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Glucose, pyruvate and lactate efflux by the perfused liver of a teleost, *Clarias batrachus* during aniso-osmotic exposure

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Abstract

Glucose, lactate and pyruvate efflux by the perfused liver of the walking catfish, *Clarias batrachus* was studied during aniso-osmotic exposure. During hypo-osmotic exposure (− 80 mOsmol l^{-1}, maintained with NaCl), glucose, lactate and pyruvate efflux by the perfused liver significantly decreased by 55, 19 and 16%, respectively. During hyper-osmotic exposure (+ 80 mOsmol l^{-1}, maintained with NaCl), efflux increased by 57, 12 and 18%, respectively. Similar effects of glucose, lactate and pyruvate efflux by the perfused liver was also seen when the anisotonicity of the medium was adjusted with mannitol instead of NaCl. The decrease of glucose, lactate and pyruvate efflux during hypo-osmotic exposure was correlated with the stimulation of glycogen synthesis and the reverse was true during hyper-osmotic exposure. These observations were supported by changes in glycogen phosphorylase *a* (GPase *a*) and glycogen synthase *a* (GSase *a*) activities. During hypo-osmotic exposure (− 80 mOsmol l^{-1}), the GPase *a* activity decreased by 1.93 fold and GSase *a* activity increased by 1.63 fold, while during hyper-osmotic exposure (+ 80 mOsmol l^{-1}), the GPase *a* activity increased by 1.58 fold and GSase *a* activity decreased by 1.95 fold. The total activity of both the enzymes were not effected by a short term exposure to aniso-osmotic conditions, suggesting that the alterations in GPase *a* and GSase *a* activity were mainly due to changes of their phosphorylation status during aniso-osmotic exposure. A direct correlation exists between glucose efflux and the hydration status of the perfused liver. These alterations of glucose metabolism are probably necessary by this walking catfish to meet the different energy demand, and also for maintenance of glucose homeostasis under osmotic stress. © 1998 Elsevier Science Inc. All rights reserved.

Keywords: Glucose; Pyruvate; Lactate; Glycogen; Carbohydrate metabolism; Glycogen phosphorylase; Glycogen synthase; Perfused liver; Aniso-osmotic conditions; Osmotic stress; *Clarias batrachus*; Hydration status

1. Introduction

Development of diverse metabolic strategies under various environmental conditions is the key to the evolution of organisms. The success of a group in a particular niche is due to its possessing appropriate adaptational flexibility in the metabolic strategies. Osmolarity change is a major problem faced regularly by various groups of organisms. Maintenance of cell volume is a prerequisite for any living organism to sustain the osmolarity changes faced regularly by the organism either in the environment or within the body. It is a phenomenon which not only occurs in vitro, but also in animal tissue cells in vivo when the organism is an osmoconformer and is subjected to changes in the environment (for review, [13]). Cell volume is frequently being challenged either due to intestinal absorption of water, to various amino acids and metabolites, or to exposure to different osmotic environments especially in case of aquatic animals.

When living cells are suddenly exposed to hypo-osmotic media, they initially swell more or less like a perfect osmometers because of water entry, but within minutes retain almost their original volume. This behaviour is considered regulatory volume decrease

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(RVD). During the phase of volume regulation, cells lose ions like K^+, Na^+, Cl^- and also some larger molecules like free amino acids (FAA), urea and/or trimethylamine oxide (TMAO), depending on the cell type [1,3,5,9,10]. Conversely, upon exposure to hyper-osmotic media, the cells shrink more or less like a perfect osmometer, but display within minutes a volume regulatory increase (RVI), which brings back cell volume almost to the normal level mostly by accumulation of various osmolytes. However, it should be noted that the initial cell volume is not restored completely following RVD or RVI, i.e. the cells remain either in a slightly swollen or shrunken state for the duration of the aniso-osmotic exposure, and this extent of cell volume deviation are known to cause alterations in various metabolic processes in rat hepatocytes, including the carbohydrate metabolism (for review, [12]).

In comparison to mammals, teleost fishes face more problems of osmotic stress primarily due to osmolarity changes to their external environment at different seasons of the year. Various studies have been carried out in fishes regarding the mechanisms of cell volume regulation [9,15,16,22], but few studies are available on alterations of different metabolic processes in relation to cell volume changes. Although it is believed that proteins and amino acids are the major sources of energy in fish, a recent review by Moon and Foster [18] has emphasised the importance of carbohydrate metabolism as an energy source especially during different environmental conditions. Carbohydrates, therefore, might play an important role for proper energy supply and also for glucose homeostasis in teleosts during osmotic stress.

