Effects of Carbohydrate Availability on Lipogenesis in Sheep

By F. J. BALLARD, O. H. FILSELL and I. G. JARRETT

*Division of Nutritional Biochemistry, Commonwealth Scientific and Industrial Research Organization,
Kintore Avenue, Adelaide, S. Austral. 5000, Australia*

(Received 2 August 1971)

1. Lipogenesis in sheep liver and adipose tissue was investigated by incorporation studies *in vitro* with radioactive glucose and acetate and by assays of key enzymes. 2. Carbohydrate availability to sheep was increased by feeding on a diet containing 70% soluble carbohydrate, by infusing glucose into the abomasum or by direct intravenous infusion of glucose. 3. Under these conditions lipogenesis from glucose and acetate was increased from very low values in liver and adipose tissue, especially in those animals where rumen fermentation was by-passed by glucose infusion. 4. Large increases in the activities of ATP citrate lyase (EC 4.1.3.8) and NADP-malate dehydrogenase (EC 1.1.1.40) occurred in both tissues when lipogenesis was increased. 5. No adaptations were found in the activities of pyruvate carboxylase (EC 6.4.1.1) in adipose tissue, glucokinase (EC 2.7.1.2) in liver or 3-hydroxybutyrate dehydrogenase (EC 1.1.1.30) in liver. It is proposed that the absence of these enzymes is not related to glucose availability. 6. The effect of glucose on liver lipogenesis was to increase conversion of acetate into lipid. 7. This effect also occurred in adipose tissue, but in this tissue glucose also became a quantitatively important precursor of triglyceride fatty acid.

In monogastric mammals given a high-carbohydrate diet, triglyceride is formed from glucose in both adipose tissue and liver. Although ruminants also eat large amounts of carbohydrate, this consists mainly of cellulose and hemicellulose, and these polymers are degraded in the rumen to yield acetate, propionate and butyrate (Elsden & Phillipson, 1948). Very little glucose is absorbed from the gut in sheep fed on a normal diet (Katz & Bergman, 1969).

One consequence of this difference between monogastric animals and ruminants is the need to produce glucose in the latter group. Glucose synthesized in the liver from propionate, amino acids, glycerol and lactate is probably used as an energy source for brain and some other tissues, but neither liver nor adipose tissue convert glucose into triglyceride for storage. The enzymes on this pathway of lipogenesis that are absent are glucokinase (Ballard & Oliver, 1964*b*), ATP citrate lyase and NADP-malate dehydrogenase in liver (Hardwick, 1966; Hanson & Ballard, 1967), and ATP citrate lyase, NADP-malate dehydrogenase and pyruvate carboxylase in adipose tissue (Hanson & Ballard, 1967).

In the present work we have examined whether lipogenesis is adaptable in sheep liver and adipose tissue and have followed with changing glucose availability the activity of those enzymes that catalyse reactions on the pathway of glucose to fatty acids. The results show marked adaptations in both tissues, especially if glucose is infused directly into the blood.

Materials and Methods

Chemicals

Lactate dehydrogenase, pyruvate kinase, malate dehydrogenase, acetate kinase, ATP, phosphoenolpyruvate, NADP⁺, NAD⁺ and NADH were purchased from C. F. Boehringer und Soehne, Mannheim, Germany. NaH¹⁴C₃, [1-¹⁴C]acetate and [U-¹⁴C]glucose were from The Radiochemical Centre, Amersham, Bucks., U.K.; acetyl-CoA, CoA, glucose oxidase and peroxidase were from Sigma Chemical Co., St Louis, Mo., U.S.A., and insulin was from Novo Industries, Copenhagen, Denmark.

Animals

Rats. The rats used were of either sex, weighed between 190 and 230g, and were given a high-carbohydrate diet containing less than 5% fat.

Control sheep. Six merino wethers were killed at between 4 and 6 months old. These animals were raised by their mothers with unrestricted access to pasture and weighed 23–24kg at the time of slaughter.

Carbohydrate-fed sheep. These animals were artificially reared from birth and were killed when 4–6 months old. During the last 4–5 weeks before slaughter the lambs were fed on a pelleted diet consisting of 1 part of crushed wheat and 1 part of a

mixture containing glucose (20g), casein (20g), corn starch (50g), skim-milk powder (10g), a salt mixture (2g) (Schwarz, 1951) and 1g of a vitamin mixture (Vidaylin; Abbott Laboratories, Kurnell, N.S.W., Australia). The energy value of this diet was 4kcal/g with 18% protein and 70% carbohydrate. By the gradual introduction of this solid diet and decrease of milk intake, a satisfactory growth rate was maintained with only occasional diarrhoea. During the last 4 weeks on this pelleted diet the mean daily intake was approx. 500g/day.

