CMLS, Cell. Mol. Life Sci. 54 (1998) 1277–1282
1420-682X/98/111277-06 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Research Article

Oxygen consumption and biosynthetic function in perfused liver from rats at different stages of development

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Received 19 June 1998; received after revision 11 September 1998; accepted 11 September 1998

Abstract. Changes in mitochondrial function were studied in perfused liver from rats aged 24–365 days. Oxygen consumption together with the rates of gluconeogenesis, urea synthesis and ketogenesis were determined. Basal mitochondrial respiration as well as the ability of the liver to synthesize glucose, urea and ketone bodies declined from 24- to 365-day-old rats. On the other hand, on transition from 24 to 60 days the liver oxidation rate of hexanoate, sorbitol and glycerol is enhanced, but not of ketone bodies or palmitate. Our results show that the transition from weaning to middle age is accompanied by defined changes in hepatic substrate oxidation. From the observed time course of the decrease in basal and substrate-stimulated oxygen consumption, it is concluded that in rat liver cells a decline in respiratory chain function, long-chain fatty acid and ketone body metabolism, gluconeogenesis and ureogenesis occurs at a relatively early life stage.

Key words. Liver perfusion; oxygen consumption; gluconeogenesis; ureogenesis; ketone bodies.

It is well known that significant changes in hepatic mitochondrial function occur in growing rats. For instance, in neonatal rats an increase in the state 3 respiration rate has been found [1], while the suckling-weaning transition is essentially characterized by a decline in mitochondrial fatty acid oxidation [2]. We have found in the transition from weaning to adolescence (60 days of age) an increase in the state 3 respiration rate in liver mitochondria using nicotinamide-adenine dinucleotide (NAD)- and flavin-adenine dinucleotide (FAD)-linked substrates [3, 4]. Finally, liver mitochondria isolated from old rats show a fall in the state 3 respiration rate and in the maximal rate of adenosine 5′-triphosphate (ATP) synthesis [5, 6]. These age-related changes were found in experiments using isolated mitochondria. However, it is feasible that the changes observed in isolated mitochondria do not reflect the mitochondrial function in intact liver cells, since mitochondrial-cytoplasmic interactions are ignored.

Isolated hepatocytes and perfused liver are more appropriate models for studies of bioenergetic activation mechanisms in developing rats. In this study we used the perfused liver system, in which the hepatocytes are embedded in their natural environment, showing the morphological and functional polarity between vascular and biliary systems with only a small surface area for exchange with blood oxygen [7]. Since oxygen supply is critical in maintaining mitochondrial function, this metabolic model appears to be more realistic than iso-

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lated organelles, which experience much higher oxygen concentrations than in vivo [8]. In isolated liver per-fused with carbogen-gassed Krebs-Henseleit bicarbonate buffer, most liver functions can be maintained for several hours [7].

Age-related changes in mitochondrial performance were studied in perfused liver from rats at different stages of development: weaning (24 days), adolescence (60 days), adulthood (180 days) and middle age (365 days). We have determined the rate of oxygen consumption which was stimulated by various substrates, together with the rate of gluconeogenesis, urea synthesis and ketogenesis, pathways which are critically dependent on mitochondrial function.

Materials and methods

Animals. Male Wistar rats bred in the animal house of Heinrich Heine University of Dusseldorf were used for the experiments. They were housed at 24 °C under an artificial circadian 12 h lightness/12 h darkness cycle, with ad libitum access to water and a standard stock diet. Livers were removed at different developmental stages of the rats (see above).

Liver perfusion. Perfusions were performed on livers from rats fasted 24 h that had free access to water. During the 24-h fasting, liver glycogen is reduced to a level that has been shown previously to minimize glucose production via glycogenolysis [9].