There are certain Indian air-breathing amphibious teleost fish which normally live in slow flowing, stagnant and polluted waters of ponds and lakes (swamps), that are believed to be more resistant to environmental changes compared with typical teleosts [20]. They also face regularly the problem of osmolarity changes of their external environment at different seasons of the year along with various other environmental stresses like higher ambient ammonia and exposure to air. During the summer, they mostly live buried in the mud to avoid total dehydration. Various adaptations related to nitrogen metabolism under different environmental conditions have been reported including the presence of a functional and regulatory ornithine-urea cycle in some species of these air-breathing teleosts [23,26–28]. Here, we report the effect of aniso-osmotic exposure on glucose, lactate and pyruvate efflux by the perfused liver of an air-breathing catfish, *Clarias batrachus* along with the changes of glycogen phosphorylase (GPase) and glycogen synthase (GSase) activities.

2. Materials and methods

2.1. Animal

Walking catfish (*C. batrachus*) weighing 60–100 g, were purchased from commercial sources and acclimatized in the laboratory for 4–6 weeks at about 30°C in plastic aquaria with 12 h: 12 h light and dark period before being used for perfusion experiments. Minced pork liver and rice bran (5% of body weight) was given as food on alternate days and water, which was collected from a natural stream, was changed regularly on alternate days. No sex differentiation of fish was done while performing these studies.

2.2. Liver perfusion technique

The fish were anaesthetised in neutralised 3-aminobenzoic acid ethyl ester (MS-222, 0.2 g l^{-1}) for 2 min before operation for liver perfusion. Livers were perfused via the portal vein in a noncirculating manner with haemoglobin-free media as used by French et al. [8] with certain modifications made by Saha et al. [28]. The blood osmolarity (determined by freezing point depression method) was found to be 265 mOsmol l^{-1}. Therefore, the osmolarity of the standard medium was also maintained the same and was prepared by mixing 119 mM NaCl, 5 mM $NaHCO_3$, 5.4 mM KCl, 0.35 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 0.81 mM $MgSO_4$ and 1.25 mM of $CaCl_2$ as a basic solution for perfusion. The media also contained 1.2 mM lactate and 0.3 mM pyruvate except in a few perfusion experiments. The medium was gassed with O_2/CO_2 (99:1, v/v) before infusing into the liver at a flow rate of 4–5 ml g^{-1} liver per min. The pH of the medium was always maintained at 7.4 after gassing since the blood pH of this walking catfish ranges between 7.4 and 7.5. The temperature of the medium was maintained at 30°C. The effluent was collected at 2-min intervals for analysis of glucose and/or lactate and pyruvate through a cannula inserted at the superior vena cava.

Hypo-osmotic medium (-80 mOsmol l^{-1}) was prepared by removing an equivalent amount of NaCl, and hyper-osmotic medium ($+80$ mOsmol l^{-1}) was prepared by adding appropriate amount of extra NaCl to the standard perfusion medium as mentioned above. In another set of experiments, where the anisotonicity of the medium was adjusted with mannitol, the hyper-osmotic medium was prepared by adding 80 mOsmol l^{-1} equivalent of mannitol to the standard medium as mentioned above. However, for hypo-osmotic experiments with mannitol, livers were initially perfused with the iso-osmotic medium where 80 mOsmol l^{-1} equivalent of NaCl was already replaced by 80 mOsmol l^{-1} equivalent of mannitol from the standard medium, followed by the withdrawal of mannitol, thereby caus-

ing no change of NaCl concentration all through the experiment.

To study the effect of anisotonicity on glucose efflux, livers were initially perfused for 20 min with iso-osmotic medium, followed by the infusion of either hypo- or hyper-osmotic solutions separately for 20 min, and then again infusion of iso-osmotic medium for 20 min. Livers were also perfused with a range of aniso-osmotic media (145, 185, 225, 245, 305, 345 and 385 mOsmol l^{-1}) to establish a correlation, if any, between glucose efflux and the hydration status of the perfused liver. It is to be noted here that none of these experimental treatments affected hepatocyte integrity since the rate of lactate dehydrogenase (LDH) leakage, which was monitored regularly, from the perfused liver was found to be negligible varying between 1 and 3 U l^{-1}.