Sheep with abomasal glucose infusions. Abomasal fistulae were inserted in three wethers aged 5 months (Jarrett, 1948). These animals received a gradually increasing glucose infusion for 7 weeks, from 50g/day over the first 4 weeks to 170g/day over the final 2 weeks. Voluntary food intake of a mixture containing 3 parts of wheaten hay chaff and 1 part of lucerne chaff averaged 750g/day throughout the whole period.

Sheep with intravenous glucose infusions. Cannulae were inserted into the jugular veins of four wethers aged 6 months and a sterile solution of glucose was infused for 3 weeks. The infusion rates were 80g/day for one week, 120g/day for the second week and 130g/day for the final week. The food intake remained at 1000g of wheaten hay chaff-lucerne hay chaff (3:1, w/w) throughout the experimental period.

Methods

Blood sampling. Blood was taken from all animals at regular intervals for the measurement of glucose concentrations. Although no marked differences in glucose concentration were evident, intravenous glucose-tolerance tests on the carbohydrate-fed sheep showed a more rapid removal of glucose. The mean time taken for the glucose concentration to fall to half the peak value (Reid, 1958) in four control animals was 100min, in four sheep on the pelleted diet it was 40min and in two animals with abomasal glucose infusion it was 50min. At the end of the experimental period the animals were lightly anaesthetized with pentobarbitone and blood samples obtained from the carotid artery and the portal vein. A portion of liver from the parietal lobe and some omental fat were taken for analysis.

Measurements of acetate and glucose in blood. Blood (3ml) was added to 5ml of 6% (w/v) HClO_4 at 0°C. The precipitated protein was removed by centrifugation and re-extracted with 2ml of 6% (w/v) HClO_4 . The combined extracts were neutralized with 3M-KOH and the precipitated KClO_4 was removed by centrifuging at 0°C. Acetate was measured in this extract by the amount of ADP produced in the presence of acetate kinase. Spectrophotometer cuvettes contained 100 μmol of tris-HCl buffer, pH 7.8, 10 μmol of ATP (adjusted to pH 7 with KOH),

20 μmol of MgCl_2 , 2.5 μmol of phosphoenolpyruvate, 0.5 μmol of NADH, 20 μg of lactate dehydrogenase and 20 μg of pyruvate kinase in a volume of 0.8ml. After incubation at 37°C to remove any ADP or pyruvate contaminants, acetate was measured as the increment in NADH oxidation on addition of 25 μg of acetate kinase.

Glucose was measured in whole blood by an automated method with glucose oxidase (Cramp, 1967).

Preparation of tissue extracts. Livers were homogenized in 9vol. of 0.25M-sucrose at 0°C. A portion of this homogenate was freeze-dried and suspended in twice the original volume of water. This was used for the assay of 3-hydroxybutyrate dehydrogenase. The remaining homogenate was centrifuged at 100000g for 30min at 2°C and the supernatant fraction was used for assays of glucokinase, hexokinase, ATP citrate lyase and NADP-malate dehydrogenase.

Sheep adipose tissue congeals irreversibly when the temperature is lowered below 30°C. To avoid this and subsequent difficulty in homogenization or tissue incubation, adipose tissue was rapidly removed from anaesthetized animals and placed in 0.15M-NaCl at 37–42°C. Tissue was homogenized in 3vol. of 0.25M-sucrose (w/v) at 37°C and a portion freeze-dried. This sample was suspended in twice the original volume of a solution containing 50mM-tris-HCl buffer, pH 7.5, 0.5mM-EDTA, 5mM-ATP and 5mM- MgSO_4 at pH 7.2. After this extract had been centrifuged at 3000g for 5min to remove the fat, the lower layers were mixed and used for the assay of pyruvate carboxylase. The remaining tissue homogenate was centrifuged at 100000g for 30min and the infranatant used for the measurement of ATP citrate lyase and NADP-malate dehydrogenase activities.

