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# Origin and utilization of volatile fatty acids and lactate in the rabbit: influence of the faecal excretion pattern

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1. Interrelations between bacterial metabolites (volatile fatty acids, lactate) in the gut contents and the blood in relation to the faecal excretory cycle (soft or hard faeces) were studied in anaesthetized rabbits.

2. It appeared that the level of organic acids in the alimentary tract varied cyclicly with the faecal excretion pattern. The lactate entering the portal circulation originates from the stomach, while the volatile fatty acids (VFA) originate from the hind-gut. Net absorption from the digestive tract and hepatic utilization of fermentation acids were greater when the rabbits produced hard faeces (hard-phase) compared with soft faeces (soft-phase). Propionate and butyrate reaching the liver were almost quantitatively removed; acetate and lactate were available for extra-hepatic tissue metabolism.

3. Whatever the excretion pattern the levels of VFA in the arterial circulation remained remarkably constant; blood lactate, however, was lower during the soft-phase.

4. Absorption of bacterial metabolites, like their metabolism in the liver, showed a circadian rhythm parallel to the changes in the activity of the adrenal glands, i.e. the activity was enhanced during the hard-phase.

The rabbit obtains up to 30% of its maintenance energy requirement from volatile fatty acids (VFA) produced by fermentation in the hind-gut (Parker, 1976; Marty & Vernay, 1984) and absorbed, to a great extent, through the caeco-colonic wall (Henning & Hird, 1972*a*; Beauville *et al.* 1974; McMillan *et al.* 1975; Leng, 1978; Vernay, 1986*a*). Rabbits produce, in a circadian rhythm, hard and soft faeces which are distinctly different in size and in chemical composition. The hard faeces are high in fibre and low in protein, water-soluble substances and vitamins, whereas the composition of the soft faeces which are ingested (caecotrophy), is very similar to that of the caecal contents (Bonnafous & Raynaud, 1970; Vernay & Raynaud, 1975; Hörnicke, 1981; Snipes *et al.* 1982; Vernay *et al.* 1984).

Several investigators have assumed that the alteration in composition of hard faeces is the result of complex absorptive, secretory and motor processes along the colon (Bonnafous & Raynaud, 1969; Ruckebusch & Hörnicke, 1977; Bonnafous, 1980; Björnhag, 1981; Ehrlein *et al.* 1983). In earlier papers on the absorption of electrolytes and VFA (Vernay *et al.* 1984) and on VFA metabolism in the hind-gut (Vernay & Marty, 1984) of the rabbit, we found that solute absorption and VFA catabolism are both enhanced during the hard-phase when the activity of the adrenal glands increases.

Until now little or no information has been reported concerning the incidence of the excretion pattern, soft-phase and hard-phase, on the level of bacterial metabolites (VFA and lactate) in the blood. Therefore the present work was designed to study the relations between VFA and lactate concentrations in the gut contents and the efferent blood, hepatic metabolism and the excretion pattern of the rabbit.

## MATERIALS AND METHODS

### *Animals*

A total of twenty-four male domestic rabbits (*Oryctolagus cuniculus*), weighing about 2·5 kg, were used. The animals were provided with oats, lucerne (*Medicago sativa*) and water *ad lib.* The experiments were carried out either between 04.00 and 07.00 hours (period of

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soft-faeces formation) or between 10.00 and 18.00 hours (period of hard-faeces formation); therefore groups were selected on the basis of their excretion pattern. Ten of the twenty-four rabbits produced soft faeces and were in soft-phase, the remaining fourteen animals produced hard faeces and were in hard-phase.

*Experimental procedure*

Anaesthesia of rabbits was achieved by administering pentobarbital solution (20 mg/kg) via the marginal ear vein. The choice of pentobarbital as the anaesthetic takes into account the findings of Bito & Eakins (1969), who reported that it induced little modification of the rabbit blood chemistry: decrease of oxygen potential is not significant, glucose and sodium values are not affected and the potassium concentration increases only slightly. After laparotomy about 0·6 ml venous blood was removed from the stomach, intestine (ileal) and caeco-colonic veins and from the inferior vena cava; arterial blood was also collected. For studies on hepatic metabolism, blood was taken from the portal and hepatic veins.