To study the effect of aniso-osmotic exposure on the activity of GPase and GSase enzymes in the perfused liver, livers were initially perfused with iso-osmotic medium for 20 min, followed by the infusion of hypo- (-80 mOsmol l^{-1}) or hyper- ($+80$ mOsmol l^{-1}) osmotic media for 20 min. Another set of liver perfused with iso-osmotic medium for 40 min served as control. The perfused liver was frozen in liquid nitrogen immediately after infusion with iso- or aniso-osmotic media, and stored in a deep freeze at -20°C until used for assaying the enzyme activity. The enzyme assays were completed within two days of freezing the tissue.

2.3. Estimations

Concentrations of glucose, lactate and pyruvate in the effluent were measured enzymatically [2]. To each 1 ml sample collected at every 2-min interval, 10 μl of 2 M perchloric acid (PCA) was added and precipitated protein removed by centrifugation, and the supernatant was neutralised by adding 10 μl of 2 M NaOH before estimation of glucose, pyruvate and lactate. The efflux of glucose, lactate and pyruvate were expressed as μmol g^{-1} wet wt of liver per min. Liver glycogen was estimated enzymatically using amyloglucosidase [24] and was expressed as μmol glucosyl U g^{-1} wet wt of liver.

2.4. Enzyme Assays

Ten percent homogenates (w/v) of the liver tissue was prepared in a homogenizing buffer containing 20 mM imidazole-HCl (pH 7.2), 100 mM NaF, 10 mM EDTA, 10 mM EGTA, 15 mM 2-mercaptoethanol and 0.1 mM phenylmethylsulphonyl fluoride (PMSF) [25]. The homogenate was centrifuged at $10000 \times g$ at 0 ± 2°C for 10 min and the supernatant was used for assay of enzymes. GPase was assayed spectrophotometrically at 340 nm following the method of Moon et al. [19]. The assay mixture contained 50 μmol potassium phosphate buffer (pH 7.0), 0.5 μmol NADP, 5 μmol glucose 1,6-biphosphate, 2.5 μmol AMP, 5 U each of phosphoglucomutase and glucose-6-phosphate dehydrogenase, 10 mg glycogen and 0.1 ml of tissue homogenate in a final volume of 1 ml. The reaction mixture also contained 10 μmol caffeine for measurement of GPase *a* (active), and no caffeine in case of total GPase ($a+b$) activity. Percent GPase *a* represents the ratio of GPase *a* to total GPase $\times$ 100. GSase was assayed spectrophotometrically at 340 nm following the method of Passonneau and Rottenberg [21]. The assay mixture contained 50 μmol imidazol-HCl buffer (pH 7.5), 150 μmol KCl, 15 μmol $MgCl_2$, 5 μmol phosphoenol pyruvate, 0.15 μmol NADH, 2 mg glycogen, 10 U each of pyruvate kinase and lactate dehydrogenase, 6 μmol UDP-glucose and 0.1 ml tissue homogenate in a final volume of 1 ml. The reaction mixture also contained 5 μmol glucose 6-phosphate (G6P) while measuring the total GSase ($a+b$) activity. Percent GSase *a* represents the ratio of GSase *a* to total GSase $\times$ 100. One unit of enzyme activity was expressed as 1 μmol of NADP reduced in case of GPase and 1 μmol of NADH oxidized in case of GSase per min at 30°C. LDH activity in the effluent was assayed following the method of Bergmeyer [2].

2.5. Measurement of water content in the perfused liver

The water content in the perfused liver was determined by oven drying. Immediately after perfusion either with iso- or aniso-osmotic media, the liver was weighed, and kept in an oven at 70–80°C for 24 h, time sufficient for complete drying of liver. The differences between the wet and the dry weight of the perfused liver was taken as water content and was expressed as the percentage water content in the perfused liver.

Data collected, were statistically analyzed and presented as mean $\pm$ standard error of the mean (SEM). Comparison of the paired mean values was made using Student's *t*-test [4] and $P < 0.05$ was taken as significant. Linear regression analysis was also done in one experiment to find a correlation [4].

