Anaerobic Cell Function: Effect of Fasting on Cation Transport in Rat Liver Slices (34632)

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Fasting initiates profound metabolic alterations in liver which could impair its ability to cope with anoxia. This might be of importance in animals subjected to severe stress. For example, liver appears to be a key factor in the survival of dogs exposed to hemorrhagic shock (1, 2). We have previously demonstrated that anaerobic liver slices from fed rats could conduct cation transport when incubated in the presence of pyruvate or oxalacetate (3, 4). It was hypothesized that these metabolites were serving as electron acceptors either in the mitochondria or in the cytoplasm. The latter would stimulate glycolysis by maintaining a high ratio of cytoplasmic NAD^+:NADH. However, in fasted rats not only is liver glycogen depleted but marked changes in the activities of glycolytic and gluconeogenic enzymes have been reported (5–8). Under such circumstances the ability of liver cells to produce energy and maintain functions under anoxia might be severely limited.

Liver slices from fed and fasted animals were incubated under anaerobiosis in various metabolite environments that should facilitate anoxic ATP production. Slices were subsequently analyzed for Na^+ and K^+ content. Cation transport was stimulated in tissues of fed animals. All attempts at metabolite reconstitution failed to induce cation transport in tissues of fasted animals.

Materials and Methods. Male Sprague-Dawley rats weighing 300–400 g were decapitated, and the livers were rapidly transferred to iced saline. Slices approximately 0.5 mm thick were prepared freehand. The details of the experimental procedure have been previously described (3, 4). Briefly, the slices were maintained in 8 ml of Krebs-Ringer bicarbonate (KRB) for 80 min at 0° under 95% O_2, 5% CO_2. At this time 12 ml of control or substrate containing KRB (at 0–1°, gassed with 95% O_2, 5% CO_2) was tipped in, and the gassing mixture was changed to 95% N_2, 5% CO_2 for the anaerobic flasks. Ten minutes later the flasks were transferred to 37° and incubated for 1 hr. At the termination of the experiment the slices were removed, blotted gently on filter paper, and dried for 48 hr at 80–100°. After the tissue was weighed, electrolytes were extracted, and Na–K content was determined in a flame photometer. Fasted animals were kept without food for 24 hr prior to the experiment and were allowed water *ad libitum*.

The sources of the chemicals used were: sodium pyruvate, β-hydroxybutyric acid, oxalacetic acid, Nutritional Biochemicals Corporation; sodium phosphoenolpyruvate, α-ketoglutaric acid, Sigma; citric acid, Fisher.

Results. Effect of pyruvate and oxalacetate. As shown previously (3, 9) incubation of liver slices at 0–1° for 90 min results in a loss of K^+ and accumulation of Na^+ (Table I). Subsequent incubation at 38° under an oxygen atmosphere brings about a decrease in Na^+ and an increase of K^+ for both fed and fasted animals. However, cation transport was consistently less effective in fasted tissue (Tables I and II). Under nitrogen, liver cells of both fed and fasted animals have no capacity to decrease Na^+ and increase K^+ concentration.

We have suggested (3, 4) that oxalacetate and pyruvate might serve as mitochondrial or cytoplasmic electron acceptors under anoxia. With this in mind, liver slices of fasted

TABLE I. Electrolyte Content in Liver Slices Incubated at 37° for 60 min after Cooling 90 min at 0°; Fed and 24-hr Fasted Rats.

Incubation condition	Fed Na+ (meq/kg dry wt)	Fed K+ (meq/kg dry wt)	Fasted Na+ (meq/kg dry wt)	Fasted K+ (meq/kg dry wt)
Cooled 90 min[a]	451 ± 11.3[b]	109 ± 3.75	509 ± 13.8	97 ± 4.21
Incubation at 37° after cooling				
Oxygen[a]	170 ± 11.0	273 ± 7.07	305 ± 11.7	220 ±18.10
Nitrogen	491 ± 27.7	123 ± 7.52	558 ± 40.0	42 ± 3.32
Pyruvate[c]	269 ± 14.7	217 ± 8.94	626 ± 25.2	78 ± 2.82
Oxalacetate	259 ± 19.8	209 ±13.30	824 ± 13.2	62 ± 2.22

[a] Gas phase 95% O_2, 5% CO_2, all others were 95% N_2, 5% CO_2.
[b] Values in the table represent averages of 10 determinations ± SEM.
[c] Concentration of each substrate in the table was 23 m*M*.

animals were incubated in media containing either oxalacetate or pyruvate even though glycogen stores were depleted (Table I). Slices of fed animals exhibited recovery of cation transport under anoxia when in the presence of oxalacetate or pyruvate (3, 4). Tissue from fasted animals showed no recovery.

