proved exceptionally unstable (cf. Bray, 1959, for a possible explanation), in all other respects the xanthine oxidase appears identical with that produced by the method of Avis *et al.* (1955).

Purity of the final product

On the basis of both the E_{280}/E_{450} ratio and ultracentrifugal analyses (Fig. 1) the purity of the final product normally lies in the range 85–95%. The chromatographic stage leads to a decrease in the E_{280}/E_{450} ratio from about 15–20 to 5.4–6.0. The method as given above represents a compromise between yield and purity: it is possible, of course, for one to be improved at the expense of the other. The lower purity value quoted may be considered adequate for some purposes, e.g. if the enzyme is to be stored for a considerable period and high stability is required. Where 'ultra-pure' enzyme is required and some 'losses' can be accepted, either the adsorption may be prolonged until only xanthine oxidase is retained on the column (virtually all other proteins being entirely displaced) or, by the use of a fraction collector, only that part of the eluate having a low and constant E_{280}/E_{450} ratio need be retained. Alternatively, the purity may be increased by a second ammonium sulphate fractionation within narrower limits than those given in Scheme 1.

SUMMARY

1. A relatively simple preparation of bovine milk xanthine oxidase is presented.

2. Yields of 70–90% have been made possible by the use of cysteine to release the enzyme from a particulate complex.

3. The average specific activity (activity/ $E_{450}^{1\text{ cm.}}$ ratio) of the product is 100.

4. The purity is 85–95% and indications are given how this may be improved at the expense of yield.

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The Effect of Short-Chain Fatty Acids on Blood Glucose Concentration in Sheep

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The energy requirements of ruminants are supplied largely by short-chain fatty acids produced by fermentation of the food in the reticulo-rumen sac and other parts of the alimentary tract. Owing to the nature of the food usually consumed

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and to these fermentations it is improbable that much sugar is normally absorbed by these animals; the only likely source is polysaccharide synthesized by bacteria and protozoa. The available evidence suggests that their carbohydrate requirements are met largely by gluconeogenesis. It can be predicted, from established metabolic reactions, that propionate is convertible into carbohydrate in the

animal body, and there is plenty of evidence that this is so (e.g. Deuel, Butts, Hallmann & Cutler, 1935).

It is much less certain whether acetate or butyrate, the other two main acids produced in the rumen, can give rise to an appreciable net synthesis of carbohydrate in animal tissues. Some authors (for reviews see Deuel & Morehouse, 1946; Weinman, Strisower & Chaikoff, 1957) have concluded that such does not occur. On the other hand, Blixenkrone-Möller (1938) obtained evidence that butyrate was converted into glucose in a perfused cat liver.

A number of workers (Reid, 1950; Schultz & Smith, 1951; Jarrett, Potter & Filsell, 1952; Johnson, 1955; Clark & Malan, 1956; Annison, Hill & Lewis, 1957; Kronfeld, 1957) have reported on the effects of injected short-chain fatty acids on blood glucose concentration in ruminants. Potter (1952) obtained particularly striking results. He found that the concentration of blood glucose in lambs which had been treated with insulin was markedly and rapidly increased by the intravenous administration of butyrate (2.5 m-moles/kg. body wt.) or propionate but not by acetate. Such an effect might conceivably be due either to gluconeogenesis from propionate and butyrate or to a breakdown of liver glycogen to glucose caused directly or indirectly by these compounds. Potter's (1952) results did not decide definitely between these possibilities, although he showed that butyrate and propionate relieved hypoglycaemic convulsions in sheep in which the splanchnic nerves had been sectioned. This indicated that the effect of these acids did not depend entirely on stimulation of the sympathetic nervous system causing a release of adrenaline from the adrenal medulla. The present work was primarily an attempt to ascertain whether the effect of butyrate is due to its conversion into carbohydrate.

A preliminary account of this work has been published (Ash, Pennington & Reid, 1959).

