The effects of feeding and acute cold exposure on the visceral release of volatile fatty acids, estimated hepatic uptake of propionate and release of glucose, and plasma insulin concentration in sheep

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1. Five sheep were given a meal while they were in a neutral environmental temperature (15–20°) and while acutely exposed to a moderately cold (1°, wind speed 2 m/s) environment.

2. Before and at various times after feeding measurements were made of hepatic portal blood flow and the concentration of volatile fatty acids (VFA) and glucose in arterial, hepatic portal and hepatic venous blood plasma. From these measurements the net rate of release of VFA from the viscera was calculated, and the uptake of propionate and output of glucose by the liver was estimated, assuming hepatic arterial blood flow to be 20% of portal flow. The concentration of insulin in arterial and portal venous plasma was also measured.

3. The change in environmental temperature did not affect the time taken by the animals to eat the meal completely.

4. After feeding, in the neutral environment, there were significant increases in portal blood flow and release of VFA into the portal bloodstream. The uptake of propionate by the liver increased, significantly, and output of glucose also increased, but not significantly. Plasma insulin concentration also increased after feeding.

5. During cold exposure portal blood flow was consistently higher, before and after feeding, than it was in the neutral environment. The release of VFA into the portal blood was also consistently greater during cold exposure, especially the release of propionate after feeding. Associated with this was an extra uptake of propionate and output of glucose by the liver. Plasma insulin concentration was slightly higher in the cold environment than the neutral environment before the animals were fed, but this difference was not apparent at any other time.

Volatile fatty acids (VFA) are major products of digestion and important substrates for oxidative metabolism in ruminant animals. In a study of substrate uptake by the shivering hind-leg in steers it was found that in animals acutely exposed to a cold environment there was increased uptake of VFA. This increased uptake was related to higher arterial VFA concentrations in the cold environment than in a neutral environment (Bell, Gardner & Thompson, 1974), suggesting an increased supply of these substrates to the peripheral tissues during cold exposure even though the animals were fed the same amount in the two environments. This possibility received support when it was found that, in fed sheep acutely exposed to a cold environment, more VFA were released from the viscera into the portal vein than normal and more VFA were taken up by the liver (Thompson, Gardner & Bell, 1975). However, this comparison between environments was limited to a single sampling time, 6 h after feeding, so the apparent difference could have been due to differences in the rates of digestion and absorption of VFA in the two environments. Therefore, in the present experiment the net release of VFA from the viscera was measured before and 1, 3 and 5 h after feeding sheep in neutral and cold environments. Particular attention was given to the release of propionate and its uptake by the liver because propionate, taken up by the liver, is an important source of glucose (Bergman, 1973) and extra glucose is oxidized during cold exposure in sheep (McKay, Young & Milligan, 1974).

Exposure of lambs and adult sheep to cold environments leads to decreased plasma insulin concentrations (Bassett & Alexander, 1971; Bassett, unpublished results) probably attributable to enhanced sympathetic activity and α-adrenergic inhibition of insulin secretion (Woods & Porte, 1974). Further, increased sympathetic activity can also stimulate glucagon secretion (Bloom, Edwards & Vaughan, 1973) leading to the possibility of substantial changes in insulin : glucagon and to consequent increases in the hepatic glucose output. However, since VFA may play some part in the regulation of insulin and glucagon release after feeding in ruminants (Bassett, 1975), an increased supply of those metabolites from the gut in the cold might have an opposite effect on insulin release.

In the present study we have therefore measured hepatic glucose output and plasma insulin concentrations in an attempt to assess the contribution of these measurements to the alterations in metabolism seen during cold exposure.

EXPERIMENTAL

Animals and diet. Five Finnish Landrace × Dorset Horn rams, weighing between 37 and 47 (average 42) kg, were used. They were housed in individual crates in a byre and given 800 g straw, 300 g rolled barley, 300 g flaked maize and molasses daily, in two meals.