The animals were then killed with an overdose of pentobarbital and the gut was immediately removed and separated into segments by ligatures. The stomach and the small intestine were each divided into three parts: fundus, corpus and antrum for the former; duodenum, jejunum and ileum for the latter. The caecum was taken as one single segment, but four segments of large intestine were prepared; one from each of the following parts of the colon: oral and aboral portion of the proximal colon, distal colon and rectum (for anatomical detail, see Snipes *et al.* 1982).

*Preparation of samples*

The material contained in each segment was weighed, homogenized and the pH was immediately measured with a pH meter (Methrohm E 532); the water content was determined by drying a portion at 110° for 24 h, the remaining wet material was extracted with an equal volume of distilled water and the suspension centrifuged for 20 min at 10000 ***g***. The supernatant fraction was filtered and the concentrations of VFA and lactate were measured in the liquid obtained. Heparinized blood samples were immediately centrifuged (Eppendorf-Zentrifuge 3200) for 2 min.

*Biochemical techniques*

The VFA were determined by gas–liquid chromatography (Intersmat IGC 120 DFL) using the techniques described by Rémésy & Demigné (1974, 1976). The lactate was measured enzymically. However, it is to be noted that the stomach of the rabbit is thought to have lactic fermentation with preferential production, accumulation (Hörnicke & Mackiewicz, 1976) and absorption (Parker & Mould, 1977) of D-lactate. Surprisingly, contrary to these observations our results (Vernay, 1985), like those of Giesecke *et al.* (1981) and of Stangassinger *et al.* (1982), showed no D-lactate either in the stomach contents or in portal blood. The absence of a measurable concentration of D-lactate in the digesta has to be interpreted as the result of a highly lactolytic activity of the gastric contents (Jilge & Meyer, 1975). Consequently, for the present investigation, only L-lactate was determined with L-lactate dehydrogenase (*EC* 1.1.1.27) from Boehringer, according to Hohorst's (1965) procedure, but with a diluted buffer (0·25 M-glycine, 0·20 M-hydrazine and 0·03 M-potassium carbonate).

*Calculations*

From values for hepatic blood supply, it was assumed that for the rabbit (Neutze *et al.* 1968) the portal vein and the hepatic artery represent 90 and 10% of the afferent blood flow respectively. Thus afferent plasma concentrations are: 0·9 (portal vein)+0·1 (aorta);

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the net hepatic uptake being the difference between the hepatic vein and the afferent plasma. The proportion of hepatic uptake is calculated from the ratio, hepatic uptake:afferent. Likewise the digestive appearance is the difference between the gut vein and the aorta, and the proportion of gut appearance is calculated as digestive appearance:aorta.

All the results are presented as means with their standard errors. Differences were evaluated statistically using paired or unpaired Student's *t* test as appropriate.

## RESULTS

### *Variations in the gut contents in relation to the faecal excretion pattern*

Values for dry matter, pH, VFA and lactate showed marked changes with the phases of the excretory cycle as well as with the site of measurement (Table 1). The changes in excretion pattern produced only small modifications both in the small intestine and in the caecal contents; in contrast important variations occurred in the composition of the gastric and colonic digesta.

The lowest pH values were found in the stomach (1·3–2·1) while in the rest of the alimentary tract the contents were at a near-neutral pH. When the rabbit excreted soft faeces the mean values of pH along the hind-gut became slightly acidic, changing from 6·4 to 6·0 ($P < 0·001$), while the pH values increased throughout the length of the colon, from 6·5 to 7·7 ($P < 0·001$), when the animals produced hard faeces.

In the gastric contents, irrespective of the faecal excretion pattern, VFA and lactate concentrations decreased from the fundus to the antrum of the stomach ($P < 0·001$). This gradient was consistently steeper during the hard-phase than during the soft-phase, e.g. the mean (with SE) decline for the VFA was 0·80 (0·056) during the hard-phase and 0·35 (0·021) during the soft-phase, while the corresponding values for lactate were 0·52 (0·030) and 0·42 (0·012). Only small quantities of VFA were found in the small intestine; although concentrations of these bacterial metabolites were higher in the ileum, they remained relatively low. The mean (with SE) molar proportions of the three fatty acids in the stomach and small-intestine contents were acetate 0·66 (0·014), propionate 0·14 (0·011), butyrate 0·20 (0·012). In contrast to VFA, lactate values were always high from the duodenum to the ileum with lower concentrations occurring in the hind-gut.