EXPERIMENTAL

Animals. Eight Cheviot sheep, 1-2 years old, were used for all the experiments described, except those of Tables 4 and 6. Sheep nos. 1, 3, 4, 5 and 6 were male and 2, 7, and 8 female. The daily food ration of each animal was 600 g. of hay and 225 g. of concentrates. No food was given for about 36 hr. before each experiment and at least 1 week elapsed after an experiment before the same animal was used again. To minimize blood sugar changes due to excitement the sheep were previously accustomed to handling and compounds were administered and blood samples taken through a polythene catheter (no. 2 Sterivac tubing) inserted into the jugular vein in the direction of blood flow, usually 2 hr. before the experiment. Blood clotting was prevented by injecting approx. 1 ml. of 0.9% NaCl con-

taining 10 units of heparin/ml. every few minutes from a syringe attached to the catheter; the catheter was flushed through with blood before sampling. Compounds were usually administered in a solution with a volume of less than 10 ml. In most of the experiments, blood samples were taken every 2 min. for the first 10 min. after the injection (with the exception of the insulin injections), then every 10 min. for the next 30 min., and thereafter at less frequent intervals if required.

For the liver-slice experiments (Table 8) tissue from miscellaneous sheep and rabbits on ordinary diets was used.

Analytical procedure. Glucose was determined by the procedure of Somogyi (1952), utilizing Nelson's arsenomolybdate reagent. Protein was precipitated with $\text{Ba}(\text{OH})_2\text{-ZnSO}_4$. Lactate was determined by the method of Barker & Summerson (1941) as modified by Pennington & Sutherland (1956), and total ketone bodies by the method of Greenberg & Lester (1944).

Radioactivity measurements. Radioactivity in blood glucose was measured by direct counting of the isolated phenylglucosazone, by the procedure of Searle & Chaikoff (1952).

Tissues were extracted with HClO_4 , by the procedure of Busch & Potter (1952). To determine the radioactivity in the neutralized extracts 0.1 ml. of 0.1 N-NaOH and 0.1 ml. of 0.1 M-sodium butyrate (unlabelled) were added to 2 ml. of extract and portions were dried on planchets on a hot-plate and counted. Control experiments showed that small amounts of radioactive sodium butyrate added to neutralized (pH 7.0) HClO_4 extracts of tissues were lost when the extracts were dried; these losses were negligible when unlabelled butyrate and alkali were added as indicated. To determine the radioactivity not volatile in acid a portion (approx. 0.1 ml.) of the neutralized HClO_4 filtrates was dried on a planchet. A drop of conc. HCl-M-sodium butyrate (1:1, v/v) was mixed with the residue and the sample dried again. Radioactive butyrate added to the samples was completely removed by this procedure.

To measure the amount of radioactive volatile fatty acid present in the blood at a certain time after injection of labelled butyrate or propionate, 200 μ moles of the appropriate fatty acid (unlabelled) were added as carrier to 10 ml. of blood, and the volatile fatty acids steam-distilled as described by Annison (1954). The neutralized steam-distillate was evaporated to a small volume and suitable portions of the liquid were dried on planchets and counted.

Radioactivity in liver lipids was measured by the procedure of Katz & Chaikoff (1954). Liver glycogen was isolated by a standard procedure (Hassid & Abraham, 1954), dialysed for 2 days against distilled water (0-4°) and reprecipitated with ethanol. After drying, weighed portions were made into slurries with ethanol on planchets, dried and counted.

All radioactivity counts were made with an end-window counter and corrected, where necessary, for self-absorption.

RESULTS

The experiments of Potter (1952) were repeated with smaller quantities of fatty acids. After the intravenous injection of insulin ('A.B.', British Drug Houses Ltd.; 0.5-2 units/kg. body wt.) the blood glucose concentration fell to a minimum

Table 1. *Effect of injected fatty acids upon blood glucose concentration in sheep previously treated with insulin*

The fatty acids (neutralized with NaOH) were infused into the jugular vein over a period of 2 min., 90 min. after the intravenous injection of insulin. In some experiments utilizing 0.25 m-mole of butyrate/kg. the injection and infusion were repeated after the concentration of blood glucose had returned to normal; both sets of figures are given.