Animal preparation. The right common carotid artery of each animal was exteriorized into a fold of skin at least 8 weeks before experiments began. This operation was carried out under general anaesthesia induced by pentobarbitone sodium (Sagatal; May and Baker Ltd, Dagenham, Essex) and maintained with a mixture of halothane (Fluothane; ICI, Macclesfield), nitrous oxide and oxygen.

At least 4 weeks before experiments began the animals were again anaesthetized and polyvinyl chloride cannulas (Portex NT3 SH80; Portland Plastics Ltd, Hythe, Kent) were placed in veins supplying and draining the liver. This involved making a vertical incision in the abdominal wall on the animal's right side. A small branch of the anterior mesenteric vein was then isolated and cannulated. Superficial branches of the hepatic portal vein and the right hepatic vein were identified in the tissue of the liver and also cannulated. The free ends of the cannulas were passed through a stab wound in the abdominal wall and the incision sewn up. The cannulas were filled with a sterile solution of trisodium citrate in water (38 g/l). Intramuscular injections of antibiotic (Streptopen; Glaxo Ltd, Greenford, England) were given for 3 d after both operations. During the postoperative recovery period animals were placed in the experimental room frequently to familiarize them with these surroundings.

Experimental procedure. Measurements were made before and after each animal was given its morning feed in the experimental room at a neutral (15–20°, still air) or a cold (1°, coat clipped and wind speed 2 m/s) environmental temperature. The latter conditions were sufficient to approximately double the metabolic rate of similar sheep (Thompson *et al.* 1975). Three of the animals were exposed to the neutral environment before the cold, and for the remaining two animals this order was reversed. At least 1 week elapsed between experiments on the same animal.

The day before an experiment a polyethylene cannula (Portex PP 90; Portland Plastics Ltd) was placed in the exteriorized carotid artery so that its tip lay just outside the heart. On the morning of an experiment the animal was placed in the experimental room 1·5 h before feeding. Immediately before feeding, blood samples were taken for the measurement of hepatic portal blood flow, the concentration of insulin in arterial and hepatic portal blood plasma, and the concentration of glucose and VFA in arterial, hepatic portal and hepatic venous plasma. Between 2 and 5 min after the beginning of eating, samples were taken for measurement of blood flow, insulin and glucose concentrations. At 1, 3 and 5 h

after the beginning of eating, samples were again taken for measurement of blood flow, insulin, VFA and glucose concentrations.

Measurements. Hepatic portal blood flow was measured by continuous infusion of a sterile indocyanine green dye solution (Cardio-Green; Hynson Westcott and Dunning, Baltimore, Maryland, USA) into the anterior mesenteric vein at a rate of 1·15 mg/min for 6 or 7 min and measuring its dilution in the hepatic portal vein. Blood was sampled from the hepatic portal vein before dye infusion began and then simultaneously from the portal vein and carotid artery, beginning 2 min after the start of the infusion. The results of pilot experiments showed that at this time there is a steady concentration of dye in the portal vein, when corrected for recirculating dye using the carotid arterial concentration. The pre-infusion blood sample was used to make a zero value and a standard solution of infused dye in blood. The extinction of dye in plasma was measured at 800 nm. Hepatic portal blood flow (ml/min) was calculated from the equation:

$$\text{portal blood flow} = \frac{\dot{I}}{(P)-(A)},$$

where $\dot{I}$ is the rate of infusion of dye (mg/min) and (P) and (A) are dye concentrations (mg/ml) in hepatic portal and carotid arterial blood respectively.

Measurements of packed cell volume in portal blood samples were made after centrifuging for 8 min using a micro-haematocrit centrifuge (Hawksley and Sons Ltd, Lancing) and these values were used to calculate plasma flow from blood flow.

