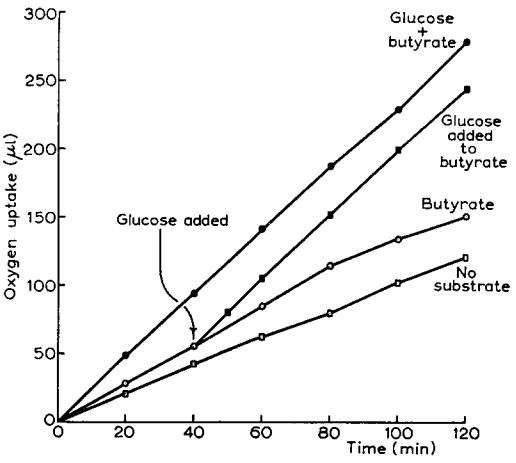

Fig. 2. Effect of the addition of glucose on the oxygen uptake by omasum tissue metabolising butyrate. Tissue (300 mg wet wt.) incubated at 38° in 2.5 ml Krebs-Ringer-phosphate buffer at pH 7.4 containing each substrate at a final concn. of 20 m*M*. At time shown, 0.4 ml 150 m*M* glucose in buffer tipped in from side arm into flask containing butyrate. Results calculated to 50 mg dry wt.

Production of $^{14}CO_2$ from [^{14}C]glucose and [1-^{14}C]butyrate

Glucose was found to increase the oxidation of [1-^{14}C]butyrate to $^{14}CO_2$ whilst butyrate usually depressed the oxidation of [^{14}C]glucose (Table II). Concns. of glucose as low as 1 m*M* were found to be effective in stimulating the oxidation of [1-^{14}C]butyrate. In 1 expt. 1 m*M* glucose had an effect equiv. to 20 m*M* glucose but neither was effective as 10 m*M*. Usually, however, the stimulation was found to increase with glucose concn.

Calculations based on the ^{14}C content of the respired CO_2 showed an oxidation of 1.5 to 3.5 % of the added [1-^{14}C]butyrate and 0.3 to 1.5 % of the added [^{14}C]glucose.

Formation of ketone bodies

Following PENNINGTON'S observations[1] on the production of ketone bodies by rumen epithelial and omasum tissue, it was of interest to investigate a possible relationship between the stimulation in oxygen consumption and the production of ketone bodies.

Total production: Ketone bodies were measured by their appearance in the

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TABLE II

EFFECT OF GLUCOSE AND BUTYRATE ON THE PRODUCTION OF $^{14}CO_2$ BY OMASUM TISSUE FROM [1-^{14}C]BUTYRATE AND [^{14}C]GLUCOSE RESPECTIVELY

Tissue (300 mg wet wt.) incubated for 1.5–2.0 h at 38° in 3.0 ml of Krebs-Ringer-phosphate buffer at pH 7.4 containing each substrate at a final concn. of 20 m*M*. Approx. $3 \cdot 10^5$ counts/min of ^{14}C substrate/flask. Gas phase 100% O_2. Results calculated to 50 mg dry wt.

Expt. No.	*Duration h*	*% Change in $^{14}CO_2$ output*	
		Effect of glucose on [1-^{14}C]butyrate	*Effect of butyrate on [^{14}C]glucose*
1	2.0	+ 30	— 42
2	1.5	+ 23	— 40
3	2.0	+ 48	— 41
4	2.0	+ 39	— 70
5	2.0	0	+ 13
6	2.0	+ 38	—
7	2.0	+ 44	—
8	2.0	+ 26	—
9	1.5	+ 6	—
10	1.5	+ 29	—

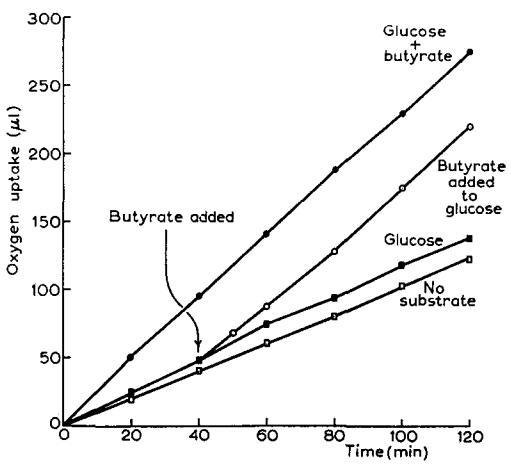

Fig. 3. Effect of the addition of butyrate on the oxygen uptake by omasum tissue metabolising glucose. Tissue (300 mg wet wt.) incubated at 38° in 2.5 ml Krebs-Ringer-phosphate buffer at pH 7.4 containing each substrate at a final concn. of 20 m*M*. At time shown, 0.4 ml 150 m*M* butyrate in buffer tipped in from side arm into flask containing glucose. Results calculated to 50 mg dry wt.