Fatty acid injected (m-mole/kg.)	Sheep no.	Concn. of blood glucose (mg./100 ml.)			
		Before insulin	90 min. after insulin	Peak value after fatty acid	Increase due to fatty acid
Acetate (1.0)	5*	53.9	20.7	22.0	1.3
	6*	70.4	16.6	16.8	0.2
	3*	54.5	19.2	23.5	4.3
	4	51.8	26.3	26.8	0.5
Propionate (1.0)	1*	58.9	19.3	38.6	19.3
	2*	52.8	22.2	52.5	30.3
Propionate (0.25)	5*	54.5	21.5	30.2	8.7
	6*	64.4	13.4	18.6	5.2
Butyrate (1.0)	1*	55.4	13.9	55.4	41.5
	2*	59.9	23.5	84.1	60.6
Butyrate (0.5)	5*	54.5	19.3	49.5	30.2
	6*	70.0	16.6	39.1	22.5
Butyrate (0.25)	1	51.5*, 52.2	15.3, 20.8	27.5, 46.0	12.2, 25.2
	2	57.4, 48.3	20.0, 19.3	58.7, 54.0	38.7, 34.7
	3	53.5	19.8	58.9	39.1
	4	50.5, 52.9	25.7, 19.3	52.5, 50.5	26.8, 31.2
	5	54.0*, 50.5	22.7, 19.8	54.0, 35.1	31.3, 15.3
	6	64.8, 63.8	30.7, 25.4	54.9, 41.5	24.2, 16.1
		61.6*, 54.5	18.7, 28.5	47.0, 35.1	28.3, 6.6
		63.8, 57.9	21.8, 21.3	44.6, 50.5	22.8, 29.2
Butyrate (0.125)	3	61.9	24.2	30.8	6.6
	4	61.9	23.2	36.9	13.7
Hexanoate (0.25)	3	47.0	17.3	57.4	40.1
	4	54.7	20.0	77.2	57.2

* Two units of insulin/kg. body wt. were injected; in all other injections of insulin only 0.5 unit/kg. was used.

Fig. 1. Effect of short-chain fatty acids on blood glucose concentration of sheep treated with insulin. Insulin (2 units/kg. body wt.) was injected intravenously at zero time. After 1½ hr. sodium salts of the following fatty acids were injected intravenously (1 m-mole/kg. body wt.): O, acetic acid; ●, propionic acid; △, butyric acid.

after 1–1½ hr. and showed little subsequent rise for a few hours. Sodium salts of the fatty acids were infused over a period of 2 min., 1½ hr. after the administration of insulin. Propionate or butyrate,

but not acetate, produced rapid and substantial rises in the blood glucose concentration; the increase was usually evident 1 min. after the end of the infusion and the peak was attained within 5–10 min. Fig. 1 shows typical results obtained with 1 m-mole of fatty acid/kg. body wt. In Table 1 the results of the administration of various quantities of fatty acids are summarized. The response increased with increasing chain length of the fatty acids. Acetate had, at the most, a very small effect on the blood glucose concentration, butyrate was more effective than equimolar amounts of propionate, and hexanoate appeared to be more potent than butyrate. For most of the experiments the commercial sodium butyrate used was first steam-distilled as butyric acid and the distillate neutralized with sodium hydroxide and evaporated to a suitable concentration. There was no indication that this altered the effectiveness of the butyrate in increasing the blood glucose concentration.

The administration of 0.25 m-mole of butyrate/kg. under these conditions to two sheep produced increases in blood ketone bodies of 33 and 98 % respectively within a few minutes.

