Origins of Blood Acetate in the Rat

By BRENDAN M. BUCKLEY* and DERMOT H. WILLIAMSON
*Metabolic Research Laboratory, Nuffield Department of Clinical Medicine, Radcliffe Infirmary,
Oxford OX2 6HE, U.K.*

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A novel enzymic cycling assay for the determination of acetate in biological material is described. Measurements of the acetate concentration in blood and liver samples from rats of various ages and nutritional states with this assay are reported. The contribution of the intestine, the liver and the rest of the body to maintaining the concentration of acetate in the circulation is examined. Evidence is presented that the gut flora constitute the main source of acetate in blood of fed adult rats, though endogenous production of acetate is of significance in other situations. The streptozotocin-diabetic rat has an elevated blood acetate concentration.

It has frequently been suggested that acetate in the circulation is produced endogenously by rat tissues. Preparations of brain (Coxon *et al.*, 1949), heart (Korkes *et al.*, 1952; Knowles *et al.*, 1974), mature erythrocytes (Hochheuser *et al.*, 1964), liver slices (Krebs & Johnson, 1937; Knowles *et al.*, 1974), liver tumours (Hepp *et al.*, 1966) and perfused liver (Seufert *et al.*, 1974a) have been reported to produce acetate from precursors of acetyl-CoA. Label from [¹⁴C]-oleate infused into obese human patients undergoing therapeutic starvation is incorporated into blood acetate (Seufert *et al.*, 1974b). In most of the work cited above it has been assumed that endogenous acetate production resulted from hydrolysis of acetyl-CoA by acetyl-CoA deacylase (EC 3.1.2.1), though Krebs & Johnson (1937) and Hochheuser *et al.* (1964) suggested that in their experimental systems it was due to the anaerobic dismutation of pyruvate. An important question is the physiological relevance of these experiments to our understanding of the control of blood acetate concentrations.

It is well established that rat liver can convert ethanol into acetate, and it is possible that ethanol produced by the microbial flora of the rat digestive tract (Krebs & Perkins, 1970) might be a source of endogenous acetate. The liver of a fed 200 g rat removes about 0.4 μ mol of ethanol/min from the portal blood (Krebs & Perkins, 1970), assuming a portal blood flow of about 10 ml/min (Dobson & Jones, 1952; Chatwin *et al.*, 1969). The total hepatic activity of acetyl-CoA synthetase (EC 6.2.1.1) is greatly in excess of this rate of ethanol uptake (Barth *et al.*, 1972; Buckley, 1974), and thus it is unlikely that appreciable quantities of acetate from ethanol oxidation escape the liver to enter the general circulation in the normal fed adult animal *in vivo*.

The observation that ethanol is produced by the gut flora (Krebs & Perkins, 1970) suggested that this site might also give rise to appreciable amounts of acetate. Elsdon *et al.* (1946) have shown that caecal contents of the pig and the rat contained high concentrations of volatile fatty acids, with acetate predominating. In addition, measurements of acetate by g.l.c. indicate that the concentration of acetate in the portal blood of the rat is higher than that in the arterial blood (Remesy & Demigne, 1974). It was decided therefore to examine the concentrations of acetate in rat blood taken from a variety of sites in the body and to study rats in different physiological conditions which might be associated with altered endogenous production of acetate.

An enzymic method was developed in the course of this study by which nanomole quantities of acetate can be measured in deproteinized extracts of blood and liver. The extensive preliminary treatment of samples involved in previous methods (Lundquist, 1963; Bergmeyer & Möllering, 1966, 1974, pp. 1520–1528) is not necessary with this technique.

Materials and Methods

Rats

Albino Wistar rats [Charles River (U.K.), Margate, Kent, U.K.] of both sexes were used, since no sexual differences in acetate concentrations were found. Pregnant rats were maintained in individual cages and newborn rats remained with their mothers until weaning (21 days). Fed animals received Oxoid breeding diet for rats and mice (Oxoid, London S.E.1, U.K.). Starved rats and those on antibiotic-supplemented diets were kept in mesh-bottomed cages to prevent coprophagy.