2.6. Chemicals

MS-222, all enzymes, coenzymes and substrates were either obtained from Sigma Chemical, St. Louis, MO, or Boehringer Mannheim, Mannheim, Germany. All other reagents used were of the highest quality obtained from indigenous sources. Deionised double glass distilled water was used for all preparations.

3. Results

3.1. Glucose efflux from the perfused liver during iso- and aniso-osmotic conditions

As shown in Figs. 1 and 2, during iso-osmotic perfu-

sion glucose efflux into the effluent by the perfused liver was approximately 1.25 μmol g^{-1} liver per min. However, when the liver was perfused with hypo-osmotic medium (-80 mOsmol l^{-1}) after the initial perfusion with iso-osmotic medium (265 mOsmol l^{-1}), glucose efflux into the effluent decreased significantly from 1.39 ± 0.1 to 0.61 ± 0.09 μmol g^{-1} liver per min ($55 \pm 3\%$; $P < 0.01$; $n = 4$) (Fig. 1Table 1). Whereas, during perfusion of liver with hyper-osmotic medium ($+80$ mOsmol l^{-1}), glucose efflux into the effluent increased significantly from 1.15 ± 0.08 to 1.86 ± 0.05 μmol g^{-1} liver per min ($57 \pm 4\%$; $P < 0.01$; $n = 4$) (Fig. 2Table 1). The effects of anisotonicity on glucose efflux persisted during hypo- and hyper-osmotic exposure and were fully reversible upon restoration of iso-osmotic perfusion condition (Figs. 1 and 2).

When the anisotonicity of the medium was adjusted with mannitol instead of NaCl, similar effects on glucose efflux from the perfused liver were observed (Table 1). When the liver was perfused with hypo-osmotic medium (-80 mOsmol l^{-1}), glucose efflux decreased significantly from 1.3 ± 0.11 to 0.64 ± 0.13 μmol g^{-1} liver per min ($51 \pm 3\%$; $P < 0.01$; $n = 4$), while during hyper-osmotic perfusion ($+80$ mOsmol l^{-1}), glucose

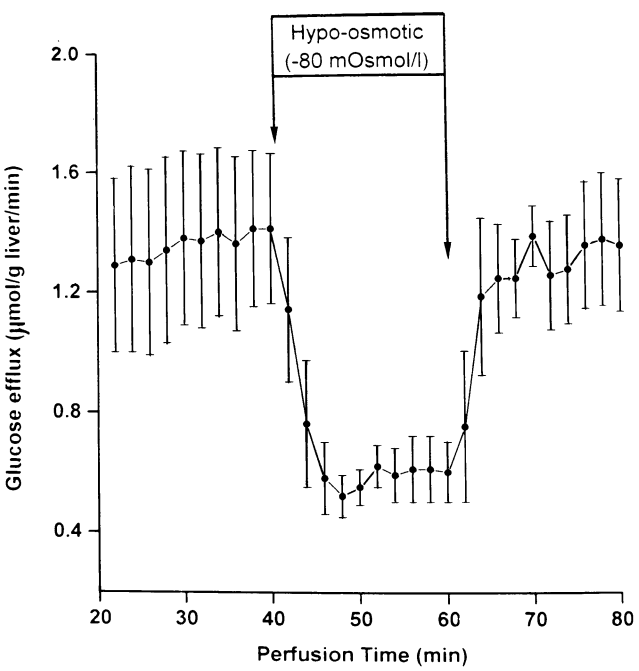

Fig. 1. The effect of hypo-osmotic (-80 mOsmol l^{-1}) exposure on glucose efflux by the perfused liver of *C. batrachus*. Values were plotted as mean $\pm$ S.E.M. ($n = 4$). The hypotonicity of the medium was obtained by withdrawing equivalent amount of NaCl from the standard medium as mentioned in Section 2. The steady state efflux of glucose between 32 and 40 min of perfusion was taken as control and was expressed as mean $\pm$ S.E.M. The hypo-osmotic induced changes of glucose efflux was taken between 52 and 60 min of perfusion when it reached the steady state and was expressed as mean $\pm$ S.E.M.