If the additional blood glucose produced by butyrate administration were simply a result of synthesis of sugar from the butyrate there would obviously be a theoretical limit to the increase in blood glucose concentration caused by administration of a definite quantity of butyrate. The limiting value would depend upon the following factors: (i) rates of conversion of butyrate into glucose; (ii) rates of passage of the glucose into and out of the blood; (iii) size of the total body glucose pool (see, for example, Steele, Wall, de Bodo & Altszuler, 1956); (iv) rate of utilization of glucose by the tissues. It was not possible to calculate with any confidence the maximum possible blood sugar rise which could result from conversion of the butyrate. Instead, an attempt was made to determine this experimentally by measuring the rise in blood glucose caused by injecting an amount of glucose equivalent to that which would be formed by total conversion of the fatty acid. The glucose was infused slowly over 8–10 min., the period usually elapsing before the blood glucose concentration reaches its maximum value after the administration of butyrate. The results are shown in Table 2. Since the only pathways at present plausible would imply formation of one molecule of glucose from two of butyrate, the results in Table 2 with 0.125 m-mole of glucose/kg. body wt. should be

compared with the effects of 0.25 m-mole of butyrate/kg., shown in Table 1. In some experiments the increase produced by this quantity of butyrate was considerably greater than after the glucose administration. This provides strong evidence that butyrate increases the blood sugar concentration by a mechanism other than by conversion into glucose itself. It does not exclude the possibility that such conversion also occurs.

To provide further evidence on this question [^{14}C]butyrate was administered to a sheep under similar conditions. At periods of 5 and 10 min. after the administration the isotope content of the blood glucose was measured. Table 3 shows the result obtained and the results of similar experiments with labelled propionate. Evidently only a very small proportion (of the order of 1%) of the extra glucose appearing in the blood after the injection of [*carboxy*- ^{14}C]butyrate contained the labelled carbon atom of the butyrate. With propionate the proportion was higher but still small. In all experiments the amount of labelled fatty acid remaining in the blood at approx. 10 min. after injection was negligible.

The possibility that large quantities of labelled intermediates in glucose synthesis or of labelled glycogen were present in the liver after administration of [^{14}C]butyrate was next considered. To test this, two lambs that had received insulin were injected with [*carboxy*- ^{14}C]butyrate. After approx. 10 min. the lambs were shot in the head and immediately bled and portions of liver and other tissues quickly removed into liquid air. Approx. 4 min. elapsed from the time of killing until the last tissue was immersed in liquid air. The radioactivity in perchloric acid extracts of duplicate tissue samples was measured as described in the Experimental section.

Table 4 shows that the same order of radioactivity was found in all the tissues examined. The volatile fraction may represent largely unmetabolized butyrate or $^{14}\text{CO}_2$ or both. That an appreciable proportion of ^{14}C would appear in carbon dioxide after 10 min. is indicated by the results of Kleiber *et al.* (1954). Only a small proportion of the injected isotope was recovered in the liver. In one

Table 2. *Increase in blood glucose concentration after injection of glucose into sheep previously treated with insulin*

Sheep nos. 1 and 2 each received 2 units and sheep nos. 5 and 6 0.5 unit of insulin. After 90 min., glucose (0.125 m-mole/kg. body wt., as 0.5M solution) was infused into the jugular vein over 8–10 min., and a blood sample taken immediately after the infusion.

Sheep no.	Concn. of blood glucose (mg./100 ml.)			
	Before insulin	90 min. after insulin	Peak value after glucose	Increase due to glucose
1	52.0	17.1	35.6	18.5
2	54.7	20.0	32.4	12.4
5	55.4	25.7	39.6	13.9
6	63.8	22.8	31.9	9.1

Table 3. *Radioactivity in blood glucose of sheep after injection of ^{14}C -labelled fatty acids*

The sodium salts of the carboxyl-labelled fatty acids (0.25 m-mole/kg. body wt.; total radioactivity about 50 μC) were injected intravenously into sheep treated with insulin (0.5 unit/kg.) 90 min. previously.