Diabetes was induced by the injection of 0.01 M-sodium citrate, pH 4.5, containing streptozotocin

* Present address: Department of Biochemistry, University College, Cork, Ireland.

(50 mg/kg body wt.) (a gift from The Upjohn Co., Kalamazoo, MI, U.S.A.), into a tail vein (Schein *et al.*, 1971). Control rats received citrate alone. Both diabetic and control rats received 5% (w/v) glucose to drink for 24 h after injection to overcome the transient hyperinsulinaemia caused by streptozotocin, and then were fed normally. All diabetic rats had a blood sugar concentration above 15 mm.

To decrease gut flora, neomycin sulphate (Glaxo Laboratories, Greenford, Middx., U.K.) was given *ad libitum* to some animals as a 7.5 g/litre solution in their drinking water. These animals also received the fungicidal agent nystatin (E.R. Squibb and Sons, Twickenham, Middx., U.K.) mixed with their solid food. Antibiotic administration in this manner was carried out for 5 days, by which time the animals developed marked gut changes, including caecal distension and gut-wall thinning, and were thus judged to possess greatly diminished gut flora.

Chemicals

Acetate kinase (EC 2.7.2.1), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), ATP, NADP⁺ and CoA were obtained from Boehringer Corp. (London), London W.5, U.K. Acyl phosphate-hexose phosphotransferase (EC 2.7.1.61) from *Aerobacter aerogenes* was a gift from Professor H. U. Bergmeyer, C. F. Boehringer und Soehne, Tutzing, W. Germany.

Blood

Mixed venous-arterial blood was obtained from the neck vessels of rats decapitated while conscious. In other experiments, blood was obtained from a hepatic vein, the hepatic portal vein, and either the left ventricle or abdominal aorta, in that sequence, from animals anaesthetized with pentobarbitone (50 mg/kg body wt.) (Abbott Laboratories, Queenborough, Kent, U.K.). A solution was prepared freshly for intraperitoneal injection by dissolving the pentobarbitone in 0.15 M-NaCl; commercially available solutions have a high ethanol content. Up to 0.3 ml of blood was removed slowly through 25-gauge needles inserted against the blood flow, from each of the sites mentioned. Blood samples were deproteinized with ice-cold 6% (w/v) HClO₄. The protein-free extract obtained by centrifugation was neutralized with 20% (w/v) KOH saturated with KCl, by using an internal indicator (Universal; British Drug Houses, Poole, Dorset, U.K.). Precipitated KClO₄ was removed by centrifugation (3000g for 10 min). The samples were kept ice-cold throughout this process to avoid volatilization of acetic acid. A perchlorate blank was always prepared concurrently with samples, by substituting water for sample.

Liver

Livers were freeze-clamped (Wollenberger *et al.*, 1960) from unanaesthetized rats killed by cervical dislocation. The frozen tissue was ground to a powder in a ceramic mortar, with frequent additions of liquid N₂, and was homogenized with 4 vol. of ice-cold 6% (w/v) HClO₄. After centrifugation (3000g for 10 min) at 4°C, the supernatant was neutralized with 20% (w/v) KOH saturated with KCl, by using an internal indicator. A perchlorate blank was always prepared concurrently, by using water instead of sample.

Acetate determination

An enzymic assay for tissue acetate was developed by using acyl phosphate-hexose phosphotransferase (Scheme 1). This enzyme catalyses the transfer of phosphate from acetyl phosphate to glucose. In the assay, acetyl phosphate is formed from acetate by acetate kinase. The product of the phosphotransferase reaction, glucose 6-phosphate, is oxidized by glucose 6-phosphate dehydrogenase and the resultant NADPH measured at 340 nm. As acetate is regenerated continually, its concentration is kept constant and the assay operates as a cycle, 1 equivalent of NADPH being formed in every revolution. The rate of the cycle is linearly related to the acetate concentration in the normal physiological range, owing to the high *K_m* of acetate kinase for acetate (0.3 M; Rose *et al.*, 1954) and the low *K_m* of acyl phosphate-hexose phosphotransferase for acetyl phosphate

Scheme 1. Sequence of reactions in cycling assay for acetate. Enzymes involved are (1) acetate kinase (EC 2.7.2.1), (2) acyl phosphate-hexose phosphotransferase (EC 2.7.1.61), (3) glucose 6-phosphate dehydrogenase (EC 1.1.1.49).