The major sites of fermentation in the alimentary tract were found to be the caecum and the proximal colon, with mean concentrations for VFA of about 74 and 38 mM respectively. At the time of production of soft faeces, the mean (with SE) VFA levels slightly decreased during transit in the colon (0·34 (0·054)), meanwhile the disappearance of VFA was consistently greater ($P < 0·001$) during the hard-phase (0.80 (0·037)). Whatever the excretion pattern, butyrate was absorbed the fastest, followed by propionate and then by acetate. In the caecum contents whichever the phase, as in the colon during the soft-phase, acetate was found to be predominant, making up about 0·73 (SE 0·016) of the total VFA with low proportions of propionate and butyrate: 0·09 (SE 0·003) and 0·18 (SE 0·011) respectively. During the hard-phase in the distal colon different values ($P < 0·001$) were found: acetate 0·93 (SE 0·014), while the proportion of propionate was 0·04 (SE 0·009) and butyrate 0·03 (SE 0·007).

### *Digestive appearance and hepatic uptake of the VFA*

Irrespective of the mode of excretion, soft faeces or hard faeces, the level of the VFA in the arterial plasma was remarkably constant (Table 2) and the means (with SE) molar proportions of acetate, propionate and butyrate were 0·89 (0·013), 0·06 (0·007) and 0·05 (0·006) respectively. The VFA were mainly found in the blood draining the hind-gut ($P < 0·001$), but there was an aborally decreasing gradient of these nutrients in the venous

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Table 1. *Dry matter (g/kg), pH values and concentrations (mM) of volatile fatty acids and lactate in the gut contents of rabbits in relation to the faecal excretion pattern: soft-phase (SP) and hard-phase (HP)*

(Mean values with their standard errors; no. of observations in parentheses)

| | Dry matter (g/kg) | | | | pH | | | | Total volatile fatty acids (mM) | | | | Lactate (mM) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SP (10) | | HP (14) | | SP (10) | | HP (14) | | SP (10) | | HP (14) | | SP (10) | | HP (14) | |
| Digestive contents from | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Stomach: | | | | | | | | | | | | | | | | |
| Fundus | 200 | 8 | 200 | 8 | 1·8 | 0·24 | 2·1 | 0·20 | 9·9 | 1·83 | 19·2* | 3·68 | 2·8 | 1·71 | 6·6* | 0·66 |
| Corpus | 190 | 7 | 200 | 6 | 1·5 | 0·13 | 1·6 | 0·09 | 5·6 | 0·83 | 10·0*** | 1·70 | 2·2 | 0·36 | 4·6** | 0·63 |
| Antrum | 190 | 7 | 190 | 5 | 1·3 | 0·05 | 1·3 | 0·03 | 3·7 | 0·53 | 5·0 | 0·92 | 1·6 | 0·22 | 3·2*** | 0·30 |
| Small intestine: | | | | | | | | | | | | | | | | |
| Duodenum | 120 | 8 | 70*** | 4 | 6·8 | 0·08 | 6·9 | 0·16 | 3·9 | 0·58 | 3·6 | 0·50 | 8·0 | 0·76 | 6·4 | 1·02 |
| Jejunum | 100 | 5 | 90 | 8 | 7·3 | 0·09 | 7·1 | 0·07 | 4·7 | 0·74 | 4·0 | 0·47 | 11·0 | 1·30 | 10·0 | 2·12 |
| Ileum | 120 | 10 | 110 | 9 | 7·6 | 0·05 | 7·4 | 0·05 | 4·7 | 0·97 | 5·6 | 0·65 | 9·6 | 1·63 | 8·6 | 1·38 |
| Caecum | 180 | 9 | 220* | 7 | 6·4 | 0·14 | 6·5 | 0·22 | 71·1 | 5·94 | 76·5 | 9·59 | 3·8 | 0·68 | 3·6 | 0·77 |
| Proximal colon: | | | | | | | | | | | | | | | | |
| Oral part | 220 | 11 | 170** | 7 | 6·2 | 0·09 | 7·2*** | 0·14 | 50·6 | 5·04 | 39·1 | 3·90 | 4·5 | 2·11 | 2·8 | 0·43 |
| Aboral part | 230 | 10 | 210 | 5 | 6·1 | 0·16 | 7·4*** | 0·09 | 43·4 | 5·75 | 20·9*** | 2·64 | 4·3 | 1·87 | 1·1 | 0·43 |
| Distal colon | 260 | 13 | 290* | 2 | 6·0 | 0·18 | 7·4*** | 0·13 | 44·6 | 3·42 | 18·8** | 6·15 | 4·3 | 1·08 | 0·6*** | 0·28 |
| Rectum | 270 | 12 | 410*** | 17 | 6·0 | 0·19 | 7·7*** | 0·10 | 46·5 | 4·12 | 14·9*** | 1·87 | 4·1 | 1·06 | 0·4*** | 0·21 |