Enzyme assays. ATP citrate lyase was measured by a minor modification (Ballard & Hanson, 1967a) of a method described by Srere (1962). NADP-malate dehydrogenase was assayed by the method of Ochoa (1955). Glucokinase and hexokinase activities were determined as described by Ballard & Oliver (1964a) but with 1mM-dithiothreitol added to the spectrophotometer cuvettes. The extinction change at 340nm in a cuvette containing 50mM-glucose but no ATP was used as a blank for glucokinase and a cuvette containing 0.5mM-glucose but no ATP was used as a blank for hexokinase. In addition, the corrected hexokinase activity was subtracted from the apparent glucokinase activity as both enzymes are measured at 50mM-glucose. Pyruvate carboxylase was assayed as described previously (Ballard & Hanson, 1967b). 3-Hydroxybutyrate dehydrogenase was measured as described by Lehninger *et al.* (1960). Test cuvettes contained 50mM-tris-HCl buffer, pH 8.5, 2.5mM-dithiothreitol, 2mM- NAD^+ , 50mM-nicotinamide, extract and 20mM-DL-3-hydroxybutyrate. Blank values with 3-hydroxybutyrate omitted from

the reaction mixture were subtracted from the observed extinction change at 340 nm.

All enzyme assays were carried out at 37°C and activities are expressed as μmol of substrate converted or product formed/min per g of tissue. Negligible enzyme activities may be caused by a decrease during extraction rather than by a truly low activity *in situ*. We have attempted to overcome this problem by using rat tissues as a standard. At the same time that each sheep was killed, samples of liver and adipose tissue from a rat were homogenized and enzymes extracted and measured in an identical manner.

Measurement of lipogenesis. Liver slices were prepared as described by Ballard & Oliver (1964a), rinsed in 0.15M-NaCl and weighed, and approx. 300mg was transferred to 25ml flasks containing 4ml of the appropriate incubation medium. This procedure was carried out in the cold with solutions at 2°C. Portions of adipose tissue (200–400mg and 2–4mm thick) were weighed and placed in similar incubation vessels containing medium at 37°C. The flasks were gassed with $\text{O}_2 + \text{CO}_2$ (95:5) and incubated with shaking for 2h at 37°C. The media used for these studies consisted of Krebs–Ringer bicarbonate, pH 7.4 (Umbreit *et al.*, 1959) containing 50mM- $[\text{U-}^{14}\text{C}]$ glucose (2.5 μCi) plus 0.1 unit of insulin, 50mM-sodium $[\text{I-}^{14}\text{C}]$ acetate (2.5 μCi), or 50mM-sodium $[\text{I-}^{14}\text{C}]$ acetate (2.5 μCi) plus 10mM-glucose with 0.1 unit of insulin. Flasks with radioactive glucose were fitted with a plastic bucket (Kontes Glass Co., Vineland, N.J., U.S.A.) containing folded filter paper (2cm $\times$ 3cm). At the end of the incubation period 0.1ml of 6M-KOH was injected on to the filter paper and CO_2 released by injection of 0.5ml of 0.5M- H_2SO_4 to the incubation medium. The flasks were shaken for an additional 30min, after which the filter papers were removed and air dried, and the radioactivity was determined by liquid-scintillation spectrometry (Buhler, 1962). With acetate as substrate the incubation was terminated with 0.5ml of 0.5M- H_2SO_4 . Liver slices or pieces of adipose tissue were removed, rinsed in 0.15M-NaCl and the lipid was extracted into chloroform–methanol (2:1, v/v). Radioactivity in fatty acid and glycerol fractions of

triglyceride was determined as described previously (Ballard & Hanson, 1967a).

Statistical analysis. Differences between means were analysed by Student's 't' test or by the Wilcoxon Rank sum test (Wilcoxon, 1945).

Results

Blood acetate and glucose concentrations

Acetate concentrations in the portal veins of all groups of sheep were approx. 1.2mM (Table 1). This represents an increase over arterial blood of 0.43mM in control sheep, 0.61mM in carbohydrate-fed sheep and 0.73mM in animals receiving glucose intravenously.

When compared with control animals the concentration of glucose in portal blood was unchanged in sheep with abomasal glucose infusion, increased in carbohydrate-fed animals and decreased in animals with intravenous glucose infusion (Table 2). These portal-vein concentrations were lower than found in arterial blood of control sheep or animals with intravenous glucose infusion. In carbohydrate-fed sheep the arterial–portal glucose concentration difference was not significant, whereas the glucose concentration in the portal vein of sheep with abomasal glucose infusion was 0.25mM greater than in arterial blood.

Lipogenesis from glucose

In liver slices from all groups of sheep the incorporation of glucose into CO_2 , glyceride fatty acid or glyceride glycerol is lower than found in rat liver slices (Table 3). The rate of incorporation of glucose into glyceride fatty acids increases from 0.17 μg -atom of carbon/g of tissue in control animals to 0.31 μg -atom/g of tissue in carbohydrate-fed sheep and 0.44 μg -atom/g of tissue in liver slices from sheep with intravenous infusions of glucose. There were also increases in the incorporation of glucose into the glycerol moiety of triglyceride in slices from carbohydrate-fed sheep and sheep with abomasal glucose infusions.