(0.5 mM; Bergmeyer & Möllering, 1974, pp. 1222–1227) and of glucose 6-phosphate dehydrogenase for glucose 6-phosphate (20 μ M; Lowry *et al.*, 1961).

The reagent mixture for the determination of acetate had the following composition: 40 mM-Tris/HCl (pH 8.5); 2 mM-MgCl₂; 0.6 mM-NADP⁺; 0.3 mM-2-mercaptoethanol; 5 mM-ATP; 2.5 mM-D-glucose; 45 nkat of glucose 6-phosphate dehydrogenase; 140 nkat of acetate kinase; 12 nkat of acyl phosphate-hexose phosphotransferase. The reagent mixture (100 ml), equilibrated at the assay temperature (30°C), was added to all cuvettes, mixed with sample (1.0 ml), and left for 5 min to permit reaction of pre-formed glucose 6-phosphate in the sample. The A_{340} was then recorded at intervals of 10 min over a period of 1 h at 30°C. Assay cycling rates were calculated as $\Delta A_{340}/\text{min}$ by determining the slope of the regression line of sample absorbance against time. The acetate concentration of each sample was calculated from a curve relating cycling rate to standard acetate concentrations determined simultaneously. Reagent blanks and acetate standards contained the same concentration of KClO₄ as samples to obviate any effect of perchlorate inhibition of the cycling rate on the assay.

This cycling assay for acetate maintained linearity of reaction rate for up to 60 min with acetate concentrations between 2.5 and 50 nmol/cuvette. Cycling rate is linearly related to acetate concentration in the range 0–50 nmol/cuvette, the linear regression coefficient for six standard concentrations (including zero) against cycling rates being always greater than 0.995 in this range. The recovery of 5 nmol of standard acetate added to blood samples of 0.05–1.0 ml/cuvette was always greater than 90%, and was usually greater than 95%. The series-to-series reproducibility of the assay as determined by the inclusion of a reference solution in each series of measurements was 96% over the set of experiments described here.

This assay has several notable advantages over previously available techniques. The indicator reaction generates NADPH rather than NADH, thereby minimizing interference by reactions of tissue metabolites with contaminating enzymes and eliminating the need for sample purification encountered by Lundquist (1963) and Bergmeyer & Möllering (1966, 1974, pp. 1520–1528). The reaction is cyclic and thus capable of an amplified response (Lowry & Passoneau, 1971). Unlike most cycling assays its progress can be observed continuously, enabling more precise calculation of results and facilitating the monitoring of linearity. The lowest limit of sensitivity of the assay is about 1 nmol/cuvette with a spectrophotometer, an order of magnitude lower than that of conventional end-point assays.

Like all cycling assays, this technique is susceptible to errors caused by activation or inhibition of the

rate-limiting reaction. Residual KClO₄ in samples prepared as described has an appreciable inhibitory influence on cycling rate. This is easily compensated for by use of blanks and standards containing KClO₄ in amounts equivalent to those present in the samples. An unknown inhibitor of the cycle is present in liver extracts, but use of small volumes of extract overcomes its influence satisfactorily, as judged by recovery studies.

Results and Discussion

Acetate concentrations in mixed arterial-venous blood

Concentrations of acetate in mixed arterial-venous blood are shown as a function of the age of the animal in Fig. 1. Suckling rats in the first 2 weeks of life had about 0.2 μ mol of acetate/ml of mixed blood, the concentration falling sharply during the third week by about 50% on day 18 of life. The acetate concentration rose again rapidly on weaning to about 0.2 μ mol/ml, and subsequently declined with age to 0.15 μ mol/ml in the fed adult. Starvation of adult rats for 48 h did not significantly affect the concentration of acetate in mixed arterial-venous blood.