Mean values for gut components in relation to the faecal excretion pattern were significantly different: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

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Table 2. *Concentrations (μM) of volatile fatty acids (acetate, propionate, butyrate) and lactate in arterial and venous plasma of rabbits in relation to the faecal excretion pattern: soft-phase (SP) and hard-phase (HP)*

(Mean values with their standard errors; no. of observations in parentheses)

| | Acetate (μM) | | | | Propionate (μM) | | | | Butyrate (μM) | | | | Lactate (μM) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SP (7) | | HP (7) | | SP (7) | | HP (7) | | SP (7) | | HP (7) | | SP (7) | | HP (7) | |
| Site | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Aorta | 2020 | 410 | 1540 | 190 | 130 | 20 | 110 | 10 | 120 | 30 | 120 | 10 | 2400 | 270 | 3600 | 580 |
| Gastric vein | 1620 | 200 | 1400 | 140 | 200 | 20 | 180 | 30 | 230** | 60 | 240** | 20 | 2710 | 300 | 5010** | 400 |
| Ileal vein | 2030 | 140 | 1580 | 169 | 170 | 20 | 140 | 20 | 190 | 30 | 200 | 10 | 3040 | 410 | 5040** | 420 |
| Caecal vein | 4060*** | 580 | 5250** | 440 | 400*** | 60 | 750*** | 50 | 860*** | 100 | 1180*** | 100 | 2280 | 190 | 3700 | 550 |
| Proximal colonic vein: | | | | | | | | | | | | | | | | |
| Oral part | 3460*** | 280 | 4070*** | 230 | 280*** | 50 | 580*** | 70 | 660*** | 140 | 890*** | 70 | 2350 | 230 | 3700 | 540 |
| Aboral part | 3020*** | 490 | 1740*** | 120 | 290*** | 70 | 120 | 10 | 300*** | 30 | 110 | 10 | 2460 | 390 | 3800 | 590 |
| Distal colonic vein | 2250 | 350 | 1780*** | 90 | 190 | 20 | 170*** | 10 | 200*** | 20 | 130 | 10 | 2400 | 250 | 3750 | 640 |
| Portal vein | 3250*** | 350 | 3890*** | 280 | 330*** | 50 | 550*** | 30 | 660*** | 40 | 870*** | 60 | 2630* | 250 | 4500** | 930 |
| Inferior vena cava | 1530* | 310 | 1200* | 120 | 130 | 10 | 110 | 10 | 120 | 20 | 120 | 20 | 1920 | 230 | 2700* | 430 |
| Afferent hepatic plasma† | 3127 | 348 | 3655 | 214 | 307 | 44 | 506 | 37 | 606 | 37 | 795 | 69 | 2607 | 230 | 4410 | 822 |
| Hepatic vein | 2440* | 310 | 2290** | 150 | 120*** | 10 | 100*** | 10 | 130*** | 30 | 120*** | 10 | 2370*** | 210 | 3650*** | 580 |

Mean values for afferent and efferent plasmas of the gut, hepatic and extra-hepatic tissues were significantly different: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

† For calculations of afferent hepatic plasma, see p. 372.