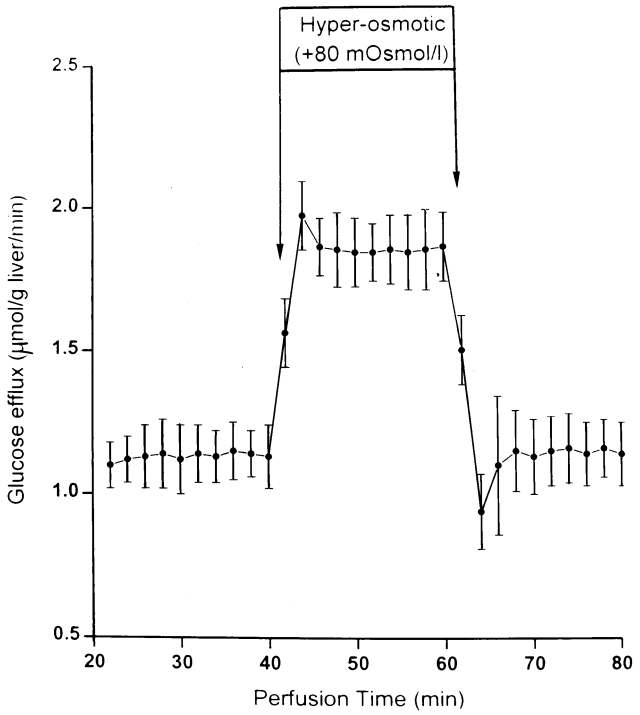

Fig. 2. The effect of hyper-osmotic ($+80$ mOsmol l^{-1}) exposure on glucose efflux by the perfused liver of *C. batrachus*. Values were plotted as mean $\pm$ S.E.M. ($n = 4$). The hypertonicity of the medium was obtained by adding equivalent amount of extra NaCl into the standard medium as mentioned in Section 2. The steady state efflux of glucose between 32 and 40 min of perfusion was taken as control and was expressed as mean $\pm$ S.E.M. The hyper-osmotic induced changes of glucose efflux was taken between 52 and 60 min of perfusion when it reached the steady state and was expressed as mean $\pm$ S.E.M.

efflux increased significantly from 1.28 ± 0.05 to 1.85 ± 0.04 μmol g^{-1} liver per min ($46 \pm 4\%$; $P < 0.01$; $n = 4$). The effects of anisotonicity on glucose efflux, which was maintained with mannitol, also persisted all through hypo- and hyper-osmotic exposure, and were fully reversible upon restoration of iso-osmotic perfusion condition (not shown).

3.2. Lactate and pyruvate efflux by the perfused liver during aniso-osmotic conditions

Lactate and pyruvate efflux into the effluent by the perfused liver were also affected by aniso-osmotic perfusion conditions (Fig. 3Table 1). When the anisotonicity of the perfusion media was adjusted with NaCl, lactate and pyruvate efflux decreased significantly from 2.72 ± 0.09 to 2.21 ± 0.12 μmol g^{-1} liver per min ($19 \pm 3\%$; $P < 0.05$; $n = 4$) and 0.86 ± 0.01 to 0.75 ± 0.02 μmol g^{-1} liver per min ($16 \pm 2\%$; $P < 0.05$; $n = 4$), respectively, during hypo-osmotic exposure and increased significantly from 3.05 ± 0.1 to 3.42 ± 0.04 μmol g^{-1} liver min^{-1} ($12 \pm 2\%$; $P < 0.05$; $n = 4$) and

Table 1
Effect of aniso-osmotic exposure on glucose, lactate and pyruvate efflux, and also the percentage water content in the perfused liver of *C. batrachus*

	Conditions	% change of efflux of			% change of water content
		Glucose	Lactate	Pyruvate	
With NaCl	Hypo-osmotic	-55 ± 3.2	-19 ± 3.1	-16 ± 2.3	$+8 \pm 1.5$
	P	<0.01	<0.05	<0.05	N.S.
	Hyper-osmotic	$+57 \pm 4.3$	-12 ± 1.8	$+18 \pm 1.9$	-6 ± 1.2
	P	<0.01	<0.01	<0.05	N.S.
With mannitol	Hypo-osmotic	-51 ± 2.7	-47 ± 4.8	-23 ± 2.6	$+7.5 \pm 1.1$
	P	<0.01	<0.01	<0.05	N.S.
	Hyper-osmotic	$+46 \pm 3.8$	$+20 \pm 1.7$	$+19 \pm 1.9$	-6 ± 1.2
	P	<0.01	<0.05	<0.05	N.S.