Acetate concentrations in blood from specific internal sites

Concentrations of acetate in blood from arterial, hepatic-vein and hepatic-portal-vein sites in rats of different ages and nutritional states are shown in Table 1. The most striking feature is the high concentration of acetate in portal-venous blood of fed

Fig. 1. Concentrations of acetate in mixed rat blood from the cervical wound

Values are means $\pm$ s.d., expressed as μ mol/ml of blood, with the numbers of observations in parentheses. The values at 8, 14 and 18 days are significantly different ($P < 0.005$) from those for the fed adult rats. The symbol 'W' refers to the time of weaning, at 21 days of age.

Table 1. *Concentrations of acetate in arterial, hepatic-portal-vein and hepatic-vein blood in rats of different ages and states*

The acetate concentrations are mean values $\pm$ s.d., expressed as $\mu\text{mol/ml}$ of blood, with the numbers of observations in parentheses. Values which are significantly different (Student's *t* test) from the appropriate value for the fed adult are shown by: * $P < 0.05$; ** $P < 0.005$.

Condition of rats	Arterial ($\mu\text{mol/ml}$)	Hepatic portal vein ($\mu\text{mol/ml}$)	Hepatic vein ($\mu\text{mol/ml}$)
Fed adult	0.25 ± 0.07 (4)	0.79 ± 0.38 (10)	0.27 ± 0.13 (11)
48 h-starved adult	0.14 ± 0.04 (8)**	0.42 ± 0.17 (7)*	0.23 ± 0.07 (7)
18-day-old suckling	—	0.08 ± 0.04 (5)**	0.10 ± 0.02 (5)**
21-day-old suckling	0.10 ± 0.02 (3)*	0.34 ± 0.16 (4)*	0.26 ± 0.08 (4)
Weanling (3 days old)	0.22 ± 0.02 (3)	0.58 ± 0.14 (6)	0.29 ± 0.09 (6)
Antibiotic-treated adult	0.14 ± 0.03 (4)*	0.15 ± 0.05 (5)**	0.25 ± 0.06 (6)
Streptozotocin-diabetes	0.77 ± 0.41 (5)*	1.56 ± 0.65 (5)*	1.68 ± 0.44 (5)**

adult and weanling rats. This indicates, in confirmation of the findings of Remesy & Demigne (1974), that a considerable amount of acetate is absorbed from the intestine. Concentrations in blood from the portal-vein and hepatic-vein sites in the 18-day-old suckling rat are lower than in the adult, whereas the concentrations 3 days later (21-day-old suckling rat) are greater, especially in the portal-vein blood. This is probably due to the difference in floral colonization of the gut between the 18-day-old suckling rat, whose diet is predominantly milk, and the 21-day-old suckling rat, whose diet contains both milk and solid high-carbohydrate rat diet. Starvation of adult rats decreases acetate concentrations significantly ($P < 0.05$) in both arterial and hepatic-portal-vein blood. Knowles *et al.* (1974) reported similar arterial acetate concentrations to those in Table 1 and found a decrease on prolonged starvation (72 h). The decrease in the portal-vein-blood acetate is likely to be due to a decreased output of acetate by the gut flora consequent on nutrient deprivation.

Acetate concentrations in blood from antibiotic-treated rats

The hypothesis that the gut flora constitute a major source of acetate in fed adult rats was tested by feeding adult rats with a normal diet supplemented with the antibacterial agent neomycin and the fungicide nystatin, and measuring acetate concentrations in blood from the three sites described above (Table 1). It is clear that abolition of the gut flora by antibiotics lowers the acetate concentration of portal-vein blood to that of the arterial blood, which in turn is significantly lower than that of normal fed rats. It is thus apparent that a decrease in the portal-vein supply of acetate greatly diminishes the size of the circulating pool. It is noteworthy that the acetate concentration in hepatic-venous blood in the treated animals did not differ from that of controls, suggesting that there is a net output of acetate from the livers of the treated group.

Production and utilization of acetate

(a) *Intestinal production of acetate.* The arterial-hepatic-portal-vein difference in acetate concentration is a measure of its uptake from the gut, and these values can be used to estimate the rates of addition of acetate to the portal-vein circulation (Table 2). In the young and the antibiotic-treated rats a number of extrapolations have been made from published data for adult rats on the relationship of blood flow to liver weight (Dobson & Jones, 1952; Chatwin *et al.*, 1969), so these estimates should be interpreted with caution.