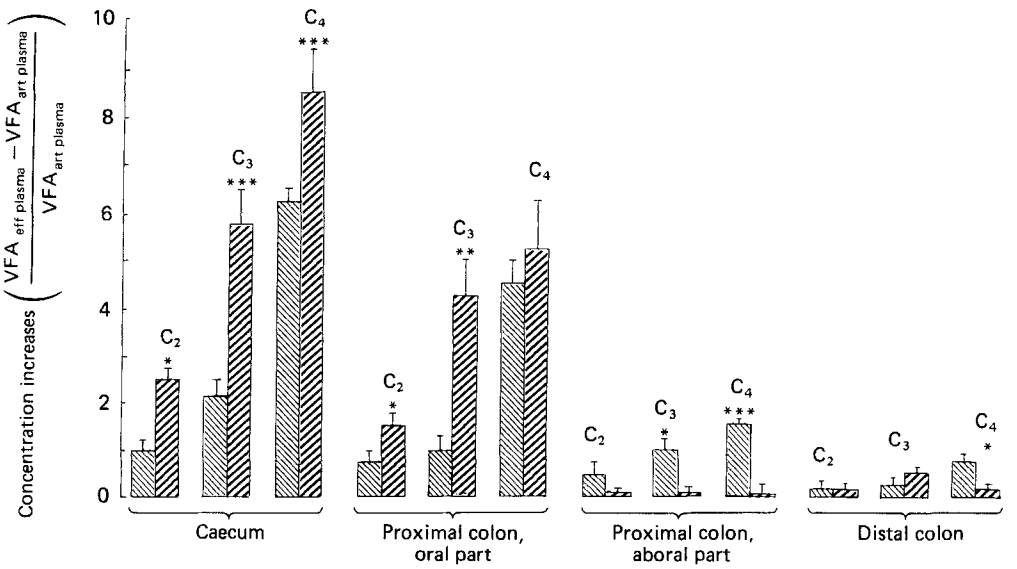


Fig. 1. Increases in volatile fatty acid (VFA) concentrations, acetate ($C_2$), propionate ($C_3$) and butyrate ($C_4$), in the efferent (eff) plasma of the hind-gut expressed as a proportion of arterial (art) concentration. Values are means with their standard errors represented by vertical bars for fourteen experiments; seven rabbits produced soft faeces (▧), the remainder produced hard faeces (▨). For calculations of volatile fatty acids increase, see pp. 372 and 373. Mean values for volatile fatty acids increase in relation to the faecal excretion pattern were significantly different: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

plasma of the large intestine (Fig. 1). On a proportionate basis, the order of appearance in the efferent blood was butyrate > propionate > acetate, except in the aboral part of the proximal colon and in the distal colon when the rabbit excreted hard faeces; the succession was propionate > acetate > butyrate. On the other hand the VFA enrichment in the blood taken from the hind-gut and portal vein was not similar in the two groups of rabbits; the blood VFA gain was greater during the hard-phase than during the soft-phase, with certain exceptions: in the second segment of the proximal colon and in the distal colon. For these latter zones there was little VFA appearance in the corresponding blood.

The means (with SE) molar proportions of the fatty acids in the portal blood of the rabbits in the soft-phase were acetate 0·75 (0·010), propionate 0·08 (0·004) and butyrate 0·17 (0·012). Different values were obtained for the rabbits in the hard-phase; acetate was significantly lower 0·69 (0·009) while propionate and butyrate were higher (0·12 (0·007) and 0·19 (0·007) respectively).

During the soft-phase the liver removed about 0·21 of the acetate reaching it, while the uptakes of propionate and butyrate were 0·61 and 0·78 respectively (Fig. 2). The hepatic uptake was greater during the hard-phase than during the soft-phase, e.g. acetate 0·37, propionate 0·80 and butyrate 0·85. So there was no obvious variation of the VFA concentrations in the hepatic vein in relation to the excretion pattern (Table 2).