The net rate of release of VFA from the visceral tissues into the portal blood stream (mmol/min) was calculated from the equation:

$$\text{visceral release} = \text{portal plasma flow } ((P)-(A)),$$

where (P) and (A) are concentrations of VFA (mmol/ml) in hepatic portal and carotid arterial plasma respectively. The release of glucose from the liver (mmol/min) was estimated using the equation:

$$\text{hepatic release} = \text{portal flow } ((V)-(P)) + \text{arterial flow } ((V)-(A)),$$

where (V), (P) and (A) are the concentrations of glucose (mmol/ml) in hepatic venous, hepatic portal and carotid arterial plasma respectively. Hepatic arterial flow was assumed to be 20% of hepatic portal flow (Katz & Bergman, 1969*a*; Thompson *et al.* 1975). This equation was modified to estimate the uptake of propionate by the liver.

The concentration of insulin in plasma was measured by radioimmunoassay using talc to separate antibody-bound from free hormone, as described by Bassett & Thorburn (1971). The concentration of glucose in plasma was measured using the glucose oxidase (*EC* 1.1.3.4) method of Huggett & Nixon (1957). The VFA (C_2–C_5) in plasma were measured by the method of Gardner & Thompson (1974). A known amount of internal standard (*n*-hexanoic acid) was added to each plasma sample and the VFA extracted, as their sodium salts, into propan-2-ol. After concentration of the salts the VFA were injected into a gas–liquid chromatograph (Model 104, Pye Unicam Ltd, Cambridge), in a diethyl ether–formic acid solution (9 : 1, v/v), and separated using a 2·1 m column packed with 170 g neopentyl glycol adipate and 30 g orthophosphoric acid/kg 60–100 mesh Embacel (May and Baker Ltd, Dagenham).

Statistics. A paired Student's *t* test was used to test the significance of the difference between values obtained in neutral and cold environments. The same test was also used to compare values obtained before feeding with those obtained at various times after feeding. Since plasma insulin concentrations do not form a normal distribution whereas their