Livers were perfused with a medium containing everything as mentioned in Section 2.
The anisotonicity was maintained in one case by adding or withdrawing equivalent amount of NaCl, and in another case by adding or withdrawing an equivalent amount of mannitol details of which are mentioned in Section 2.
The steady state efflux of glucose, lactate and pyruvate obtained between 32 and 40 min of initial perfusion with iso-osmotic medium were taken as control values and were set to 100% in individual set of experiments.
Data on aniso-osmotic induced changes of glucose, lactate and pyruvate efflux were taken between 52 and 60 min of perfusion when it reached the steady state and was calculated the percentage changes of efflux from control values.
The method for calculation of percentage change of water content are mentioned in Fig. 4.
Positive values indicate increase and negative values indicate decrease of respective parameters.
N.S., not significant.

0.87 ± 0.08 to 1.03 ± 0.1 μmol g^{-1} liver per min ($18 \pm 2\%$; $P < 0.05$; $n = 4$), respectively, during hyper-osmotic exposure. Similarly, when the anisotonicity of the media were adjusted with mannitol, lactate and pyruvate efflux decreased significantly from 2.62 ± 0.18 to 1.38 ± 0.05 μmol g^{-1} liver per min ($47 \pm 5\%$; $P < 0.01$; $n = 4$) and from 0.85 ± 0.11 to 0.65 ± 0.04 μmol g^{-1} liver per min ($23 \pm 3\%$; $P < 0.05$; $n = 4$), respectively, during hypo-osmotic exposure and increased significantly from 3.62 ± 0.15 to 4.35 ± 0.4 μmol g^{-1} liver per min ($20 \pm 2\%$; $P < 0.05$; $n = 4$) and from 1.35 ± 0.1 to 1.6 ± 0.06 μmol g^{-1} liver per min ($19 \pm 2\%$; $p < 0.05$; $n = 4$), respectively, during hyper-osmotic exposure (Table 1). The effects of anisotonicity on lactate and pyruvate efflux persisted all through the period of aniso-osmotic exposure and were fully reversible upon restoration of iso-osmotic perfusion condition. (Fig. 3).

3.3. Changes of GPase and GSase enzymes activity during aniso-osmotic exposure

As shown in Table 2, perfusion of the liver with aniso-osmotic media for 20 min caused a significant change of activity of only the active forms of GPase and GSase enzymes, while not having any significant effect on the total activity of either enzymes. GPase *a*, which constituted about 38% of the total GPase ($a + b$) activity during iso-osmotic condition, decreased significantly ($P < 0.01$) by $48 \pm 4.2\%$ during hypo-osmotic condition and increased significantly ($P < 0.01$) by $58 \pm 5.1\%$ during hyper-osmotic condition compared with iso-osmotic condition, thereby changing the percentage of GPase *a* activity to 19.7 ± 3.7 and $56.7 \pm 6.1\%$, respectively. Whereas, the GSase *a* activity, which constituted about 55% of the total GSase ($a + b$) activity during iso-osmotic condition, increased significantly ($P < 0.01$) by $63 \pm 5.4\%$ during hypo-osmotic condition, and decreased significantly ($P < 0.05$) by $30.5 \pm 4.6\%$ during hyper-osmotic condition, thereby changing the percentage of GSase *a* activity to 78.8 ± 7.6 and $30.5 \pm 4.6\%$, respectively.

3.4. Glucose efflux and the hydration status of the perfused liver

As shown in Fig. 4, a direct relation of percentage increase or decrease of glucose efflux exists with percentage increase or decrease of water content in the perfused liver of *C. batrachus*. During iso-osmotic condition the water content in the perfused liver was found to be $74 \pm 1.5\%$ ($n = 10$). However, with an increase of hypotonicity of the perfusion medium, the water content in the perfused liver increased linearly, which was accompanied by a linear decrease of glucose efflux with relation to control. In contrast, with the increase of hypertonicity of the perfusion medium, the water content in the perfused liver decreased linearly, which was again accompanied by a linear increase of glucose efflux with relation to control. Linear regression analysis of the data presented revealed the following relationship between the water content and the glucose efflux by the perfused liver: $Y = 6.895 + 5.303x$ ($n = 7$; $r = 0.95$).