Rats were anaesthetized with sodium pentobarbital (Nembutal, Sanofi, Bad Segeberg, Germany) by intraperitoneal injection of 12 mg/100 g body weight in isotonic solution. Before opening the abdomen, heparin (250 international units (IU)/100 g) was injected into the femoral vein. Livers were perfused 'in situ' with Krebs-Henseleit bicarbonate buffer [10], pH 7.4, in a nonrecirculating system [11]. The fluid was pumped through a temperature-regulated (37 °C) membrane oxygenator supplemented with 95% O_2/5% CO_2, v/v, prior to entering the liver via a cannula inserted into the portal vein. The arterial oxygen concentration, maintained constant by the oxygenator, was measured before and after each experiment. The effluent perfusate was collected via a cannula placed in the vena cava. It passed by a platinum electrode for continuous monitoring of the venous oxygen concentration, and perfusate samples were collected every 1–2 min for metabolite analyses. The flow rate was adjusted to the metabolic activity of the liver as judged from the venous oxygen concentration. It varied from experiment to experiment between 5 and 6 ml $\times$ min^{-1} $\times$ g^{-1}, but it was constant in each individual experiment. Substrates were added to the perfusion fluid before it entered the liver. The final concentrations of substrates were: β-hydroxybutyrate 0.5 mM + acetoacetate 0.5 mM, lactate + pyruvate 2 mM at a ratio lactate/pyruvate of 7, NH_4HCO_3 1.2 mM, hexanoate 0.5 mM, hexanoate 0.5 mM + lactate 11 mM, palmitate 0.5 mM, glycerol 12 mM and sorbitol 10 mM. Palmitate was dissolved in a bovine serum albumin (BSA) solution: 1 g of palmitate was slowly added to 170 ml of albumin solution (20 g/100 ml) under gentle stirring which was continued for a further 2 h at room temperature.

Before addition of the substrates the liver was perfused for 10–15 min until a steady state was reached, that is, the oxygen consumption rate remained constant. Following the addition of each substrate, the liver was allowed to reach steady state before further additions (ca. 10–15 min).

Following perfusion, the wet weight of the liver was determined.

Metabolic rates. Glucose, ammonia, urea, lactate, pyruvate, β-hydroxybutyrate and acetoacetate concentrations were measured in percloric acid extracts of the perfusate samples using enzymatic analysis by spectrophotometric procedures coupled to the appearance or disappearance of nicotinamide-adenine dinucleotide (NADH) [12]. The steady-state metabolic rates were calculated from the arteriovenous concentration differences of oxygen, glucose, urea, β-hydroxybutyrate and acetoacetate, the flow rate, and the liver wet weight.

Statistical analysis. Data are given as means $\pm$SEM of different rats. Statistical analyses were performed by one-way analysis of variance (ANOVA). Post-hoc comparisons between group pairs were made with the Tuckey test after ANOVA had established significant differences among groups. Probability values <0.05 were considered to indicate a significant difference.

Materials. β-hydroxybutyrate, acetoacetate, lactate, pyruvate, NH_4HCO_3, hexanoate, palmitate, glycerol, sorbitol, β-hydroxybutyrate dehydrogenase, NAD, NADH, ATP, ADP, nicotinamide-adenine dinucleotide phosphate (NADP), lactate dehydrogenase, glucose-6-phosphate dehydrogenase, hexokinase, glutamate dehydrogenase, urease, and fatty acid free BSA were from Boehringer (Mannheim, Germany) or Sigma (Munich, Germany). Other chemicals were from Merck (Darmstadt, Germany). All reagents were of the highest purity commercially available.

Results

In each experiment, the liver was perfused 'in situ' in the absence of exogenous substrates, and basal oxygen consumption was measured. Then various combinations of substrates were added. The addition of substrates to perfused liver caused a rapid (<1 min) increase in oxygen consumption (ΔO_2).

Basal oxygen consumption (table 1), as well as ΔO_2 with hydroxybutyrate + acetoacetate or palmitate as

Table 1. Oxygen consumption and metabolic rates in perfused livers from rats at different ages.