It is to be noted that the differences between the venous and arterial metabolite concentrations allow interesting comparisons of metabolite fluxes in identical physiological situations. However, comparison of metabolite fluxes in different physiological situations would also necessitate measurements of blood flow. To our knowledge there is no reference concerning possible blood flow variations in relation to the phase of the excretory cycle of the rabbit. Nevertheless it has been shown for the ruminant (Dobson & Phillipson, 1956; Bensadoun *et al.* 1962; Sellers, 1965), the dog (Kvietys & Granger, 1981) and the rat

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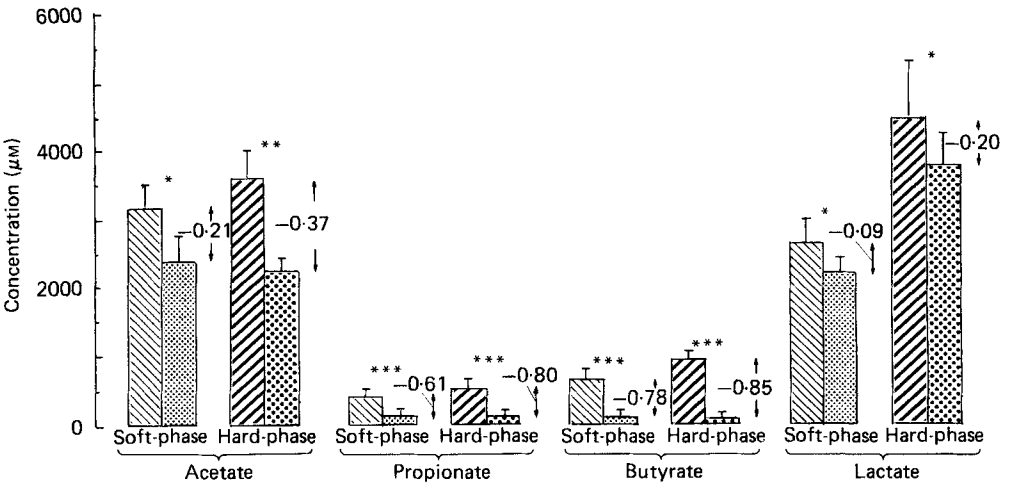


Fig. 2. Concentrations (μM) in afferent and efferent hepatic plasma and hepatic uptake of volatile fatty acids (acetate, propionate, butyrate) and lactate in relation to the faecal excretion pattern; soft-phase: afferent (▩) and efferent (▨) plasma; hard-phase: afferent (▨) and efferent (▩) plasma. Values are means with their standard errors represented by vertical bars for fourteen experiments: seven rabbits excreted soft faeces, the remainder excreted hard faeces. For calculations of hepatic uptake see p. 373. Mean values for afferent and efferent plasma concentrations were significantly different: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

(Demigné & Rémésy, 1985) that an increase in the VFA level in the mesenteric circulation enhances the blood flow. If this is the case for the rabbit, the evaluation of the hepatic uptake that we propose is underestimated for the animal in the hard-phase.

### *Digestive appearance and hepatic uptake of lactate*

The mean concentrations of lactate in blood taken from the artery were significantly higher ($P < 0{\cdot}001$) when the animal eliminated hard faeces compared with soft faeces (Table 2). Whatever the excretion pattern the results obtained suggest that the lactate entering the portal circulation does not originate from the hind-gut, since the differences in the levels of this organic acid in the arterial and venous plasmas of the caeco-colon were not significant; in fact the lactate originates from the foregut (stomach, small intestine). The enrichment of the portal blood was greater during the hard-phase than during the soft-phase, the mean (with SE) values were 0·20 (0·070) and 0·09 (0·040) respectively, while the corresponding hepatic uptakes were 0·18 (0·030) and 0·07 (0·010) as reported in Fig. 2.

### *Extra-hepatic VFA and lactate uptake*

The inferior vena cava was sampled to determine whether blood from the posterior portion of the body differs greatly from that in the aorta. As shown in Table 2, these extra-hepatic tissues removed arterial acetate and lactate ($P < 0{\cdot}05$). The mean (with SE) removal was acetate 0·19 (0·040) to 0·26 (0·071) and lactate 0·21 (0·029) to 0·25 (0·015); the higher values were obtained when the subject eliminated hard faeces.