incubation medium. PENNINGTON[1] had previously found that about 10% of the total ketone bodies produced by rumen epithelial tissue remained in the tissue and values obtained by this author were corrected by this amount. Such a correction would serve little purpose with the present work and has not therefore been made.

Table III shows the production of ketone bodies by pieces of omasum tissue in the presence of various concns. of butyrate. The value of 9.8 µmoles for 20 m*M* butyrate represents about 16% of the total butyrate added. As only about 2% of the original butyrate was oxidised to CO_2 in this expt., the metabolism of butyrate by this tissue is therefore concerned predominantly with the production of ketone bodies.

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TABLE III

THE EFFECT OF CONCENTRATION OF BUTYRATE AND GLUCOSE PLUS BUTYRATE ON KETONE BODY PRODUCTION BY OMASUM TISSUE

Tissue (300 mg wet wt.) incubated for 1.25 h at 38° in 3.0 ml of Krebs-Ringer-phosphate buffer at pH 7.4. Gas phase 100% O_2. Results calculated to 50 mg dry wt.

Substrate	*Ketone bodies*	
	Total (μmoles)	*Reduced / Oxidised*
1 m*M* Butyrate	2.1	0.1
5 m*M* Butyrate	6.1	0.1
10 m*M* Butyrate	7.2	0.1
20 m*M* Butyrate	9.8	0.1
20 m*M* Butyrate + 1 m*M* Glucose	11.4	0.3
20 m*M* Butyrate + 10 m*M* Glucose	13.3	0.6
20 m*M* Butyrate + 20 m*M* Glucose	11.8	0.9

TABLE IV

THE EFFECT OF GLUCOSE AND BUTYRATE ON THE TOTAL AND TYPE OF KETONE BODY PRODUCTION BY OMASUM TISSUE

Tissue (300 mg wet wt.) incubated at 38° in 3.0 ml of Krebs-Ringer-phosphate buffer at pH 7.4 containing each substrate at a final concn. of 20 m*M*. Results calculated to 50 mg dry wt.

Expt. No.	*Duration h*	*No substrate Ketone bodies*		*Glucose Ketone bodies*		*Butyrate Ketone bodies*		*Glucose + Butyrate Ketone bodies*	
		μmoles	*Red/Oxid*	*μmoles*	*Red/Oxid*	*μmoles*	*Red/Oxid*	*μmoles*	*Red/Oxid*
1	2.0	0.7	0.0	0.6	0.3	5.6	0.0	14.3	0.5
2	2.0	0.4	0.0	0.7	0.3	2.9	0.1	6.9	0.8
3	1.5	0.8	0.1	1.6	0.8	3.8	0.3	8.6	0.9
4	1.5	0.4	0.3	0.4	0.9	2.6	0.1	4.6	0.8
5	2.0	0.6	1.1	0.7	1.1	3.7	0.3	7.8	1.0
6	1.5	0.6	2.0	1.1	4.0	3.2	0.4	7.1	1.4
7	1.5	0.6	—	0.9	1.0	6.9	0.1	9.4	0.8
8	2.0	0.4	0.9	0.9	1.2	4.1	0.2	9.9	0.8
9	2.0					9.8	0.1	11.8	0.9
10	2.0					5.4	0.0	7.9	0.8
11	1.5					6.2	0.1	11.0	0.9
12	2.0					3.8	0.3	7.2	1.0

Glucose, however, contrary to its usual behaviour as an antiketogenic agent, was found to cause a marked rise in the ketone body production when butyrate was metabolised in its presence. Even concns. as low as 1 m*M* can have a significant effect (Table III). In Table IV the results of a number of expts. are given and the marked stimulatory effect of glucose can be seen. In many cases the ketone body production is doubled.