Addition	24 days	60 days	180 days	365 days
	μmol/h per gram wet liver			
O_2 consumption				
None	164 ± 5	134 ± 2*	124 ± 2*	88 ± 2*†‡
L + P	240 ± 7	193 ± 3*	174 ± 3*†	122 ± 3*†‡
L + P + N	205 ± 5	158 ± 3*	144 ± 2*	104 ± 3*†‡
Gluconeogenesis				
None	10.4 ± 0.4	9.6 ± 1.1	11.3 ± 0.3	13.2 ± 1.2†
L + P	71 ± 2.8	59 ± 1.9*	51.9 ± 1.3*†	42.8 ± 1.2*†‡
Urea synthesis				
None	27 ± 2	12 ± 2*	16 ± 2*	13 ± 1*
L + P + N	91 ± 9	62 ± 2*	63 ± 4*	51 ± 1*

Results are expressed as mean ± SEM of 6 to 11 experiments. L, lactate; P, pyruvate; N, NH_4HCO_3. *$P < 0.05$ compared with 25 days. †$P < 0.05$ compared with 60 days. ‡$P < 0.05$ compared with 180 days.

substrate significantly decreased in 60-, 180- and 365-day-old rats compared with rats of 24 days of age (fig. 1). Using hexanoate as substrate, ΔO_2 significantly increased in 60-day-old rats compared with 24-day-old rats; in 180-day-old rats it returned to the value exhibited by 24-day-old rats, and it decreased further in 365-day-old rats (fig. 1). When lactate was added to perfusion medium containing hexanoate, a further increase in ΔO_2 was achieved (by about 70% in 24-day-old rats and 45% in 60-, 180- and 365-day-old rats) (fig. 1). Lactate is a gluconeogenic substrate which increases ADP availability and, thus, respiration. However, ΔO_2 was lower in perfused liver from 180- and 365-day-old rats than in that from younger animals (fig. 1). As with hexanoate, ΔO_2 with sorbitol and glycerol increased in 60- and 180-day-old rats compared with 24-day-old rats and decreased in 365-day-old rats compared with 60- and 180-day-old rats (fig. 1).

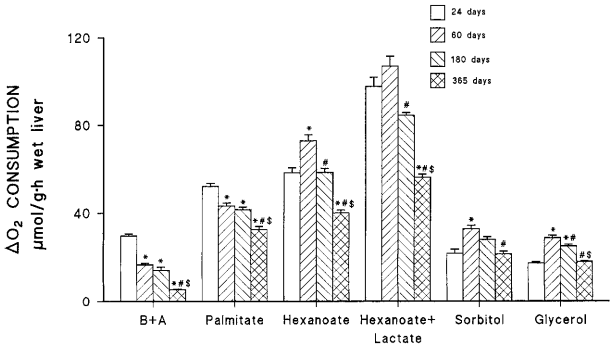

Figure 1. Substrate-induced increase in oxygen consumption (ΔO_2) measured in perfused liver from rats at different ages. Values are the mean ± SEM of 6 to 11 different rats. *$P < 0.05$ compared with 25 days. #$P < 0.05$ compared with 60 days. \$$P < 0.05$ compared with 180 days.

Gluconeogenesis and oxygen consumption were measured both without substrates and following addition of lactate + pyruvate, in the presence of 1 mM hydroxybutyrate + acetoacetate to saturate respiration (table 1). It should be noted that hydroxybutyrate + acetoacetate did not interfere with gluconeogenesis; in fact, the addition of hydroxybutyrate + acetoacetate to Krebs-Henseleit buffer had no effect on gluconeogenesis (not shown). Gluconeogenesis and oxygen consumption with lactate + pyruvate significantly decreased with age, whereas basal gluconeogenesis did not significantly change (table 1).

The rate of urea synthesis in the absence of exogenous substrates was significantly lower in perfused liver from of 60-, 180- and 365-day-old rats than in 24-day-old rats (table 1). To get a reasonable high rate of urea synthesis, it is necessary to add a nitrogen source together with a precursor for oxalacetate. Therefore, ammonia and lactate + pyruvate were added to prevent a possible shortage of intermediates and energy. The production of urea was increased in all groups, but it also significantly declined in rats older than 24 days (table 1). Finally, the rate of basal ketogenesis did not change with age, whereas the hydroxybutyrate/acetoacetate ratio significantly increased in 365-day-old rats only (table 2). Although hexanoate and palmitate enhanced ketone body production in all groups, it declined successively from 60 days to 365 days. With fatty acids the redox ratio of hydroxybutyrate to acetoacetate significantly increased only in 365-day-old rats with a transient decrease at 60 days and 180 days (significant with palmitate, table 2).