## DISCUSSION

The results of the present study indicate that in the adult rabbit, microbial fermentation mainly occurs in the large intestine but also occurs in the stomach after caecotrophy. In the latter compartment the food starch is exposed to salivary amylase, to food amylase and

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to bacterial amylases from the autochthonous flora or the ingested faeces, or both (Griffiths & Davies, 1963; Hörnicke & Mackiewicz, 1976). The soft faeces (caecotrophes) are swallowed without chewing and are stored in the fundus of the stomach; the mucous envelope withstands mechanical and chemical action for up to 8 h. During this period the caecotrophe pellets act as small fermenters (Hörnicke & Mackiewicz, 1976; Hörnicke & Björnhag, 1980; Vernay, 1985) and the bacteria produce large quantities of lactate and VFA by fermentation of dietary carbohydrates (Alexander & Chowdhury, 1958; Griffiths & Davies, 1963; Vernay, 1985). Then the bacterial metabolites are rapidly absorbed from the stomach into the blood-stream, essentially during the hard-phase. For the VFA there was no concentration difference between arterial and venous blood which indicates that the VFA absorbed are completely metabolized by the gastric wall.

On passing into the small intestine the sudden increase in pH caused by the pancreatic and intestinal secretions and the antiseptic effect of the bile salts stop the acidophilic fermentation, so VFA and lactate production are both negligible (Vernay, 1985). The large quantities of lactate appearing in the duodenum mainly originate from the bile and intestinal secretions (Le Bars *et al.* 1969; Vernay, 1986*b*); then lactate is removed from the ileal contents. Generally diffusion of lactate out of the lumen of the gut is assumed, but recent observations in vivo (Vernay, 1985) and in vitro (Hildmann *et al.* 1980) suggest the existence of an electroneutral L-lactate–sodium co-transport system in the rabbit ileum.

The large volume of the caecum and the stable conditions in this compartment are very favourable for the development of a flora adapted to the substrates coming from the small intestine. As a result, concentrations of fermentation acids tend to remain rather constant throughout the day when the subjects are kept in stable conditions. So, in the rabbit (Beauville *et al.* 1974; McMillan *et al.* 1975; Bonnafous & Raynaud, 1978; Marty & Vernay, 1984), as in other simple-stomach animals such as the rat (Mottaz & Worbe, 1972; Rémésy & Demigné, 1976), pig (Friend *et al.* 1964; Imoto & Namioka, 1978), dog (Banta *et al.* 1979) and human (Dawson *et al.* 1964; Cummings *et al.* 1979), the main site for VFA production and absorption is the large intestine. However, it is interesting to note that for the rabbit, the concentration of the VFA in the digesta and in the gut blood varies markedly with the excretion pattern as well as with the site from which the samples are obtained. At the time of the production of soft faeces there is little modification of the caecal material during transit through the colon, whereas when hard faeces are being formed substantial amounts of VFA are removed at a rate which increases with their chain-length and there is a striking rise of their level in the blood draining the caecum and the oral part of the proximal colon. These results agree with those of preceding works (Henning & Hird, 1972*a*; Vernay & Raynaud, 1975; Bonnafous & Raynaud, 1978; Leng, 1978; Vernay *et al.* 1984; Vernay, 1986*a*). The VFA appear to provide a regular source of energy for the colonocyte. It is now well established that these bacterial metabolites are partly metabolized in the rabbit colonocyte in the order: butyrate > propionate > acetate; their complete oxidation to carbon dioxide is often the main catabolic pathway (Marty & Vernay, 1984; Marty *et al.* 1985; Vernay & Marty, 1984). A preferential utilization of butyrate by the large intestine in the human, rat (Roediger, 1980, 1982), guinea-pig (Wirthensohn & Engelhardt, 1981; Wirthensohn *et al.* 1981) and pig (Rérat *et al.* 1985) has also been suggested.

After intestinal absorption, VFA and lactate have to pass through the liver before entering the general circulation. It is to be noted that in the mesenteric blood of the rabbit (Henning & Hird, 1972*b*; Beauville *et al.* 1974; McMillan *et al.* 1975; Marty & Vernay, 1984), the butyrate molar proportion (170 mmol/mol) is higher than that (15–50 mmol/mol) observed in other simple stomach (Friend *et al.* 1964; Rémésy & Demigné, 1976) and ruminant species (McClymont, 1951; Cook & Miller, 1965; Bergman & Wolff, 1971). Under our experimental conditions, propionate and butyrate were largely removed