Glucose when metabolised alone only produced small quantities of ketone bodies but this production was practically always greater than the level with no substrate (Table IV)

The results for butyrate alone agree with those of PENNINGTON[1] for omasum tissue in that there is considerable production of ketone bodies when butyrate is metabolised. PENNINGTON AND PFANDER[6], however, found that, in rumen epithelial

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tissue, glucose caused a small (10 %) decrease in the ketone body production when metabolised with butyrate. In the present work in 2 out of 4 preliminary expts. with rumen epithelium, glucose has been found to stimulate markedly ketone body formation from butyrate. In the other 2 there was no effect.

The above results were obtained when the tissue was incubated in an atmosphere of oxygen with alkali in the centre well of the manometer flasks to remove any C_{O_2} evolved. However, the presence of carbon dioxide in the gas phase (95 % O_2; 5 % CO_2), had no effect on the total production of ketone bodies from butyrate or on the ketogenic effect of glucose.

Ratio of reduced to oxidised form of ketone bodies: The molar ratio of the β-hydroxybutyrate fraction to the acetone plus acetoacetate fraction of the total ketone bodies has been found to vary in an interesting way. When butyrate alone is metabolised the molar ratio is low *i.e.* the ketone bodies produced are mostly in the form of acetoacetate (Tables III and IV). However, the metabolism of glucose and butyrate together leads to a marked increase in the molar ratio *i.e.* in the proportion of β-hydroxybutyrate. Associated with this change in the molar ratio is the large increase in ketone body production referred to previously. Most of this increase is due to β-hydroxybutyrate with generally only a small increase in the "acetone plus acetoacetate" fraction.

The metabolism of glucose therefore, besides increasing the production of ketone bodies in the presence of butyrate and the oxidation of butyrate to CO_2, apparently leads to a supply of reduced coenzyme (DPNH?) for the conversion of acetoacetate to β-hydroxybutyrate.

Origin of the carbon atoms in the ketone bodies: Having observed this marked increase in ketone body production when butyrate was metabolised in the presence of glucose, it was of interest to determine the origin of the carbon atoms involved. By determining the radioactivity of the ketone bodies it has been possible to show that they mostly originate from butyrate.

It can be seen from the results given in Table V that the contribution of [^{14}C]glucose to the radioactivity (counts/min.) found in the ketone bodies shows little change when butyrate is added, even though there is a many fold change in the total ketone body production. Likewise the specific activity (counts/min/μmole) of the ketone bodies shows a decrease proportional to the rise in the total amount of ketone bodies.

TABLE V

EFFECT OF BUTYRATE AND GLUCOSE ON THE INCORPORATION OF ^{14}C FROM [^{14}C]GLUCOSE AND [1-^{14}C]BUTYRATE RESPECTIVELY INTO KETONE BODIES BY OMASUM TISSUE

Tissue (300 mg wet wt.) incubated for 2.0 h at 38° in 3.0 ml of Krebs-Ringer-phosphate buffer at pH 7.4 conta ning each substrate at a final concn. of 20 m*M*. $420 \cdot 10^3$ counts/min [^{14}C]glucose or $260 \cdot 10^3$ counts/min [1-^{14}C]butyrate flask. Only carbon atoms 2, 3 and 4 of acetoacetate or β-hydroxybutyrate can be counted, as carbon atom one is lost during the estimation of ketone bodies. Results calculated to 50 mg dry wt.

Substrate	*Ketone bodies (μmoles)*	*counts/min in ketone bodies*	*Specific activity of ketone bodies counts/min/μmole*
[^{14}C]Glucose	0.7	850	1,210
[^{14}C]Glucose + Butyrate	7.8	1,050	135
[1-^{14}C]Butyrate	3.7	2,550	690
[1-^{14}C]Butyrate + Glucose	7.8	4,950	640

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It follows that this large increase in ketone bodies results from the metabolism of the added butyrate.

A similar conclusion is reached by a consideration of the activity of the ketone bodies formed in the presence of [1-^{14}C]butyrate. The results in Table V show that the increase in total ketone bodies when glucose is added is approx. paralleled by the increase in the counts/min found in the ketone bodies. In the same way the specific activity of the ketone bodies remains about the same. Such results have been repeatedly obtained.

A comparison of the specific activities (in this case counts/min/μatom C) of the [^{14}C]glucose and of the ketone bodies formed in its presence has shown that roughly 40 % (range 24–58 %) of the ketone bodies with glucose as the substrate have their origin in the carbon skeleton of glucose, the remaining 60 % arising from the metabolism of endogenous substrates.