The calculations show that the addition of acetate to the blood from the gut of the fed rat is considerable, a 250 g adult rat receiving almost $10 \mu\text{mol}$ of acetate/min from this source (Table 2). The 48 h-starved rat receives about 35% of this amount. Antibiotic-treated rats show a negligible addition of acetate to the portal-vein blood, as do 18-day-old rats. Production of acetate by the gut contents appears to begin at about the time of weaning.

(b) *Hepatic uptake and output of acetate.* Portal-vein-hepatic-vein differences in blood acetate concentration are shown in Fig. 2, as a function of the portal-vein concentration of acetate. The portal-hepatic-venous difference is linearly related to the portal-vein concentration, with a correlation coefficient $r = 0.92$ for 43 data points from observations on fed, starved, suckling, weaned and antibiotic-treated rats. An important feature is that the results show that the liver sheds acetate into the hepatic vein when portal-vein concentration is below a minimum value of about $0.2 \mu\text{mol/ml}$, which is close both to the concentration of acetate in the fed adult liver (see below) and to the K_m for acetate of hepatic acetyl-CoA synthetase (Sladek, 1971; Buckley, 1974).

An estimate of the rate of hepatic uptake of acetate may be made by using the relationship:

$$\text{Uptake } (\mu\text{mol/min}) = (A_p \times F_p) + (A_a \times F_a) - (A_v \times F_v)$$

Table 2. Differences in acetate concentrations in blood taken from different sites, and estimated rates of acetate uptake or output by gut, liver and peripheral tissues, in rats of different ages and states

Concentration differences for acetate are mean values $\pm$ s.d., expressed as $\mu\text{mol/ml}$, with the numbers of observations in parentheses. Estimates of flux rates are expressed as $\mu\text{mol/min}$ per 100 g body wt., and have been calculated as described in the text. The rates are merely estimates, and so are presented without statistical analysis. The concentration differences are compared with values for the fed adult; values which are significantly different (Student's *t* test) are shown by: **P* < 0.05; ***P* < 0.005. Each observation represents measurements from a single rat. Negative values for the concentration difference denote higher acetate concentrations at the input site than at the output site. Negative uptake values denote output of acetate.

Condition of rats	Arterial-portal differences ($\mu\text{mol/ml}$)	Rate of output from gut ($\mu\text{mol/min}$ per 100 g body wt.)	Portal-vein-hepatic vein difference ($\mu\text{mol/ml}$)	Rate of uptake by liver ($\mu\text{mol/min}$ per 100 g body wt.)	Hepatic-vein-arterial difference ($\mu\text{mol/ml}$)
Fed adult	0.8 ± 0.3 (3)	3.6	-0.51 ± 0.34 (10)	2.3	-0.12 ± 0.07 (5)
48 h-starved adult	0.3 ± 0.2 (6)*	1.3	-0.19 ± 0.16 (7)*	0.7	-0.12 ± 0.08 (5)
Antibiotic-treated adult	0.0 ± 0.0 (5)**	0.0	0.09 ± 0.09 (5)**	-0.6	-0.10 ± 0.08 (4)
18-day-old suckling	—	—	0.01 ± 0.04 (5)*	-0.1	—
21-day-old suckling	—	—	0.01 ± 0.01 (5)*	0.05	-0.09 ± 0.06 (3)
Weanling (3 days old)	0.3 ± 0.1 (5)	1.2	-0.29 ± 0.14 (6)*	1.2	-0.13 ± 0.03 (4)
Streptozotocin-diabetes	0.8 ± 0.6 (5)	3.3	0.11 ± 0.52 (5)*	-3.3	-0.91 ± 0.48 (5)*

Fig. 2. Correlation of hepatic-portal-vein-hepatic-vein differences of acetate concentration with the acetate concentration in portal-vein blood of rats of various ages and nutritional states
The individual values are from the experiments described in Table 1. The regression coefficient *r* = 0.92 (*n* = 43).