Discussion

In this study we have investigated oxygen uptake, urea synthesis, gluconeogenesis and ketone body production

Table 2. Ketogenesis and B/A ratio in perfused livers from rats at different ages.

Addition		24 days	60 days	180 days	365 days
		μmol/h per gram wet liver			
None					
	A + B	28.6 ± 0.7	24.1 ± 1.1	25.3 ± 1.5	28.1 ± 1.1
	B/A	0.18 ± 0.03	0.21 ± 0.01	0.21 ± 0.01	0.27 ± 0.02*
Hexanoate					
	A + B	200 ± 1	140 ± 6*	138 ± 3*	109 ± 1.5*†‡
	B/A	1.08 ± 0.11	0.99 ± 0.15	1.44 ± 0.16	1.73 ± 0.07*†
Palmitate					
	A + B	92.2 ± 1.1	56.1 ± 2.9*	66.8 ± 3.4*†	64.9 ± 0.9*
	B/A	1.64 ± 0.21	0.49 ± 0.03*	0.77 ± 0.11*	1.98 ± 0.11*†‡

Results are expressed as mean ± SEM of five to eight experiments. B, β-hydroxybutyrate; A, acetoacetate. *$P < 0.05$ compared with 25 days. †$P < 0.05$ compared with 60 days. ‡$P < 0.05$ compared with 180 days.

in perfused liver from rats at different stages of development. These metabolic pathways are dependent on liver cell mitochondrial function.

The 24-day-old rats represent animals just after weaning. The two intermediate age groups (60 and 180 days) represent animals in the young to young adult stage of lifespan. Finally, the 365-day-old rats represent animals in middle age. These developmental stages allow us to detect adaptive changes in mitochondrial performance without complications due to the late ageing process and accompanying diseases.

Basal respiration. Oxygen consumption was determined to elucidate the adaptation of energy supply during development. In fact, livers from fasted rats have only one ATP-producing system, the mitochondrial respiratory chain, due to absence of glycolysis. The results show that basal oxygen consumption, measured without added substrates, decreased with age (table 1). This decrease could reflect (i) lower energy requirements, and/or (ii) a failure in substrate supply to the electron transport chain and/or (iii) an age-dependent decrease in respiratory chain activity.

Additional information on the bionergetic state of liver cells can be obtained considering the NADH/NAD redox state, which is closely related to respiratory activity [13]. We used the hydroxybutyrate/acetoacetate ratio measured in the effluent perfusate as an index of mitochondrial NADH/NAD ratio (table 2) [14]. This is possible because ketone bodies are thought to pass freely through the cellular and mitochondrial membranes [15]. The mitochondrial redox state was always higher in older rats compared with 24-day-old rats, although the increase became significant only in 365-day-old rats (table 2). The increase in mitochondrial redox state is not due to cellular hypoxia, since a membrane oxygenator gave an efficient oxygenation (about 90% saturation of the medium) [7], and, additionally, the flow rate was adjusted to the metabolic activity of the liver. From the higher hydroxybutyrate/acetoacetate ratio in older rats we can exclude the possibility that the decreased basal respiration is due to a decrease in mitochondrial NADH production. Rather, it appears that NADH oxidation by the respiratory chain is reduced. This reduction in NADH oxidation is probably due to lower energy requirements [e.g. the decrease in basal urea synthesis found in older rats compared with 24-day-old rats (table 1)] at least in 60-day-old rats, since in these rats we have previously found an increase in the state 3 respiration rate in isolated liver mitocondria using NAD- and FAD-linked substrates [3, 4]. The further decrease in basal respiration found in 365-day-old rats could also indicate an impairment in respiratory chain activity.