Table 1. *Acetate concentration in sheep blood*

Acetate was measured in blood obtained from the portal vein and carotid artery of anaesthetized sheep. Acetate concentrations are expressed as $\mu\text{mol/g}$ of blood. Values are the means $\pm$ S.E.M. with the numbers of animals in parentheses. Values that are statistically different ($P < 0.05$) from the controls are indicated by an asterisk.

Animal treatment	Arterial blood	Portal-vein blood	Difference (portal vein–artery)
Controls (6)	0.68 $\pm$ 0.05	1.11 $\pm$ 0.08	0.43 $\pm$ 0.07
Carbohydrate-fed (9)	0.64 $\pm$ 0.11	1.28 $\pm$ 0.23	0.61 $\pm$ 0.11
Intravenous glucose infusion (4)	0.43* $\pm$ 0.09	1.16 $\pm$ 0.23	0.73* $\pm$ 0.14

Table 2. *Glucose concentrations in sheep blood*

Glucose was measured in blood samples taken from anaesthetized sheep and is expressed as $\mu\text{mol/g}$ of blood. Values are the means $\pm$ S.E.M. with the numbers of animals in parentheses. Values that are statistically different from the controls ($P < 0.05$) are indicated by an asterisk.

Animal treatment	Arterial blood	Portal vein blood	Difference (portal vein—artery)
Controls (6)	3.66 $\pm$ 0.22	3.48 $\pm$ 0.21	-0.18 $\pm$ 0.05
Carbohydrate-fed (9)	4.21* $\pm$ 0.16	4.19* $\pm$ 0.16	-0.03* $\pm$ 0.04
Abomasal glucose infusion (3)	3.17 $\pm$ 0.20	3.43 $\pm$ 0.23	+0.25* $\pm$ 0.06
Intravenous glucose infusion (4)	2.75* $\pm$ 0.22	2.45* $\pm$ 0.15	-0.29 $\pm$ 0.20

Table 3. *Glucose incorporation into CO₂, glyceride fatty acid and glyceride glycerol in liver slices*

Incorporation of [U-¹⁴C]glucose is expressed as μg -atoms of carbon/g of tissue per 2h incubation at 37°C. Values are means $\pm$ S.E.M. with the numbers of animals in parentheses. With sheep liver slices values that are significantly different from controls ($P < 0.05$) are indicated by asterisks.

Animals	Treatment	Glucose incorporated into radioactive product		
		CO ₂	Fatty acid	Glycerol
Sheep	Controls (6)	8.2 $\pm$ 1.0	0.17 $\pm$ 0.04	2.1 $\pm$ 0.2
	Carbohydrate-fed (10)	8.2 $\pm$ 0.7	0.31* $\pm$ 0.03	3.6* $\pm$ 0.5
	Abomasal glucose infusion (3)	11.6* $\pm$ 0.6	0.32 $\pm$ 0.11	3.6* $\pm$ 0.2
	Intravenous glucose infusion (4)	9.2 $\pm$ 1.6	0.44* $\pm$ 0.05	3.0 $\pm$ 0.4
Rats (8)		14.7 $\pm$ 1.5	1.91 $\pm$ 0.51	6.8 $\pm$ 0.7

Adipose tissue from control sheep shows a very low rate of incorporation of glucose into all products measured (Table 4). Of the ten sheep fed on the high-carbohydrate diet, four (group A) showed no adaptation, but in six animals (group B) there was a tenfold increase in glucose incorporation into fatty acid and a lesser increase into CO₂ and glyceride glycerol. Fatty acid labelling from glucose was markedly increased in adipose tissue from sheep with glucose infusions, from 0.24 μg -atom of carbon/2h per g of tissue in control animals to 4.44 and 8.10 respectively in tissue from sheep with abomasal or intravenous glucose infusions.

Lipogenesis from acetate

All carbohydrate treatments increased hepatic lipogenesis from acetate (Table 5). The greatest effect was found in liver slices from sheep with intravenous glucose infusions. In these animals the incorporation of acetate into glyceride fatty acids was 10.8 μg -atoms of carbon/2h per liver, compared with a value of 1.18 in slices from control sheep. This increased rate of lipogenesis was fourfold greater than found in rat liver slices. The addition of 10mm-glucose plus 0.1 unit of insulin did not affect acetate incorporation in either sheep or rat liver slices.