where *A_p*, *A_a* and *A_v* denote portal-venous, arterial and hepatic-venous acetate concentrations respectively, and *F_p*, *F_a* and *F_v* denote portal-venous, arterial and hepatic-venous blood flows. Estimates of hepatic acetate uptake and output based on haemodynamic data of Dobson & Jones (1952) and Chatwin *et al.* (1969) are presented in Table 2. Uptake is

Fig. 3. Correlation of hepatic vein-arterial differences of acetate concentration with the acetate concentration in the hepatic vein
Values shown (○) are for non-diabetic rats of various ages and nutritional states; ■ indicate values for diabetic rats. Regression coefficient, *r* = 0.90 (*n* = 20).

considerable in the fed adult and is decreased in the starved adult. The developmental pattern suggests a possible change-over from hepatic acetate production to utilization in the late suckling period as the pup nears weaning and begins to eat solid food. Antibiotic-treated adult rats show a significant hepatic acetate output.

(c) *Peripheral uptake of acetate.* The difference in acetate concentrations in arterial and hepatic-vein blood is significantly correlated with the hepatic-vein concentration, *r* = 0.90 for 20 observations (Fig. 3). If the hepatic vein is considered as the input route of acetate to the peripheral tissues, and the arteries as the return route from these tissues back to the liver, the hepatic-venous-arterial difference in acetate

concentration may be considered to be an indicator of peripheral acetate uptake.

Acetate concentrations in the streptozotocin-diabetic rat

The liver in the diabetic rat oxidizes fatty acids at a high rate. Rats made diabetic with streptozotocin eat a certain amount of food (Blackshear & Alberti, 1974) and thus support a gut flora. The activity of acetyl-CoA synthetase in diabetic rat liver is decreased (Kornacker & Lowenstein, 1965a; Murthy & Steiner, 1972). The animal thus represents a state in which portal-vein supplies of acetate are likely to be high, acetate production from endogenous hepatic sources may be elevated, and the capacity of the liver to utilize acetate is limited. It was thus expected that acetate concentrations in diabetic-rat blood would be greatly elevated, and that the relationships of uptake, production and concentration obtaining in the normal rat would be disturbed.

Concentrations of acetate in blood from the portal and hepatic veins and the aorta of diabetic rats are shown in Table 1, together with the corresponding values for fed adult controls for comparison. It is clear that acetate concentrations are indeed greatly elevated in blood from all sites in the diabetic rats. The large variation in individual values, as shown by the standard deviations, for the diabetic animals probably reflects individual differences in food intake and severity of the diabetes.

Use of these data permits estimation of the production and utilization of acetate by various tissues in the diabetic rat. Differences in acetate concentration across the portal bed, and estimates of exchange rates of acetate between blood and organs at these sites, are shown in Table 2 for control and diabetic rats. An indication of peripheral utilization is given by hepatic-vein–arterial differences, which are also shown. Acetate uptake from the gut is similar in diabetic rats and in controls. However, the diabetic liver sheds acetate at about $1.5 \mu\text{mol/min}$ per 100 g body wt., unlike livers of normal fed rats, which remove it. Data from diabetic animals lie well outside 99%-confidence limits for the linear regression of hepatic–portal-vein–hepatic-vein differences against hepatic–portal-vein concentration for non-diabetic rats (Fig. 2), indicating that diabetes alters the characteristics of acetate exchange between blood and liver, though a linear relationship may obtain in diabetes ($r = 0.84$) between hepatic–portal-vein acetate concentration and hepatic uptake or output. A marked increase in peripheral utilization of acetate in diabetes is indicated by the 3-fold elevation of hepatic-vein–arterial concentration differences. However, data for diabetic rats fit the regression of these concentration differences against hepatic-vein concentration for non-diabetic animals (Fig. 3), suggesting

that increased peripheral uptake in diabetes is simply due to increased circulating concentrations.

The data presented above on the marked elevation of circulating acetate concentrations in streptozotocin-diabetic rats are in agreement with findings of elevated plasma acetate concentrations in alloxan-diabetic rats by Hochheuser *et al.* (1964), elevated production of acetate by the perfused alloxan-diabetic rat liver (Seufert *et al.*, 1974a) and elevated blood acetate concentrations in diabetic humans (Seufert *et al.*, 1974b). Knowles *et al.* (1974), however, observed only a slight increase in blood acetate concentrations in alloxan-diabetics.