Sheep no.	Fatty acid	Concn. of blood glucose ($\mu\text{moles}/100\text{ ml.}$)			^{14}C in blood glucose ($\mu\text{g.atoms}/100\text{ ml. of blood}$)	
		Initial	5 min.	10 min.	5 min.	10 min.
2	Butyrate	110	227	284	0.5	1.6
5	Propionate	128	156	164	1.5	1.7
6	Propionate	124	134	138	1.6	1.6

lamb the liver lipids contained a small amount of radioactivity (0.3 % of the injected dose) but in the other experiment no radioactivity could be detected in the liver lipids. The liver glycogen contained negligible radioactivity in both experiments. In view of the relatively large mass of skeletal muscle it would appear that much the largest amount of the isotope recovered was present in this tissue, if the two muscles assayed are representative.

In Table 5 the effect of a number of other compounds upon the blood sugar of insulin-treated sheep is shown. In two out of three sheep β -hydroxybutyrate gave little or no response; in the third the rise and fall of the concentration of blood glucose was rather erratic. Neither lactate nor succinate, both of which are well established as

potential carbohydrate precursors in the animal body, produced appreciable increases in blood glucose concentration.

In view of the increasing effect with increase in chain length of the acids (Table 1) the possibility was considered that the effect on blood glucose was due to the surface activity of the fatty acids. Sodium dodecyl sulphate was injected to test this; the amount used (10 μ moles/kg.) was sufficient to cause slight haemolysis. However, little or no effect upon the blood glucose concentration was observed.

Adrenaline hydrochloride (Parke, Davis and Co. Ltd., Hounslow, Middx.) at a dose of 25 μ g./kg. produced responses of the same order as was usually produced by 0.25 m-mole of butyrate/kg. The time-course of the response was also very similar.

Table 4. *Acid-soluble radioactivity in tissues of lambs after injection of [carboxy- 14 C]butyrate*

Sodium butyrate (0.25 m-mole/kg. body wt.) was injected intravenously into two lambs (14.5 and 21.0 kg. respectively) 90 min. after treatment with insulin (2 units/kg.). The lambs were killed 10 min. later. In the first experiment the blood glucose concentration increased by 66 μ moles/100 ml. after the injection of butyrate but only 0.16 μ g.atom of 14 C/100 ml. appeared in the blood glucose. In the second experiment the corresponding figures were 74 μ moles and 0.30 μ g.atom. Each figure in parentheses is the amount of 14 C (non-volatile or volatile) in the whole organ expressed as a percentage of the dose injected. The total weights of the skeletal muscles were not recorded.

14 C in tissue (μ g.atom/g.)									
Liver		Kidney		Heart		Semitendinosus muscle		Psoas muscle	
Non-volatile	Volatile	Non-volatile	Volatile	Non-volatile	Volatile	Non-volatile	Volatile	Non-volatile	Volatile
0.26 (1.8)	0.05 (0.4)	0.47 (1.0)	0.20 (0.4)	0.22 (0.8)	0.08 (0.3)	0.10	0.14	0.09	0.10
0.24 (1.7)	0.05 (0.3)	0.80 (2.1)	0.28 (0.4)	0.24 (0.7)	0.07 (0.2)	0.09	0.08	0.10	0.07

Table 5. *Effect of various compounds on blood glucose concentration in sheep previously treated with insulin*

All compounds were injected intravenously. Insulin: 0.5 or 1 unit/kg. body wt. The organic acids were administered as sodium salts.