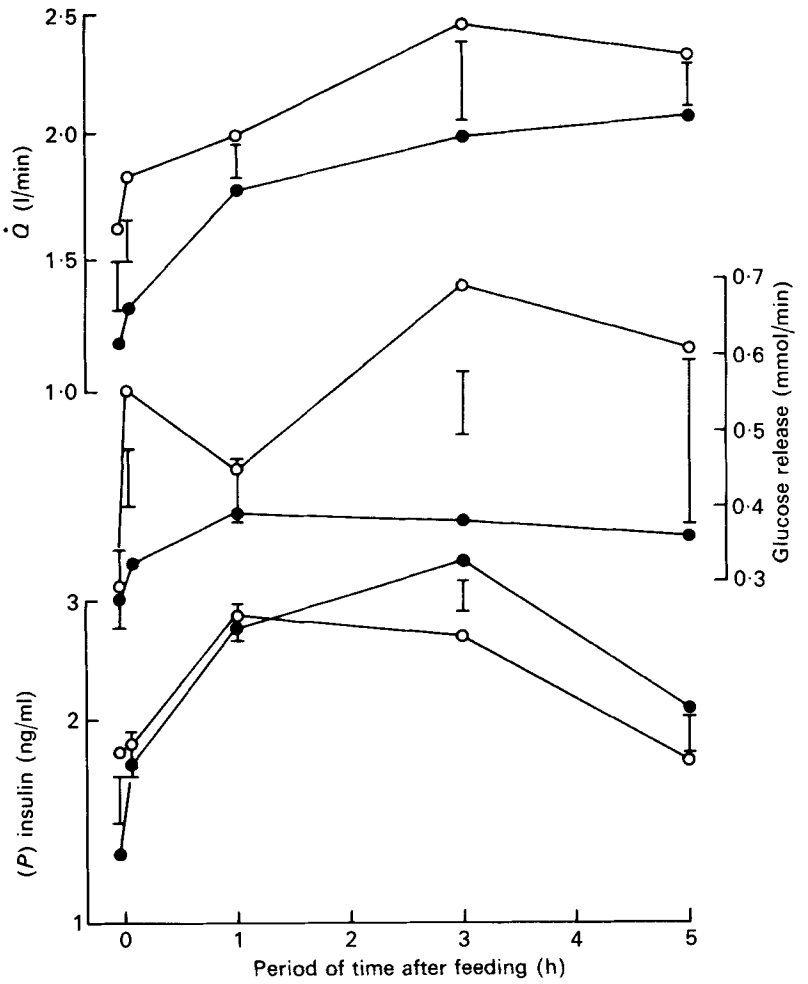

Fig. 1. The effect of feeding in neutral (●) and cold (○) environments on hepatic portal blood flow ($\dot{Q}$ l/min), hepatic release of glucose (mmol/min), and portal venous ((*P*)) plasma insulin concentration (ng/ml). The standard error of the mean change due to environment is represented by the vertical bar. The mean values with their standard errors of the insulin values are plotted on a logarithmic scale. For details of experimental procedures see p. 202.

logarithms do, concentrations of insulin were converted to logarithms before calculations of means and standard errors (Bassett, 1971).

RESULTS

The animals usually finished eating 30–60 min after food was offered. Small amounts of food were occasionally left for up to 2–3 h but all animals ate the entire ration within this period. There was no subjectively noticeable difference in the period of time taken to eat the meal completely in the two environments.

Feeding, in the neutral environment, increased the hepatic portal blood flow rate above the prefeeding value by (mean ± SE of difference; ml/min) 614 ± 192 (n 5, $P < 0{\cdot}05$), 841 ± 154 ($P < 0{\cdot}01$), and 948 ± 193 ($P < 0{\cdot}01$) 1, 3 and 5 h after the beginning of feeding respectively (Fig. 1). Feeding, in the neutral environment, also increased the rate of release of VFA

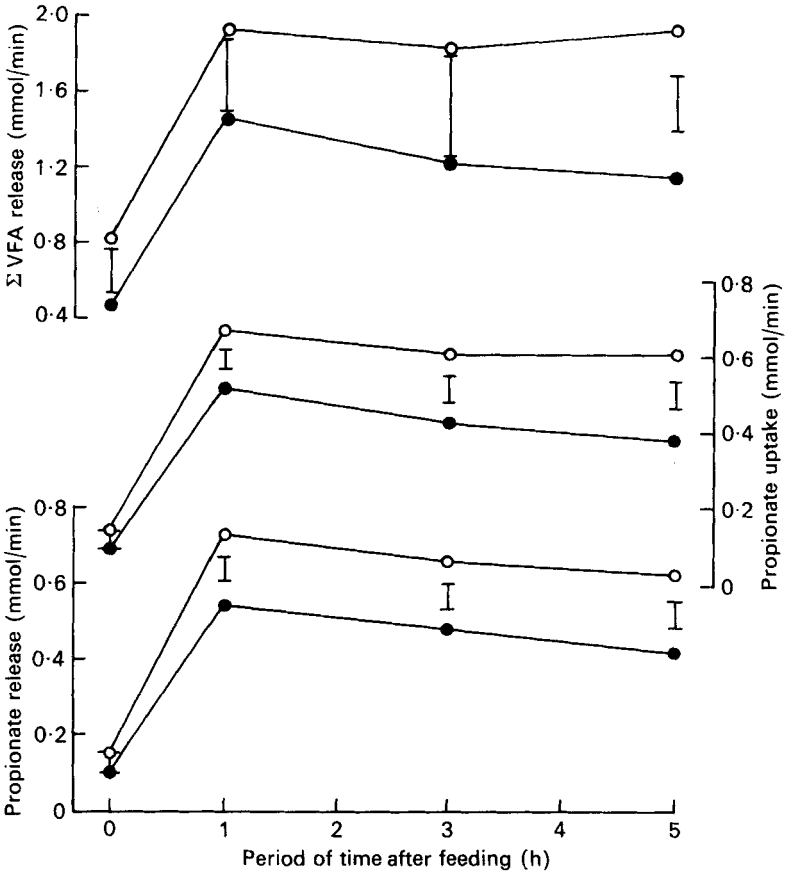

Fig. 2. The effect of feeding in neutral (●) and cold (○) environments on total visceral release of volatile fatty acids (ΣVFA) into the portal blood stream (mmol/min), the visceral release of propionate (mmol/min), and the hepatic uptake of propionate (mmol/min). The standard error of the mean change due to environment is represented by the vertical bar. For details of experimental procedures, see p. 220.