Reconstitution with glucose and α-ketoglutarate. Because of the decrease in liver glycogen after a 24-hr fast, it was felt that replenishment with glucose might be esesntial if oxalacetate and pyruvate were serving to stimulate glycolysis (Table II). Since a diminution of other endogenous metabolites might occur, experiments were also conducted with αketoglutarate since this might serve as a source for mitochondrial substrate level phosphorylation. No stimulatory effect was obtained after fasting with glucose alone or in combination with oxalacetate or pyruvate. Addition of α-ketoglutarate was also ineffectual. Results obtained on tissue of fed animals were identical to those reported in Table I.

Preincubation at 37° with O_2, glucose, and other metabolites. Preincubation of liver slices under O_2 stimulates anaerobic glycolysis (10–12). Prior to cooling at 0°, tissue incubation was conducted at 37° for 60 min under 95% O_2, 5% CO_2 in the presence or absence of metabolite. The incubation flasks were then cooled to 0°, and the experiments were continued in the usual way (Table III).

Preincubation with oxygen was sufficient to stimulate anaerobic cation transport in slices of fed animals ($p < .05$ for both Na^+

TABLE II. Influence of Reconstitution with Glucose and α-Ketoglutarate on Cation Transport in Fasted Livers.

Incubation condition	Fed Na+ (meq/kg dry wt)	Fed K+ (meq/kg dry wt)	Fasted Na+ (meq/kg dry wt)	Fasted K+ (meq/kg dry wt)
Cooled 90 min[a]	449 ± 18.4[b]	112 ± 7.77	559 ± 19.9	98 ± 3.44
Incubation at 37° after cooling				
Oxygen[a]	169 ± 7.8	277 ± 4.34	342 ± 20.0	223 ± 6.47
Nitrogen	526 ± 39.6	109 ± 12.00	742 ± 14.0	63 ± 2.19
Glucose[c]	516 ± 44.2	113 ± 14.70	702 ± 11.7	62 ± 2.50
+ Oxalacetate	266 ± 10.5	240 ± 6.00	800 ± 37.2	77 ± 4.30
+ Pyruvate	267 ± 16.4	212 ± 8.39	613 ± 22.6	86 ± 4.80
Oxalacetate + α-ketoglutarate	—	—	766 ± 31.5	64 ± 1.06
Pyruvate + α-ketoglutarate	—	—	596 ± 22.6	70 ± 2.55

Symbol footnotes same as Table I.

TABLE III. Recovery of Cation Transport in Tissue Preincubated at 37° for 60 min with O_2 Prior to Cooling and Rewarming.

Incubation condition	Fed		Fasted	
	Na^+ (meq/kg dry wt)	K^+ (meq/kg dry wt)	Na^+ (meq/kg dry wt)	K^+ (meq/kg dry wt)
Preincubation at 37°[a]	145 ± 6.9[b]	281 ± 3.6	270 ± 14.5	230 ± 5.1
Cooled 90 min after preincubation[a]	301 ± 11.6	173 ± 8.7	350 ± 9.2	175 ± 5.0
Incubation at 37° after cooling				
Oxygen[a]	162 ± 7.1	298 ± 1.8	323 ± 11.3	221 ± 5.9
Nitrogen	261 ± 13.4	229 ± 7.6	561 ± 18.9	100 ± 4.6
Oxalacetate[c]	241 ± 11.4	272 ± 5.1	581 ± 19.6	113 ± 4.5
Pyruvate	213 ± 10.8	270 ± 6.3	519 ± 12.9	120 ± 3.3

Symbol footnotes same as Table I.

and K^+). This did not require the presence of glucose in the oxygen preincubation nor was the presence of oxalacetate or pyruvate necessary under anoxia to obtain significant cation transport. However, these substrates did serve to increase the effectiveness of the tissue to conduct cation transport.

In oxygenated fasted tissue there was no significant decrease in Na^+ but K^+ concentration was significantly increased ($p < .001$) after rewarming at 37°. No anoxic stimulation of cation transport occurred under any circumstance in fasted liver.

Preincubation of tissues from fasted animals was also conducted with metabolites anticipating the possibility that one of these might serve to elevate the necessary endogenous metabolite pool for anoxic ATP production. The metabolites used were glucose, α-glycerophosphate, pyruvate, β-hydroxybutyrate, malate, oxalacetate, citrate, α-ketoglutarate, and succinate. These data are not presented since the results were comparable to preincubation in O_2 alone (Table III); no anoxic cation transport being obtained in any of these studies.