Compound injected (amount/kg.)	Sheep no.	Concn. of blood glucose (mg./100 ml.)		
		Before insulin	90 min. after insulin	Peak value after compound
DL- β -Hydroxybutyrate (0.25 m-mole)	3	51.7	19.3	29.7
	5	52.3	22.0	23.8
	6	59.5	24.3	23.3
DL-Lactate (0.25 m-mole)	5	52.5	23.3	23.5
	6	57.4	19.3	19.8
Succinate (0.25 m-mole)	5	55.9	29.0	29.4
	6	69.0	26.7	26.7
Dodecyl sulphate (10 μ moles)	7	47.5	16.8	15.1
		64.8	31.7	34.6
	8	56.4	19.3	18.3
Adrenaline hydrochloride (5 μ g.)		77.2	30.7	28.7
	3	51.5	19.8	31.7
	4	50.5	19.3	30.7
Adrenaline hydrochloride (25 μ g.)	5	39.8	20.3	46.0
	6	60.9	20.8	37.6

Administration of adrenaline almost invariably caused the sheep to begin ruminating; this is probably due to the stimulation of receptors in the rumen wall (Kay, 1959). Rumination was not observed after injections of any of the fatty acids.

In a few experiments blood lactate was determined before and after the administration of fatty acids or adrenaline. Increases were obtained in all cases (Table 6). The last column shows the peak values, obtained about 10 min. after the injection.

The effects of butyrate and adrenaline upon the blood concentrations of glucose and lactate in sheep not given insulin are shown in Table 7. Peak values of both glucose and lactate were obtained 5-10 min. after the injections. Compared with the previous experiments adrenaline appeared to be relatively more effective than butyrate in influencing the glucose concentration. Butyrate had little or no effect on the lactate concentration.

It is conceivable that the fatty acids may increase the blood sugar concentration directly by causing an increase in glycogenolysis in the liver. To test this possibility liver slices were incubated in the presence and absence of butyrate and the amount of glucose appearing in the medium was determined. Rat-liver slices produce more glucose in a medium in which Na^+ ion rather than K^+ ion is the predominant univalent cation, probably by activation of phosphorylase by Na^+ ion (Cahill, Ashmore, Zottu & Hastings, 1957). Rabbit-liver

slices produce more glucose in the presence of adrenaline, which activates phosphorylase (Sutherland, 1950). Table 8 shows that both of these effects are reproducible in sheep-liver slices. However, the presence of a high concentration of butyrate did not significantly stimulate the release of glucose by liver slices from either sheep or rabbit.

DISCUSSION

A consideration of all the data presented above leaves little doubt that the increase in blood glucose produced by butyrate in sheep previously treated with insulin is not due to gluconeogenesis from butyrate and cannot therefore be considered as evidence that butyrate can provide carbohydrate for the animal. The fact that considerably larger increases in blood glucose concentration were obtained after the administration of butyrate (0.25 m-mole/kg.) than when glucose (0.125 m-mole/kg.) was given (Tables 1 and 2) implies that butyrate increases the blood glucose concentration by a mechanism other than conversion into glucose.

The results of the experiments with labelled butyrate show that little of the carboxyl carbon of the administered butyrate was incorporated into glucose. These results also make it appear unlikely that a substantial proportion of the butyrate is converted into glucose by a pathway involving loss of the carboxyl carbon atom (e.g. oxidation to acetoacetate, decarboxylation of the latter and gluconeogenesis from the acetone formed; cf. Sakami & Lafaye, 1951). Liver and kidney are the only organs known to be capable of gluconeogenesis and it seems probable from Table 4 that relatively little of the butyrate was metabolized in these tissues and that the greater part was metabolized in muscle.

In view of the fact that rather similar results were obtained with propionate (Table 3) it does not seem likely that the gluconeogenic potential of propionate, which is well established, is primarily responsible for its effect upon the blood glucose concentration. In fact the failure of lactate and succinate to increase blood glucose would suggest that the effect of a compound upon the concn

Table 6. *Effect of injected fatty acids and adrenaline on blood lactate concentration in sheep previously treated with insulin*

Compound injected (amount/kg.)	Sheep no.	Concn. of blood lactate (mg./100 ml.)	
		Before	After
Acetate (1 m-mole)	5	9.7	12.2
	6	7.7	9.2
Propionate (1 m-mole)	1	8.8	12.5
	2	7.5	16.5
Butyrate (0.25 m-mole)	5	7.4	15.8
	6	9.0	11.0
Adrenaline (5 $\mu\text{g.}$)	3	8.0	17.1
	4	12.6	23.6