Substrate-dependent increase in respiration. Oxygen consumption was also measured with added substrates to involve different dehydrogenases, different carriers and different sites of entry of reducing equivalents into the respiratory chain (fig. 1). When the livers are perfused with hydroxybutyrate + acetoacetate, the observed increase in oxygen consumption is due only to an increase in NADH production resulting from hydroxybutyrate oxidation to acetoacetate. However, in 24-day-old rats, we measured not only the highest ΔO_2 (fig. 1) but also a ketone body consumption rate of 45.4 ± 3.4 μmol/g per hour, indicating that in 24-day-old rats, ketone bodies are converted back into acetyl coenzyme A (CoA). This suggests that in young rats the enzyme which catalyses the rate-limiting step of the degradation of ketone bodies, 3-oxoacid-CoA-transferase, appears to be present. This result is quite unexpected; in fact, it is known that only foetal liver has the capability to oxidize ketones [16], whereas 3-oxoacid-CoA transferase is not present in liver of adult rats [17, 18]. Therefore, the failure of the liver to metabolize the ketone bodies may be peculiar to adult animals.

ΔO_2 with hexanoate was significantly increased in 60-day-old rats compared with 24-day-old rats (fig. 1). Hexanoate enters the mitochondrial matrix independent

from the carnitine system [19, 20]; therefore, the greater ΔO_2 following hexanoate addition could be due to an increase in the intramitochondrial β-oxidation pathway and/or respiratory chain activity. The latter possibility is more likely: in fact, ketogenesis with hexanoate decreased in 60-day-old compared with 24-day-old rats (table 2), and we have previously found increased oxygen consumption with NAD- and FAD-linked substrates in liver mitochondria isolated from 60-day-old rats. When respiration was stimulated by palmitate, ΔO_2 in 60-day-old rats significantly decreased compared with younger animals (fig. 1), despite the probable improvement in the activity of the respiratory chain (see above), suggesting a decline in the acyl-CoA synthetase and/or carnitine acyltransferase. Since we previously found an increase in the state 3 respiratory rate using palmitoyl-CoA + carnitine + malate as substrate in isolated liver mitochondria from 60-day-old rats compared with 24-day-old rats, there seems to be no reason to suggest any decline in carnitine acyltransferase. In preliminary experiments carried out in liver homogenate, we showed no variation in peroxisomal fatty acid oxidation using palmitoyl CoA in 60-day-old rats compared with 24-day-old rats. Therefore, we assume that the above-mentioned decrease in ΔO_2 is not caused by a failure in peroxisomal fatty acid oxidation.

The additional decrease in ΔO_2 with hexanoate or palmitate found in older rats (fig. 1) may then be caused by a fall in the activity of the respiratory chain, in line with the observation of an increased hydroxybutyrate/acetoacetate ratio found in these rats (table 2). Our findings about hepatic fatty acid degradation are consistent with the general view that the capacity of the liver to degrade long-chain fatty acids is higher in very young animals than at other stages of life [21]. Accordingly, the highest rate of ketogenesis from hexanoate or palmitate was found in 24-day-old rats (table 2). Preferential channelling of fatty acids through the triacylglycerol synthetic pathway may increase with age, since long-chain acyl-CoA synthetase activity was found to be decreased in liver mitochondria and increased in liver microsomes in old rats [22].

As far as glycerol and sorbitol are concerned, whose oxidation is known to be linked mainly to FADH oxidation [23], as with hexanoate, an increase in ΔO_2 was found in 60-day-old rats compared with 24-day-old rats (fig. 1). This finding can be explained taking into account that in previous work we have found a significant increase in the activity of the respiratory chain from complex II onwards [4] and cytochrome content [3] in isolated mitochondria from 60-day-old rats compared with 24-day-old rats. On the other hand, ΔO_2 with glycerol and sorbitol, like that with hexanoate or palmitate, decreased in older rats (fig. 1).

Gluconeogenesis and urea synthesis. We also measured the rate of biochemical pathways that are critically dependent on mitochondrial function, like gluconeogenesis and urea synthesis.