In sheep adipose tissue the addition of glucose and

insulin to the incubation medium produces a 4–13-fold increase in lipogenesis, compared with a fivefold effect in rat adipose tissue (Table 6). The smallest effect was in tissue from control animals. The rate of lipogenesis in the presence of glucose and insulin was increased from 3.5 μg -atoms of carbon incorporated/2h per g of tissue to about 14 in carbohydrate-fed sheep and to 35.8 and 41.6 respectively in sheep with abomasal and intravenous infusions of glucose. The carbohydrate-fed animals are separated into the two groups defined in Table 4. With acetate as lipogenic precursor, however, these groups show no statistical differences from each other.

Enzyme measurements

The activities of ATP citrate lyase, NADP-malate dehydrogenase, glucokinase and 3-hydroxybutyrate dehydrogenase in liver from control sheep were 4, <2, 2 and 2% respectively of the values in rat liver (Table 7). Of these enzymes only ATP citrate lyase and NADP-malate dehydrogenase increased in activity in sheep either fed on or infused with carbohydrate. There was considerable variability in these enzyme assays, probably caused by the low activities in sheep liver. Hexokinase was present in sheep liver at about 20–25% of the activity in the rat, but was

Table 4. *Glucose incorporation into CO₂, glyceride fatty acid and glyceride glycerol in isolated adipose tissue*

Incorporation of [U-¹⁴C]glucose is expressed as $\mu\text{g-atoms}$ of carbon/g of tissue per 2h incubation at 37°C. Values are means $\pm$ S.E.M. with the numbers of animals in parentheses. The grouping of carbohydrate-fed animals is explained in the text. Values with sheep adipose tissue that are significantly different from controls are indicated by * ($P < 0.05$) and ** ($P < 0.01$).

Animals	Treatment	Glucose incorporated into radioactive product		
		CO ₂	Fatty acid	Glycerol
Sheep	Controls (6)	0.9 $\pm$ 0.2	0.24 $\pm$ 0.06	1.9 $\pm$ 0.2
	Carbohydrate-fed (A) (4)	0.8 $\pm$ 0.3	0.37 $\pm$ 0.07	2.5 $\pm$ 0.5
	Carbohydrate-fed (B) (6)	2.4** $\pm$ 0.2	2.42** $\pm$ 0.54	2.7* $\pm$ 0.2
	Abomasal glucose infusion (3)	4.8** $\pm$ 0.7	4.44** $\pm$ 1.02	2.3 $\pm$ 0.2
	Intravenous glucose infusion (4)	5.6** $\pm$ 0.7	8.10** $\pm$ 1.26	2.7* $\pm$ 0.3
Rats (8)		18.4 $\pm$ 2.6	44.2 $\pm$ 5.8	7.6 $\pm$ 0.8

Table 5. *Acetate incorporation into glyceride fatty acids in liver slices*

Incorporation of [1-¹⁴C]acetate is expressed as $\mu\text{g-atoms}$ of carbon/g of tissue per 2h incubation at 37°C. Values are the means $\pm$ S.E.M. with the numbers of animals in parentheses. Values with sheep liver that are statistically different from controls are indicated by * ($P < 0.05$) and ** ($P < 0.01$).

Animals	Treatment	Additions ...	Acetate incorporated	
			None	Glucose, insulin
Sheep	Controls (6)		1.18 $\pm$ 0.22	1.42 $\pm$ 0.24
	Carbohydrate-fed (10)		4.52** $\pm$ 0.46	4.32** $\pm$ 0.62
	Abomasal glucose infusion (3)		4.56** $\pm$ 0.40	3.26* $\pm$ 1.04
	Intravenous glucose infusion (4)		10.80** $\pm$ 0.76	9.24** $\pm$ 1.34
Rats (8)			2.30 $\pm$ 0.48	2.78 $\pm$ 0.30

not altered under any of the experimental conditions.

Of the three enzyme activities measured in adipose tissue, ATP citrate lyase showed the greatest adaptation (Table 8). This enzyme activity was not increased above control activities in the group of carbohydrate-fed animals (group A) that showed no increase in lipogenesis from glucose (Table 4), but in group B there was a large increase, from 0.005 to 0.066 unit/g of tissue. With intravenous infusion of glucose the ATP citrate lyase activity increased to 0.220 unit/g, a value comparable with that found in rat adipose tissue (0.280 unit/g). NADP-malate dehydrogenase activity was increased in adipose tissue of all groups except those with abomasal glucose infusion, where one animal had an activity similar to the controls. Although NADP-malate dehydrogenase activity in sheep with intravenous glucose infusion represented a ninefold increase over control values, this was still less than 10% of the enzyme activity in rat adipose tissue. Pyruvate carboxylase activity was low and variable in adipose tissue from all groups of sheep. There were no statistically signi-

ficant increases in activity with carbohydrate feeding or infusion.