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from the portal blood during this passage, leaving acetate and lactate as the only organic acids present in a significant concentration in the peripheral blood. As shown by previous findings with rabbits, propionate is an efficient precursor of substrates for gluconeogenesis (Jean-Blain & Martin, 1980; Marty & Vernay, 1984; Vernay, 1987), while butyrate is poorly ketogenic and is utilized for energy production in liver mitochondria or for lipogenesis (Marty & Vernay, 1984; Vernay & Marty, 1984). From the present study it appears that the liver of the rabbit is a more efficient site of acetate uptake than that of ruminant animals as it removes 0·21–0·37 of the acetate reaching it, while the corresponding values for the ruminant are 0·04–0·10 (Annison *et al.* 1957; Cook & Miller, 1965; Bergman & Wolff, 1971; Baird *et al.* 1975; Pethick *et al.* 1981); acetate uptake by the liver of the rat is 0·50 (Rémésy *et al.* 1980). In contrast to ruminants (Ballard *et al.* 1969; Knowles *et al.* 1974), there is an active cytosolic acetyl-CoA synthetase (*EC* 6.2.1.1) in the livers of rabbits (Woodnutt & Parker, 1978) and rats (Hanson & Ballard, 1967; Knowles *et al.* 1974; Scholte & Groot, 1975), so acetate metabolism seems to be mainly cytosolic for lipogenesis and cholesterogenesis.

Whatever the excretion pattern the lactate molecule is partly taken up by the liver and utilized by the hepatocyte. However, unlike the majority of species, e.g. cow (Baird *et al.* 1977), rat (Phillips & Hird, 1977; Rémésy *et al.* 1980; Rémésy & Demigné, 1982), guinea-pig and chick (Sarkar, 1971), the conversion of lactate to glycogen in the rabbit hepatocyte is of secondary importance, the lactate being mainly oxidized (Drury & Wick, 1965) or excreted via the bile, or both (Le Bars *et al.* 1969; Vernay, 1986*b*). In arterial plasma, acetate and lactate are the only organic acids present in significant concentrations, the metabolism of these molecules occurs in peripheral tissues (Drury & Wick, 1965; Beauville *et al.* 1974; Jones & Parker, 1977, 1981). For the rabbit, as for the ruminant, acetate is mainly oxidized into carbon dioxide and it is a preferential fatty acid precursor as compared with glucose (Smith, 1975; Vézinhet & Nouguès, 1977).

A review of the literature suggests that the levels of bacterial metabolites in the blood of the rabbit are dependent on the animal's food supply. For example, in animals fed on commercial food the concentrations (mM) of VFA and lactate are 0·7–1·1 and 1·1–1·6 respectively (McMillan *et al.* 1975; Le Bars, 1976; Parker & Mould, 1977); in animals provided with oats and lucerne the amounts are higher: VFA 1·7–1·9 (Beauville *et al.* 1974; Bonnafous & Raynaud, 1978; Vernay & Marty, 1984) and lactate 3·9–8·8 (Alexander & Chowdhury, 1958; Kinsey, 1961; Stangassinger *et al.* 1982). This fact and the use of different experimental procedures (e.g. conscious or anaesthetized rabbits) may explain some of the discrepancies between our results and those from other authors, since it is admitted that anaesthesia affects blood flow (Katz & Bergman, 1969). However, according to the present investigation it is also apparent that the levels of bacterial metabolites in the blood of the rabbits depends on the periodicity of hard- and soft-faeces production.

For the rabbit there is a relation between the VFA absorption in the alimentary tract, the plasma level of aldosterone and the circadian pattern of the faecal excretion (Vernay *et al.* 1984). After administration of aldosterone to animals or in the course of hard-faeces production, during which the plasma concentration of aldosterone increased twofold, the hind-gut absorption of VFA and their metabolism, in the colonocyte and in the hepatocyte, are both enhanced (Vernay & Marty, 1984; Vernay *et al.* 1984; Vernay, 1985, 1986*c*). These observations may go a long way towards explaining the present results: VFA enrichment in portal blood is more important when the subject produces hard faeces and simultaneously hepatic uptake increases.

It can be concluded that under *ad lib.* feeding conditions, bacterial metabolite absorption and catabolism and the faecal excretion pattern all depend on the same circadian clock.

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