into the portal blood above the prefeeding value by (mean ± SE of difference; mmol/min) 0·99 ± 0·09 ($P < 0·001$), 0·74 ± 0·26 ($P < 0·05$) and 0·69 ± 0·09 ($P < 0·01$) 1, 3 and 5 h after the beginning of feeding respectively (Fig. 2). The total concentration of all the VFA in arterial plasma averaged 0·60 mmol/l before feeding, and increased above this by (mean ± SE of difference; mmol/l) 0·79 ± 0·14 ($P < 0·05$), and 0·90 ± 0·20 ($P < 0·02$) 3 and 5 h after the beginning of feeding respectively. The estimated uptake of propionate by the liver significantly increased after feeding in the neutral environment (Fig. 2) by (mean ± SE of difference; mmol/min) 0·43 ± 0·04 ($P < 0·001$), 0·34 ± 0·06 ($P < 0·01$), and 0·29 ± 0·05 ($P < 0·01$) 1, 3 and 5 h after feeding respectively. The estimated release of glucose from the liver consistently, but not significantly, increased after feeding in the neutral environment (Fig. 1). There was no statistically significant change in arterial plasma glucose concentration after feeding; before feeding the mean value was 3·79 mmol/l and after feeding for 4 min, 1, 3 and 5 h the mean values were 4·15, 3·79, 3·75 and 3·57 mmol/l respectively. Feeding, in the neutral environment, significantly increased the hepatic portal and arterial plasma insulin concentrations. Fig. 1 shows the insulin concentration in portal plasma,

which increased significantly after 1 and 3 h of feeding (increase in the $\log_{10}$ value of (ng/ml) $0{\cdot}33 \pm 0{\cdot}06$, $P < 0{\cdot}01$ and $0{\cdot}43 \pm 0{\cdot}08$, $P < 0{\cdot}01$ respectively).

In the cold environment portal blood flow was consistently faster than in the neutral environment, before and after feeding (Fig. 1), but the difference between environments was statistically significant at one time interval only; 4 min after feeding it was 507 ± 139 ml/min (n 5, $P < 0{\cdot}05$) faster in the cold. The release of VFA into the portal blood was also consistently faster when the animals were exposed to the cold environment, before and after feeding (Fig. 2), but the difference between environments was statistically significant at one time interval only; 5 h after feeding it was $0{\cdot}76 \pm 0{\cdot}25$ mmol/min ($P < 0{\cdot}05$) faster in the cold.

Acetate was the major VFA to be released but its release was variable. Values for the release of propionate were less variable and, in the fed animal, propionate release was significantly faster in the cold than the neutral environment (Fig. 2), by (mean ± SE of difference; mmol/min) $0{\cdot}17 \pm 0{\cdot}04$ ($P < 0{\cdot}02$) and $0{\cdot}21 \pm 0{\cdot}06$ ($P < 0{\cdot}05$) 1 and 5 h after feeding respectively. The estimated hepatic uptake of propionate in the fed animal was also greater in the cold environment than the neutral environment (Fig. 2) by (mean ± SE of difference; mmol/min) $0{\cdot}15 \pm 0{\cdot}05$ ($P < 0{\cdot}05$), $0{\cdot}18 \pm 0{\cdot}06$ ($P < 0{\cdot}05$), and $0{\cdot}22 \pm 0{\cdot}06$ ($P < 0{\cdot}05$) 1, 3 and 5 h after feeding respectively. Cold exposure had no effect on the concentration of total VFA or propionate in arterial plasma. The estimated release of glucose from the liver increased after feeding in the cold environment much more than it did in the neutral environment (Fig. 1) so that the difference between the two environments averaged (mean ± SE of difference; mmol/min) $0{\cdot}22 \pm 0{\cdot}07$ ($P < 0{\cdot}05$) and $0{\cdot}31 \pm 0{\cdot}08$ ($P < 0{\cdot}02$) 4 min and 3 h after the beginning of feeding respectively. Cold exposure consistently increased the arterial plasma glucose concentrations above those measured in the neutral environment before and after feeding, and 1 h after feeding the difference between environments, $0{\cdot}68 \pm 0{\cdot}21$ mmol/l, was statistically significant ($P < 0{\cdot}05$). The portal plasma insulin concentration was significantly higher before feeding in the cold environment than it was before feeding in the neutral environment (difference in $\log_{10}$ values of $0{\cdot}15 \pm 0{\cdot}05$ ng/ml, $P < 0{\cdot}05$), however this difference was no longer apparent 4 min after feeding or at any time up to 5 h after feeding (Fig. 1).