Discussion

Normal sheep

The four groups of sheep in this study were selected because of differences in carbohydrate availability to tissues. Normal animals obtain very little, if any, glucose from the diet, since carbohydrate is converted into volatile fatty acids by rumen micro-organisms (Elsden & Phillipson, 1948). The higher concentration of acetate and lower concentration of glucose in the portal blood of these animals compared with the arterial blood is consistent with this rumen fermentation, and contrasts with the situation in monogastric animals. The low rates of hepatic or adipose-tissue lipogenesis from glucose emphasize the minimal importance of this pathway to the ruminant animal. As reported previously (Ballard *et al.*, 1969) the citrate-cleavage pathway probably limits fatty acid synthesis from glucose in both tissues. This limitation

Table 6. *Acetate incorporation into glyceride fatty acids in adipose tissue*

Incorporation of [$1\text{-}^{14}\text{C}$]acetate is expressed as μg -atoms of carbon/g of tissue per 2h incubation at 37°C . The grouping of carbohydrate-fed animals is explained in the text. Values are the means $\pm$ S.E.M. with the numbers of animals in parentheses. Values with sheep adipose tissue that are statistically different from controls are indicated by * ($P < 0.01$).

Animals	Treatment	Additions ...	Acetate incorporated	
			None	Glucose, insulin
Sheep	Controls (6)		0.92 $\pm$ 0.32	3.5 $\pm$ 1.5
	Carbohydrate-fed (A) (4)		1.02 $\pm$ 0.40	13.2* $\pm$ 3.3
	Carbohydrate-fed (B) (6)		2.18 $\pm$ 0.68	15.0* $\pm$ 3.5
	Abomasal glucose infusion (3)		6.08* $\pm$ 1.00	35.8* $\pm$ 10.0
	Intravenous glucose infusion (4)		4.88* $\pm$ 1.22	41.6* $\pm$ 1.6
Rats (8)			3.16 $\pm$ 0.74	16.3 $\pm$ 1.1

Table 7. *Enzyme activities in liver*

All enzyme activities are expressed as μmol of substrate converted or product formed/min per g of tissue at 37°C . Values are the means $\pm$ S.E.M. for the numbers of animals indicated in parentheses. Values with sheep liver that are statistically different from controls are indicated by * ($P < 0.05$) and ** ($P < 0.01$).

Animals	Treatment	ATP citrate	NADP-malate	Hexokinase	Glucokinase	3-Hydroxy-
		lyase	dehydrogenase			butyrate
Sheep	Controls (6)	0.062 $\pm$ 0.019	0.18 $\pm$ 0.12	0.33 $\pm$ 0.05	<0.1	0.60 $\pm$ 0.11
	Carbohydrate-fed (10)	0.149** $\pm$ 0.04	0.49 $\pm$ 0.09	0.27 $\pm$ 0.04	<0.1	0.57 $\pm$ 0.08
	Abomasal glucose infusion (3)	0.056 $\pm$ 0.015	0.32 $\pm$ 0.16	0.29 $\pm$ 0.04	<0.1	0.68 $\pm$ 0.22
	Intravenous glucose infusion (4)	0.150* $\pm$ 0.037	1.09* $\pm$ 0.46	0.26 $\pm$ 0.04	<0.1	0.92 $\pm$ 0.20
Rats (8)		1.64 $\pm$ 0.25	6.20 $\pm$ 0.24	1.18 $\pm$ 0.24	4.76 $\pm$ 0.67	33.6 $\pm$ 2.8

is expressed through the very low activities of ATP citrate lyase and NADP-malate dehydrogenase in liver, and the additional effects of negligible pyruvate carboxylase activities in adipose tissue.

It is unlikely that the low activities of hexokinase and glucokinase in sheep liver regulate fatty acid synthesis from glucose, because this precursor is oxidized to CO_2 and converted into glyceride glycerol at substantial rates. However, these rates are lower than in rat liver slices and probably reflect the physiological role of sheep liver to produce glucose in both the fed and the starved states (Bergman *et al.*, 1970).