Effect of phosphoenolpyruvate. In addition to depletion of glycogen, several studies have shown decreased activity of glycolytic enzymes upon fasting (5, 7, 8). One of these is pyruvate kinase, which converts phosphoenolpyruvate to ATP and pyruvate. Utilization of phosphoenolpyruvate would provide a direct energy source and an opportunity to determine if reduction of pyruvate kinase activity is sufficient to result in impairment of anaerobic energy production.

Liver slices were placed directly at 0° in KRB, with and without phosphoenolpyruvate to facilitate permeation of tissue and cells by the substrate. Otherwise, the protocol is identical to that described under Methods. A moderate but significant stimulation of cation transport occurred only in tissues of fed animals (Table IV).

Discussion. The present investigation shows that a difference in physiological response exists between livers of fed and fasted rats. Anoxic liver slices of fed animals can maintain cation transport when incubated in oxalacetate, pyruvate, or phosphoenolpyruvate, or when preincubated under O_2. Liver slices of fasted animals do not have this capacity to function under anoxia either under the above-mentioned conditions or when incubated in addition with glucose or other metabolites.

Maintenance of Na–K gradients specifically requires ATP as an immediate source of energy (13). In most mammalian tissues, cation transport appears to require respiration since generation of ATP is insufficient by other means (9, 14–17). However, in liver slices cation transport does occur under anoxia, providing that electron-accepting metabolites are present in the medium (3, 4). In the present study this has been extended to include incubation in phosphoenolpyruvate and

TABLE IV. Cation Transport in Tissue Incubated in Phosphoenolpyruvate.

Incubation condition	Fed		Fasted	
	Na^+ (meq/kg dry wt)	K^+ (meq/kg dry wt)	Na^+ (meq/kg dry wt)	K^+ (meq/kg dry wt)
Cooled 90 min[a]	478 ± 3.9[b]	91 ± 2.9	551 ± 10.0	93 ± 2.9
Incubation at 37° after cooling				
Oxygen[a]	168 ± 3.9	271 ± 3.5	322 ± 11.3	221 ± 5.1
Nitrogen	593 ± 26.9	95 ± 7.9	790 ± 14.6	61 ± 2.2
Phosphoenolpyruvate[c]	369 ± 19.5	156 ± 9.5	772 ± 27.3	56 ± 1.8

Symbol footnotes same as Table I.

preincubation in O_2. Both of these procedures might be comparable, since preincubation in O_2 probably leads to accumulation of phosphorylated intermediates. Anoxic liver slices show increased production of lactate when preincubated in oxygen (10–12). Therefore, it appears that stimulation of glycolysis is responsible for anoxic cation transport. Possibly both pyruvate and oxalacetate also stimulate glycolysis by regenerating cytoplasmic NAD^+.

The activities of gluconeogenic and glycolytic enzymes are especially sensitive to diet (6, 8, 18). The responses, however, are not universally obtained in all animals (19, 20) or in all tissues (7). In the 24-hr starved rat there is a pronounced diminution in the activity of all three glycolytic enzymes; glucokinase (20), phosphofructokinase (8), and pyruvic kinase (5, 7, 8,). Although the glycolytic enzymes are all present after a 24-hr fast, their activities apparently are sufficiently depressed so that energy-requiring cell functions cannot be maintained. Incubations in glucose as well as phosphoenolpyruvate were completely ineffective in stimulating cation transport. This is not the result of an impaired ability to conduct cation transport, since oxygenated tissue had this capacity. It is not surprising, therefore, that when fasted and fed rats are subjected to trauma (21), hemorrhage (22), or anoxia (23) survival time is significantly decreased in the fasted animals.

Anoxic energy-producing reactions might be of general importance in other tissues. Electron-accepting metabolites significantly stimulated heart rate over that obtained with glucose alone when the isolated rat heart was perfused under anoxia (24). Infusion of rabbits with electron-accepting metabolites during hemorrhagic shock significantly increased survival compared to those animals infused with NaCl or glucose (25). These reactions could play a critical role in enabling the animal to survive an acute stress.

Summary. Rat liver slices from fed or fasted animals do not have the ability to conduct cation transport when subjected to anoxia. However, anoxic cation transport can be stimulated in liver slices of fed animals when slices are preincubated under O_2. In addition, anoxic cation transport can be stimulated by incubation of slices in pyruvate, oxalacetate, or phosphoenolpyruvate. These conditions did not stimulate anoxic cation transport in liver of fasted animals. All attempts at metabolite reconstitution also failed to elicit anoxic cation transport in liver of fasted animals.

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