Our experiments show an age-related decline in the ability of the liver to synthesize glucose from lactate + pyruvate (table 1), suggesting that the gluconeogenic pathway may be altered during development. Also, ΔO_2 with hexanoate + lactate or lactate + pyruvate (fig. 1, table 1) as substrates diminished with increasing age, consistent with the general notion that the liver declines in its ability to maintain blood glucose homoeostasis with age [24, 25]. Both effects, diminished gluconeogenesis and diminished respiration, can indicate either inhibition of respiration and/or inhibition of gluconeogenesis. Thus it has been reported that phosphoenolpyruvate carboxykinase (PEPCK) becomes limiting during ageing; however, this observation has been reported only for older animals (22 months of age) [26, 27].

To test if other mitochondrial functions were affected by increasing age, we determined urea synthesis, which is an exclusive biosynthetic function of the liver. We found in the youngest rats a high synthetic capacity which significantly decreased in 60-day-old rats and then remained constant (table 1). This finding is in agreement with previous investigations in isolated hepatocytes which showed that urea synthesis was not affected by ageing in rats [28].

Conclusions

Our present data show that in perfused liver the most important stages of development are associated with defined changes in liver metabolic pathways involving mitochondrial function probably in order to satisfy actual body needs. Whereas basal oxygen consumption gradually declines with age accompanied by a decrease in energy-consuming pathways such as urea synthesis and gluconeogenesis, substrate oxidation via the respiratory chain increases during the first 2 months of life and then continuously decreases. This suggests an increasing impairment of respiratory enzymes with age, which could in turn inhibit mitochondrial energy-consuming pathways, such as gluconeogenesis, although a decrease in gluconeogenic enzymes cannot be excluded.

The rate of mitochondrial free-radical generation is dependent on the activity of the electron transport chain, and it increases as complete reduction of oxygen is impaired [29]; thus a decrease in respiration at a relatively early rat life stage would further enhance the rate of free-radical production, giving rise to the cellular macromolecular damage that is responsible for cellular ageing.

Acknowledgements. The authors wish to thank Prof. Antonio Barletta for helpful discussions and Sonja Grundel for technical assistance. This work was supported by a grant from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica of Italy.