Liver and adipose tissue from young sheep can convert acetate into fatty acid at appreciable rates, suggesting that fatty acid synthesis is not limited by the sequence acetyl-CoA to triglyceride (Table 9). Under all manipulations acetate is a better precursor for fatty acid synthesis than glucose. This is not the case for fatty acid synthesis in rat adipose tissue.

Adaptations to increased glucose availability

Several authors have measured the activities of NADP-malate dehydrogenase and ATP citrate lyase in tissues from cattle fed on high-carbohydrate diets (Baldwin & Ronning, 1966; Baldwin *et al.*, 1966; Young *et al.*, 1969). These experiments show little if any adaptation to the diets in either liver or adipose tissue and both enzyme activities remain very low compared with rat tissues.

In the present study we found it surprising that of the ten animals fed on a high-carbohydrate diet, four showed no changes in conversion of glucose into fatty acid or in ATP citrate lyase activities in adipose tissue, whereas both parameters were increased ten-fold in the other six animals. This difference could not be related to weight gain, food intake, differences in the volatile fatty acid content of rumen fluid or the glucose tolerance of the animals. Further, there were no differences in acetate incorporation, NADP-

Table 8. *Enzyme activities in adipose tissue*

All enzymes are expressed as μmol of substrate used or product formed/min per g of adipose tissue at 37°C . Values are the means $\pm$ S.E.M. for the numbers of animals indicated in parentheses. The grouping of carbohydrate-fed animals is explained in the text. Values that are statistically different from controls are indicated by $*$ ($P < 0.05$) and $**$ ($P < 0.01$).

Animals	Treatment	ATP citrate lyase	NADP-malate dehydrogenase	Pyruvate carboxylase
Sheep	Controls	0.005 $\pm$ 0.003 (6)	0.032 $\pm$ 0.019 (6)	0.009 $\pm$ 0.006 (4)
	Carbohydrate-fed (A)	0.006 $\pm$ 0.004 (4)	0.104* $\pm$ 0.030 (4)	0.025 $\pm$ 0.012 (4)
	Carbohydrate-fed (B)	0.066** $\pm$ 0.025 (6)	0.128* $\pm$ 0.022 (6)	0.028 $\pm$ 0.010 (4)
	Abomasal glucose infusion	0.037* $\pm$ 0.015 (3)	0.106 $\pm$ 0.058 (3)	0.041 $\pm$ 0.026 (3)
	Intravenous glucose infusion	0.220** $\pm$ 0.026 (4)	0.280** $\pm$ 0.038 (4)	0.012 $\pm$ 0.005 (4)
Rats		0.280 $\pm$ 0.065 (8)	3.03 $\pm$ 0.74 (8)	0.80 $\pm$ 0.14 (8)

Table 9. *Relative rates of conversion of acetate and glucose into fatty acid*

Values are the ratios of acetate incorporation into fatty acid in the presence of glucose and insulin to glucose incorporation into fatty acid, and are calculated from means in Tables 3–6.

Animals	Treatment	Liver	Adipose tissue
Sheep	Control	8.4	14.6
	Carbohydrate-fed (A)	13.9	35.7
	Carbohydrate-fed (B)	—	6.2
	Abomasal glucose infusion	10.2	8.1
	Intravenous glucose infusion	21.0	5.1
Rats		1.5	0.4

malate dehydrogenase or pyruvate carboxylase in adipose tissue, no differences in any liver parameters, and no indication of differences in net glucose uptake by the portal bed.

The infusion studies were aimed at supplying the young sheep with at least the amount of glucose that they normally synthesize via gluconeogenesis. Results collected by Ballard *et al.* (1969) indicate an average glucose entry rate of 80 g/day per 30 kg sheep. The finding that blood glucose concentrations did not increase in sheep given 170 g of glucose/day via abomasal fistula or 130 g of glucose/day intravenously can only mean that glucose utilization has increased. This would be so even if gluconeogenesis was completely suppressed. The radioisotope-incorporation studies with isolated adipose tissue showed an 18-fold increase in fatty acid synthesis from glucose in sheep with abomasal infusions of glucose and a 34-fold increase when glucose was infused intravenously. The effects in liver were small, and lipogenesis from acetate was increased. It appears that glucose infusion caused a different effect in liver from that in adipose tissue. In liver, the primary effect was to stimulate acetate metabolism. Although this certainly occurred in adipose tissue, the ability to utilize glucose was increased relative to lipogenesis from acetate (Table 9). This conclusion is also

consistent with the enzyme-activity measurements. The enzymes of the citrate-cleavage pathway show much greater adaptations in adipose tissue than in liver.