Acetate concentrations in liver

The concentrations of acetate found in freeze-clamped liver from fed adult rats and 21-day-old suckling rats, together with values previously reported by other workers, are given in Table 3. Values found in the present study are in best agreement with those of Hochheuser *et al.* (1964). In the reports by Bergmeyer & Möllering (1966) and Murthy & Steiner (1973) it is not clear whether the livers were freeze-clamped. Knowles *et al.* (1974) draw attention to difficulties experienced with their assay in liver extracts. The similarity between acetate concentrations found in the present study in livers from fed adult rats and 21-day-old sucklings, in contrast with the large differences in blood concentrations (see above), suggests that the hepatic acetate concentration is regulated by intracellular mechanisms rather than by the availability of acetate in the circulation.

Conclusions

The data presented above suggest that substantial amounts of acetate are provided in the fed adult rat

Table 3. *Concentrations of acetate in rat liver*

The concentrations of hepatic acetate found in the present study are expressed as mean values $\pm$ S.D., with the numbers of observations in parentheses. Each observation represents the mean of duplicate measurements on an individual rat.

Condition	Hepatic [acetate] of rats ($\mu\text{mol/g}$ wet wt.)	Reference
21-day-old suckling	$0.21 \pm 0.04(4)$	Present study
Fed adult	$0.21 \pm 0.04(4)$	
Fed adult	0.17	Hochheuser <i>et al.</i> (1964)
Fed adult	0.47	Bergmeyer & Möllering (1966)
Fed adult	0.40	Murthy & Steiner (1973)
Fed adult	0.63	Knowles <i>et al.</i> (1974)

by the gut flora and that endogenous production of acetate by tissues, in particular the liver, is of less importance in this situation, contrary to prevailing belief (Hepp *et al.*, 1966; Ballard, 1972; Knowles *et al.*, 1974; Seufert *et al.*, 1974a,b). The estimated uptake of acetate by the fed liver (about 1 $\mu\text{mol}/\text{min}$ per g wet wt.) could account for an appreciable proportion of the substrate supply for fatty acid synthesis, which proceeds in rats fed on a standard commercial diet at a rate of about 3 μmol of C_2 units/min per g wet wt. (Brunengraber *et al.*, 1973). Acetate produced by the gut flora must therefore be considered to be a physiological substrate in the fed rat. Hepatic acetate production was observed in 18-day-old suckling rats and in fed adults treated with antibiotics. Such production indicates that a certain amount of acetate formation from endogenous precursors occurs even in the fed liver. However, this endogenous acetate only contributes to circulating concentrations when the concentration gradient between blood and liver is favourable. The present study is thus in agreement with reports by Hepp *et al.* (1966), Knowles *et al.* (1974) and Seufert *et al.* (1974a,b) insofar as the ability of rat liver to form acetate endogenously is concerned, but it is our contention that such formation is only of importance when the availability of acetate from the portal blood is low. Factors which regulate the net release of acetate from the liver when the portal-vein-blood concentration of acetate is low include the relative activities of acetyl-CoA hydrolase (EC 3.1.2.1) and acetyl-CoA synthetase (EC 6.2.1.1) and the rate of production of acetyl-CoA from endogenous sources other than acetate. In the diabetic liver, both the concentration of acetyl-CoA (Knowles *et al.*, 1974) and the activity of acetyl-CoA hydrolase (Seufert *et al.*, 1974a) are increased, and activity of acetyl-CoA synthetase is decreased (Kornacker & Lowenstein, 1965a,b; Murthy & Steiner, 1972). Thus hepatic generation of acetate is elevated and its utilization is decreased. The fact that the acetyl-CoA hydrolase of rat liver may be an artifact represented by the combined action of carnitine acetyltransferase (EC 2.3.1.7) and acetyl-carnitine hydrolase (Costa & Snoswell, 1975) does not alter the above considerations.

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