Table 7. *Effect of butyrate and adrenaline on concentrations of glucose and lactate in sheep not given insulin*

Compound injected (amount/kg.)	Sheep no.	Concn. of glucose (mg./100 ml.)		Concn. of lactate (mg./100 ml.)	
		Before	After	Before	After
Adrenaline (25 $\mu\text{g.}$)	1	51.0	80.2	—	—
	3	61.0	107.6	3.8	22.1
	5	53.2	104.0	6.2	19.6
Butyrate (0.25 m-mole)	4	55.3	71.3	5.4	5.7
	6	64.1	73.3	5.4	6.2

Table 8. *Effects of butyrate and of other factors on glucose production by liver slices*

Liver slices (about 100 mg. wet wt.) were shaken for 1 hr. under O_2 at 38° in the media indicated (each in triplicate). Medium A: 0.4 ml. of 0.1M-potassium phosphate buffer (pH 7.4) + 1.6 ml. of 0.15M-NaCl. Medium B: 0.4 ml. of 0.1M-potassium phosphate buffer (pH 7.4) + 1.6 ml. of 0.15M-KCl. Butyrate, where added, replaced an equivalent amount of chloride. After incubation, the slices were removed, dried at 105° and weighed. The medium was treated with $Ba(OH)_2$ and $ZnSO_4$ and glucose determined on the filtrate.

Expt. no.	Species	Medium	Glucose released (mg./100 mg. dry wt. of tissue)
1	Sheep	A	6.4
		A + adrenaline (1 μ g./ml.)	9.3
		A + butyrate (2.5 mM)	6.2
		A + butyrate (10 mM)	6.5
		A + butyrate (50 mM)	5.9
2	Sheep	A	5.2
		B	3.3
		A + butyrate (10 mM)	5.2
		B + butyrate (10 mM)	3.5
		B + adrenaline (1 μ g./ml.)	5.3
3	Sheep	B	3.2
		B + butyrate (2.5 mM)	3.0
		B + butyrate (10 mM)	3.4
		B + adrenaline (1 μ g./ml.)	5.0
4	Rabbit	A	9.5
		A + adrenaline (1 μ g./ml.)	16.2
		A + butyrate (2.5 mM)	9.2
		A + butyrate (10 mM)	8.7

tration of blood glucose under these conditions bears no relationship to its potentiality as a carbohydrate precursor.

It would seem most likely therefore that the injection of butyrate in some way induces an increased release of glucose from the liver. An obvious possibility is that it stimulates the adrenal medulla to release adrenaline. The glycogenolytic activity of the latter is well known. In the present experiments with insulin-treated sheep 25 μ g. of adrenaline/kg. produced a response similar in time and magnitude to 0.25 m-mole of butyrate/kg. Pollak (1922) observed that subcutaneous injection of certain amino acids and short-chain fatty acids into rabbits caused increases in the blood sugar concentration, which he attributed to adrenaline release; however, propionate had a large effect, but butyrate had hardly any. Weil-Malherbe (1949) obtained some evidence that the action of glutamic acid in terminating hypoglycaemic coma was due to adrenaline release. On the other hand, Table 7 shows that butyrate, in contrast with adrenaline, failed to increase the blood lactate concentration in sheep not given insulin.

A direct glycogenolytic effect of butyrate upon the liver does not seem a likely explanation in view of the results obtained with liver slices (Table 8). Butyrate had no effect upon glucose release, under conditions in which the glycogenolytic action of adrenaline or Na^+ ions could be demonstrated.