Ballard & Hanson (1967b) have proposed that pyruvate carboxylase is an essential component of the citrate-cleavage pathway in adipose tissue, since any pyruvate formed from the cleavage of citrate via ATP citrate lyase, NAD-malate dehydrogenase and NADP-malate dehydrogenase must be converted into oxaloacetate for subsequent condensation with acetyl-CoA within the mitochondria. However, NADP-malate dehydrogenase and ATP citrate lyase are adaptable in sheep adipose tissue whereas the pyruvate carboxylase activity remains at extremely low values with all dietary treatments. It is possible that this low activity is sufficient to account for the flux through the citrate-cleavage pathway. Alternatively, NADP-malate dehydrogenase, instead of converting malate into pyruvate, may catalyse the carboxylation of pyruvate to malate and this malate may be the source of mitochondrial oxaloacetate.

In this project on adaptation caused by increasing the availability of carbohydrate, we wished to obtain evidence showing whether the lower activities of several enzymes in sheep were caused by diet or by other factors. Certainly ATP citrate lyase and NADP-

malate dehydrogenase adapt to the dietary stimulus. These two enzymes are also present in foetal ruminant liver at activities many times greater than in normal adults (Hanson & Ballard, 1968; Muramatsu *et al.*, 1970) and thus change with age towards the particular metabolic pattern of mature ruminants. Glucokinase and 3-hydroxybutyrate dehydrogenase in liver and pyruvate carboxylase in adipose tissue have insignificant activities under all conditions tested. The absence of these enzymes may be part of an intrinsic pattern in ruminant tissues.

We thank Mr. T. L. J. Mann and Mr. H. B. Wright for maintenance of the animals, Mr. G. H. Pfitzner, Mr. S. E. Knowles and Miss J. M. Robinson for technical assistance and Mr. W. B. Hall for advice on statistical procedures.

References

- Baldwin, R. L. & Ronning, M. (1966) *J. Dairy Sci.* **49**, 688
- Baldwin, R. L., Ronning, M., Radanovics, C. & Plange, G. (1966) *J. Nutr.* **90**, 47
- Ballard, F. J. & Hanson, R. W. (1967a) *Biochem. J.* **102**, 952
- Ballard, F. J. & Hanson, R. W. (1967b) *J. Lipid Res.* **8**, 73
- Ballard, F. J. & Oliver, I. T. (1964a) *Biochem. J.* **90**, 261
- Ballard, F. J. & Oliver, I. T. (1964b) *Biochem. J.* **92**, 131
- Ballard, F. J., Hanson, R. W. & Kronfeld, D. S. (1969) *Fed. Proc. Fed. Amer. Soc. Exp. Biol.* **28**, 218
- Bergman, E. N., Katz, M. L. & Kaufman, C. F. (1970) *Amer. J. Physiol.* **219**, 785
- Buhler, D. R. (1962) *Anal. Biochem.* **4**, 413
- Cramp, D. G. (1967) *J. Clin. Pathol.* **20**, 910
- Elsden, S. R. & Phillipson, A. T. (1948) *Annu. Rev. Biochem.* **17**, 705
- Hanson, R. W. & Ballard, F. J. (1967) *Biochem. J.* **105**, 529
- Hanson, R. W. & Ballard, F. J. (1968) *Biochem. J.* **108**, 705
- Hardwick, D. C. (1966) *Biochem. J.* **99**, 228
- Jarrett, I. G. (1948) *J. Counc. Sci. Ind. Res.* **21**, 312
- Katz, M. L. & Bergman, E. N. (1969) *Amer. J. Physiol.* **216**, 953
- Lehninger, A. L., Sudduth, H. C. & Wise, J. B. (1960) *J. Biol. Chem.* **235**, 2450
- Muramatsu, M., Ambo, K. & Tsuda, T. (1970) *J. Biochem. (Tokyo)* **67**, 727
- Ochoa, S. (1955) *Methods Enzymol.* **1**, 699
- Reid, R. L. (1958) *Aust. J. Agr. Res.* **9**, 788
- Schwarz, K. (1951) *Proc. Soc. Exp. Biol. Med.* **77**, 818
- Srere, P. A. (1962) *Methods Enzymol.* **5**, 641
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1959) *Manometric Techniques*, p. 149, Burgess Publishing Co., Minneapolis
- Wilcoxon, F. (1945) *Biometrics* **1**, 80
- Young, J. W., Thorp, S. L. & de Lumen, H. Z. (1969) *Biochem. J.* **114**, 83