In the case of [1-^{14}C]butyrate, however, the only ketone bodies which can be counted as ^{14}C are those formed by a recombination of acetyl CoA such that carbon one of butyrate ends up as carbon 3 of acetoacetate. Any ^{14}C in carbon atom 1 of the ketone bodies is lost as carbon dioxide during the procedures used in their estimation. Because the specific activity of the ketone bodies formed from [1-^{14}C]butyrate shows little change when glucose is added, it follows that glucose has little effect on the random or otherwise recombination of acetyl CoA to give acetoacetate.

If this recombination is random, then only half the recombined molecules of acetoacetyl CoA contain ^{14}C in carbon 3. The percentage recombination of acetoacetyl CoA leading to relocation of carbon atom 1 to carbon 3 is therefore given by the equation:

$$\frac{2 \times \text{specific activity ketone bodies}}{\text{specific activity [1-}^{14}\text{C]butyrate}} \times 100$$

The values found vary from about 14 to 33 % (Table VI) so that about three-quarters of the acetoacetyl CoA formed from butyrate is converted to free acetoacetate before it can be split into 2 molecules of acetyl CoA.

TABLE VI

RELOCATION OF CARBON ATOM 1 OF BUTYRATE TO CARBON ATOM 3 OF KETONE BODIES BY BREAKDOWN AND RECOMBINATION OF KETONE BODY PRECURSORS

Expt. No.	*Recombination (%)*
1	33
2	22
3	20
4	14
5	18
6	26
7	28
8	19

The equation given is only approximate because the total ketone bodies estimated includes a proportion derived from endogenous substrates which cannot be exactly determined. The total oxygen consumption in the presence of butyrate is much less than the sum of the endogenous respiration (no substrate) plus butyrate respiration

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calculated from data on $^{14}CO_2$ and ketone body production. This means that the utilisation of endogenous substrates is inhibited in the presence of butyrate and that any error based on endogenous ketone body production is a maximum error. In the case of butyrate alone the proportion of ketone bodies produced from endogenous substrates to those produced from butyrate ranges from 9–20 %. The error in the calculations based on the above equation will therefore be less than 20 %.

DISCUSSION

It is quite clear from the results obtained that glucose affects the metabolism of butyrate in a number of ways. However, the stimulation of oxygen consumption and the increase in carbon dioxide and ketone body production from butyrate probably have a common basis. It is not at all clear which or how many rate-limiting steps are abolished by the addition of glucose. One possibility is that the glycolytic metabolism of glucose provides the necessary adenosine triphosphate for the entry of butyrate into the tissue and/or for its activation to initiate its metabolism. A further possible rate-limiting step might be the supply of oxaloacetate for the metabolism of the acetyl coenzyme A derived from butyrate. We are as yet unable to evaluate the relative importance of these possibilities.

The results show that the increase in the conversion of butyrate to ketone bodies caused by the addition of glucose is 5 to 15 times the increase in the conversion to carbon dioxide. Most of the increased quantity of acetoacetyl CoA formed is therefore converted to ketone bodies and not to carbon dioxide. Assuming that conversion to carbon dioxide is mediated by the TCA cycle this could indicate that the enzymes responsible for the conversion of acetoacetyl CoA to acetoacetate are not nearly saturated as are those responsible for the oxidation of acetyl CoA. However, there may also be spatial reasons within the cell that could account for the observed effects.

The ketone body formed from butyrate in the absence of glucose is in the oxidised form, acetoacetate. This means, that under these conditions the reduced coenzymes coming from the tissue's metabolism are not effective as reducers of acetoacetate. On the other hand the increase in ketone body due to the addition of glucose to the butyrate system is in the reduced form, β-hydroxybutyrate. It is therefore possible that the reducing agent (DPNH?, TPNH?) involved has its origin from a non-mitochondrial source connected with one or both of the metabolic pathways of glucose. This point is being investigated in more detail.