A third possibility (Randle, Garland, Hales & Newsholme, 1963), that the short-chain fatty acids increase the blood glucose concentration by blocking the uptake of glucose by the tissues, was also considered. Dunn, Friedmann, Maass, Reichard & Weinhouse (1957) found that glucose was removed from the blood of dogs during insulin hypoglycaemia at a rate of 1.4 mg./100 ml. of blood/min. The rate of rise of blood glucose after administration of butyrate to sheep was much greater than this in most cases. At present therefore no satisfactory explanation for the effect of the short-chain fatty acids on the blood sugar concentration can be given.

SUMMARY

1. Intravenous administration of 0.25 m-mole of butyrate/kg. to insulin-treated sheep caused rapid increases in blood glucose concentration of up to about 40 mg./100 ml. Hexanoate was at least as effective and propionate less so, whereas acetate, succinate, lactate or 10 μ moles of sodium dodecyl sulphate/kg. had little or no effect.

2. Administration of glucose (0.125 m-mole/kg.) produced increases of less than 20 mg. of glucose/100 ml.

3. The fatty acids also increased blood lactate concentration under these conditions.

4. After the injection of carboxyl-labelled butyrate or propionate the blood glucose contained only a small proportion of the ^{14}C injected.

5. Carboxyl-labelled butyrate was injected into lambs, which were killed after approx. 10 min. Only about 2% of the administered ^{14}C was found in the acid-soluble fraction of the liver. The concentration of radioactivity in skeletal muscle, heart and kidney was of the same order as that in liver.

6. The release of glucose by sheep- or rabbit-liver slices was not significantly affected by the presence of butyrate in the medium, although adrenaline accelerated glucose release.

7. It is concluded that the rise in blood glucose produced by the fatty acids is not due to gluconeogenesis from the fatty acids. Possible mechanisms for the rise are discussed.

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The Role of Glucokinase in the Phosphorylation of Glucose by Rat Liver

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In view of the long-recognized role of the liver in the control of the blood sugar concentration in animals (Soskin, Essex, Herrick & Mann, 1938; Soskin & Levine, 1952; Cahill, Ashmore, Renold & Hastings, 1959) and the appreciation of the part played by glucose 6-phosphatase (D-glucose 6-phosphate phosphohydrolase, EC 3.1.3.9) in the production of glucose by the liver (for reviews, see Ashmore, 1959; Weber, 1959), the study of the nature of the enzymic system for the phosphorylation of glucose by the liver has started rather late. Various experimental procedures (see, for example, Slein, Cori & Cori, 1950; Long, 1952; Renold, Hastings, Nesbitt & Ashmore, 1955; Bekina & Petrova, 1957; Vester, 1959) gave glucose-phosphorylating activities too low to account for the observed rates of conversion of glucose into carbon dioxide and glycogen (Renold *et al.* 1955) by liver slices.

DiPietro & Weinhouse (1960) described a greatly improved system for the assay of glucose phosphorylation. Using this system and dialysed samples of preparations of the supernatant fraction

of liver homogenates, Walker (1962) demonstrated the presence in adult guinea-pig liver of two enzymes, both of which catalysed the phosphorylation of glucose to glucose 6-phosphate but with widely differing apparent affinities for glucose. Extending this work, Walker (1963*b*) showed by kinetic analysis that adult rat liver also contains two enzymes having apparent K_m values of about 10 mM and 37 μ M for glucose respectively. This has received confirmation in a preliminary report (Viñuela, Salas & Sols, 1963) of the appearance of the two enzymes in different ammonium sulphate fractions of a liver-cell supernatant preparation. These observations correlated many reports (Cahill, Hastings, Ashmore & Zottu, 1958*b*; Spiro, 1958*b*; DiPietro, Sharma & Weinhouse, 1962; Lange & Kohn, 1961) on the nature of the hepatic glucose-phosphorylating system. The two enzymes have been tentatively designated as a more-specific glucokinase (i.e. of type ATP-D-glucose 6-phosphotransferase, EC 2.7.1.2) and a non-specific hexokinase (i.e. of type ATP-D-hexose 6-phosphotransferase, EC 2.7.1.1) (Viñuela *et al.* 1963;