1 Aprille J. R. and Asimakis G. K. (1980) Postnatal development of rat liver mitochondria: state 3 respiration, adenine nucleotide translocase activity and the net acccumulation of adenine nucleotides. Arch. Biochem. Biophys. **201:** 564–575
2 Quant P. A., Robin D., Robin P., Ferre P., Brand M. D. and Girard J. (1991) Control of hepatic mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase during the foetal/neonatal transition, suckling and weaning rats. Eur. J. Biochem. **195:** 449–454
3 Lionetti L., Iossa S., Liverini G. and Brand M. D. (1998) Changes in the mitochondrial respiratory system in the transition from weaning to adulthood in rats. Arch. Biochem. Biophys. **352:** 240–246
4 Iossa S., Mollica M. P., Lionetti L., Barletta A. and Liverini G. (1998) Rat liver mitochondrial respiratory capacities in the transition from weaning to adulthood. Mech. Ageing Dev. **100:** 59–66
5 Kim J. H., Woldgiorgis G., Elson C. E. and Shrago E. (1988) Age-related changes in respiration coupled to phosphorylation. I. Hepatic mitochondria. Mech. Ageing Dev. **46:** 263–277
6 Tummino P. J. and Gafni A. (1991) A comparative study of succinate-supported respiration and ATP/ADP translocation in liver mitochondria from adult and old rats. Mech. Ageing Dev. **59:** 177–188
7 vom Dahl S. and Haussinger D. (1997) Experimental methods in hepatology. Guidelines of the German Association for the Study of the Liver (GASL). Liver perfusion-technique and applications. Z. Gastroenterol. **35:** 221–226
8 Soboll S. (1995) Regulation of energy metabolism in liver. J. Bioenerg. Biomembr. **27:** 571–582
9 Winder W. W. and Holman R. T. (1982) Endurance training attenuates stress hormone response to exercise in fasted rats. Am. J. Physiol. **243:** R179–R184
10 Krebs H. A. and Henseleit K. (1932) Untersuchungen über die Harnstoffbildung in Tierkörpern. Hoppe-Seyler's Z. Physiol. Chem. **210:** 33–66
11 Scholz R., Hansen W. and Thurman R. G. (1973) Interaction of mixed function oxidation with biosynthetic processes. Eur. J. Biochem. **38:** 64–72
12 Bergmeyer H. U. (ed.) (1974) In: Methoden der Enzymatischen Analyse, vol. 3, Verlag Chemie, Weinheim
13 Chance B. and Williams C. M. (1956) The respiratory chain and oxidative phosphorylation. Adv. Enzymol. **17:** 65–134
14 Sies H. (1977) Redox compartmentation; a survey with emphasis on current problems. In: Alcohol and Aldehyde Metabolizing System, vol. III, pp. 47–64, Thurman R. G., Williamson J. R. and Drott H. B. (eds), Academic Press, New York
15 Demigné F. P. and Rémésy C. (1987) Mechanisms involved in ketone body relase by rat liver cells: influence of pH and bicarbonate. Am. J. Physiol. **252:** G200–G208
16 Shambaugh G. E., III, Koehler R. R. and Yokoo H. (1978) Fetal fuels III: ketone utilization by fetal hepatocytes. Am. J. Physiol **235:** E330–E337
17 Williamson D. H., Bates M. W., Page M. A. and Krebs H. A. (1971) Activities of enzymes involved in acetoacetate utilization in adult mammalian tissues. Biochem. J. **121:** 41–47
18 Ganapathi K., Kwon M., Haney P. M., McTiernan C., Javed A. A., Pepin R. A. et al. (1987) Cloning of rat brain succinyl-CoA: 3-oxoacid CoA-transferase cDNA. Biochem. J. **248:** 853–857
19 McGarry J. D. and Foster W. (1980) Regulation of hepatic fatty acid oxidation and ketone body production. Ann. Rev. Biochem. **49:** 395–420
20 Berry M. N., Gregory R. B., Grivell A. R. and Wallace P. G. (1983) Compartmentation of fatty acid oxidation in liver cells. Eur. J. Biochem. **131:** 215–222
21 Girard J., Ferré P., Regorier J. P. and Dueé P. H. (1992) Adaptations of glucose and fatty acid metabolism during perinatal period and suckling-weaning transition. Physiol. Rev. **72:** 507–562
22 Mori S., Morisaki N., Satio Y. and Yoshisa S. (1988) Age-related changes of long-chain fatty acid metabolism in rat liver. Mech. Ageing Dev. **44:** 175–83
23 Berry M. N., Kuhn E. and Werner H. V. (1973) Regulatory role of reduction-equivalent transfer from substrate to oxygen in the hepatic metabolism of glycerol and sorbitol. Eur. J. Biochem. **33:** 407–417
24 Podolin D. A., Gleeson T. T. and Mazzeo R. S. (1994) Role of norepinephrine in hepatic gluconeogenesis: evidence of aging and training effect. Am. J. Physiol. **267:** E680–E686
25 Podolin D. A., Pagliassotti M. J., Gleeson T. T. and Mazzeo R. S. (1994) Influence of endurance training on the age-related decline in hepatic gluconeogenesis. Mech. Ageing Dev. **75:** 81–93
26 Podolin D. A., Gleeson T. T. and Mazzeo R. S. (1996) Hormonal regulation of hepatic gluconeogenesis: influence of age and training. Am. J. Physiol. **270:** R365–R372
27 Pagliassotti M. J. and Prach P. A. (1997) Increased net hepatic glucose output from gluconegenic precursor after high-sucrose diet feeding in male rats. Am. J. Physiol. **272:** R526–R531
28 Sastre J., Pallardò F. V., Plà R., Pellin A., Juan G., O'Connor J. E. et al. (1996) Aging of the liver: age-associated mitochondrial damage in intact hepatocytes. Hepatology **24:** 1199–1205
29 Yu B. P. and Yang R. (1996) Critical evaluation of free radical theory of aging. Ann. N.Y. Acad. Sci. **15:** 1–11