DISCUSSION

The present experiments confirm the observations that feeding, in sheep, increases hepatic portal blood flow (Bensadoun & Reid, 1962), the flow of VFA into the portal bloodstream (Kiddle, Marshall & Phillipson, 1951), the uptake of propionate by the liver (Cook & Miller, 1965), the release of glucose from the liver (Katz & Bergman, 1969*b*), and increases plasma insulin concentration (Bassett, 1974).

The present experiments also provide more evidence for the suggestion (Thompson *et al.* 1975) that a few hours of exposure to a cold environment increases the flow of VFA into the portal bloodstream in fed sheep. An increased visceral release of acetate could originate from incomplete oxidation of long-chain free fatty acids by the visceral tissues but the increased flow of propionate must originate from microbial fermentation of carbohydrate in the rumen. There are three likely explanations for this phenomenon: (1) when sheep with a clipped coat are exposed to a cold environment they initially produce an excessive amount of body heat and rectal temperature increases (Webster, 1966). Such a response may also increase the temperature of the rumen and the rate of fermentation in it; (2) acute exposure to a cold environment increases the motility of the reticulum in sheep (Westra & Christopherson, 1976). The long-term effect of this is to increase the rate at which food passes through the rumen and reduce its digestibility (Kennedy, Christopherson & Milligan, 1976) but the initial effect could be to mix the rumen contents and temporarily increase VFA

absorption; (3) some of the VFA produced in the rumen is taken up and metabolized by rumen epithelial tissue (Pennington, 1952) without entering the portal circulation. Rumen epithelial tissue may also metabolize longer-chain free fatty acids (Taylor & Jackson, 1968) which are likely to be supplied by the arterial circulation and not through the rumen wall. During cold exposure there is an increased circulating level of free fatty acids (Bost & Dorleac, 1965) and an apparent increase in fat catabolism in sheep (Graham, Wainman, Blaxter & Armstrong, 1959). In the rumen epithelium a greater metabolism of free fatty acids may partially substitute for VFA metabolism, allowing the passage of more VFA into the portal blood.

The estimated increase in hepatic uptake of propionate during cold exposure, in the fed animal, agrees with the previous observations, on all VFA, by Thompson *et al.* (1975). It has been reported previously that when sheep are acutely exposed to a cold environment this increases their plasma glucose concentration and the rate at which they oxidize glucose (McKay *et al.* 1974). In the present experiments, estimates of the release of glucose from the liver suggest that this increases during acute cold exposure. In the period 1–5 h after feeding in the cold the extra uptake of propionate by the liver, due to cold exposure, averaged 0·18 mmol/min. If all this propionate were converted to glucose in the liver it would account for less than half of the extra glucose that was released from the liver, which averaged 0·21 mmol/min for the same period.

Further it is interesting to note that the increases in hepatic glucose output observed after feeding occurred despite increases in the plasma insulin concentration and are presumably indicative of concomitant increases in blood glucagon concentrations at this time as observed by Bassett (1972). These findings re-emphasize the significance of insulin: glucagon in controlling glucose metabolism in ruminants (Bassett, 1975). The greater increase in hepatic glucose output in the cold environment is suggestive of a relatively greater increase in glucagon in this environment, consistent with the opposite effects of sympathetic stimulation on secretion of the two hormones.

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