The close relationship between the specific activities of the ketone bodies and the specific activity of the butyrate added, leaves no doubt that the major part of the ketone bodies has its origin in the carbon chain of butyrate. The relocation of radioactive carbon atoms from carbon 1 of butyrate to carbon 3 of the ketone bodies indicates that there is a continual breakdown and resynthesis of acetoacetyl-coenzyme A through the intermediate acetyl-coenzyme A. The breakdown and recombination of one quarter of the carbon atoms also means that the rate of conversion of acetoacetyl-coenzyme A to acetoacetate is much faster than the thiolase reaction which in turn is faster than the removal of acetylcoenzyme A by the tricarboxylic acid cycle; a conclusion in agreement with the one derived above from a consideration of ketone body and CO_2 production.

Butyrate has been shown by PENNINGTON[1] to be a potent precursor of ketone

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bodies in the rumen, omasum and the liver of the sheep. The results for the omasum obtained in the present work and unpublished results on the rumen confirm these results. Among the fatty acids butyrate is unique in that during its oxidation the complete molecule passes through the form acetoacetyl-coenzyme A. This substance is now known to react with acetyl-coenzyme A to form β-hydroxy-β-methylglutaryl coenzyme A, the immediate precursor in the synthesis of acetoacetate[14,15].

In non-ruminant animals the liver seems to be the active tissue in the production of ketone bodies and there is evidence to show that the production of ketone bodies is related to the supply of glucose (oxaloacetate?). In the ruminant animals so far studied[1,2] it seems that the special digestive organs, the rumen and omasum, also have a great capacity for the formation of ketone bodies. In these organs the production of ketone bodies would depend on the supply of the precursors (butyrate) and on the presence of the 2 enzymes discovered by BACHHAWAT *et al.*[15] and by LYNEN *et al.*[14] and not on a low glucose status of the tissue. Indeed the present work has shown that the addition of glucose markedly enhances ketone body production. There is no information to relate quantitatively the importance of the special digestive organs to ketosis although it would seem likely that in the diseased state the major part of the ketosis has its origin elsewhere (for review see SHAW[16]). The high activity of these special organs with respect to ketone body formation is probably of significance and may be related to their close contact with a digestion mass rich in fatty acids.

As the oxidation of hydrogen atoms of butyrate is quantitatively greater than the oxidation of carbon atoms, the oxidising system is of interest. If one assumes that the usual coenzymes and oxidative phosphorylating ratios are involved then it can be shown that, for adenosine triphosphate production, the oxidation of butyrate to acetoacetate is much less efficient, on an oxygen basis, than its complete oxidation to carbon dioxide and water. For a given requirement of energy therefore, more butyrate must be metabolised than in the case of a substrate oxidised mostly to carbon dioxide.

It seems reasonable therefore that the oxidation of fatty acids to ketone bodies as end products ought to be regarded as a normal and useful metabolic pathway in ruminant as well as non-ruminant animals. Under conditions of stress it is probably of even more importance as a means by which useful energy is produced.

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STUDIES OF DEOXYRIBONUCLEIC ACID SYNTHESIS AND CELL GROWTH IN THE DEOXYRIBOSIDE REQUIRING BACTERIA, *LACTOBACILLUS ACIDOPHILUS*

II. DEOXYRIBONUCLEIC ACID SYNTHESIS IN RELATION TO RIBONUCLEIC ACID AND PROTEIN SYNTHESIS*

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SUMMARY

1. Depletion of the deoxyriboside requirement of *Lactobacillus acidophilus* R-26 inhibits almost completely DNA synthesis and cell division, but little affects the synthesis of RNA and protein.

2. Omission of uracil from the medium causes a parallel inhibition of RNA and protein formation; but the synthesis of DNA is stimulated in the absence of uracil.

3. Addition of excess thymidine to the cells previously starved of both uracil and deoxyriboside causes a remarkable accumulation of acid-soluble deoxyribosidic compounds and active synthesis of DNA, with little or no concurrent increase of protein, RNA or cell number. Simultaneous addition of chloramphenicol and thymidine abolishes the small increase in protein observed under such conditions, with little effect on DNA synthesis.

4. Despite the fact that DNA is synthesized actively in the absence of RNA and protein increase, omission of amino acids from the uracil-deficient as well as complete medium strongly inhibits the increase of DNA.

INTRODUCTION

Syntheses of replicating materials such as DNA**, RNA and protein in the cell may be the most essential processes involved in cell growth. The question whether or not

* A preliminary account of some of the results reported in this paper has been published[1].
** The following abbreviations are used: deoxyribonucleic acid, DNA; ribonucleic acid, RNA.

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