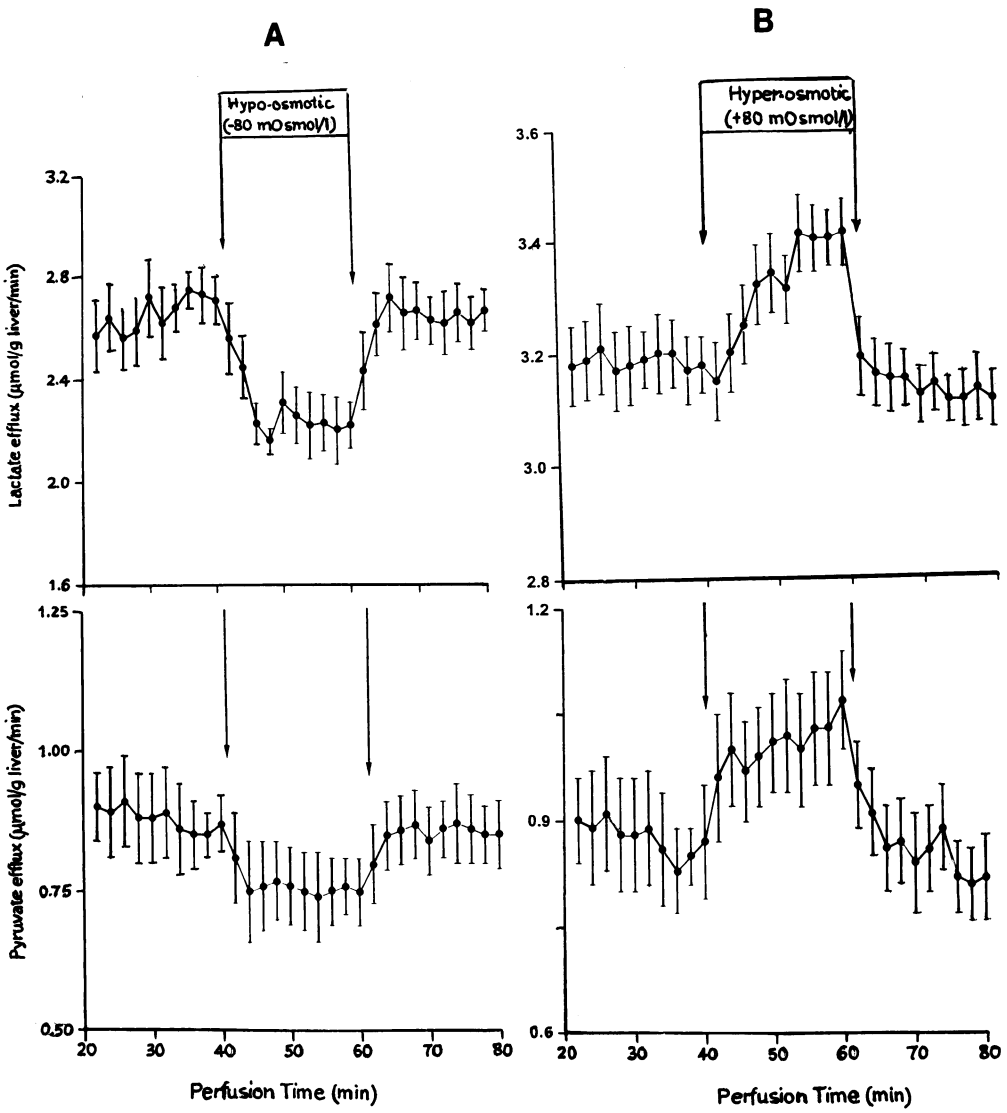

Fig. 3. The effect of hypo-osmotic (A) and hyper-osmotic (B) exposure on lactate and pyruvate efflux by the perfused liver of *C. batrachus*. Values were plotted as mean ± S.E.M. ($n = 4$). The anisotonicity of the media were obtained by either withdrawing or adding equivalent amount of extra NaCl into the standard medium as mentioned in Section 2 containing lactate (1.2 mM) and pyruvate (0.3 mM). The steady state efflux of lactate and pyruvate between 32 and 40 min of perfusion were taken as control and were expressed as mean ± S.E.M. The aniso-osmotic induced changes of lactate and pyruvate efflux were taken between 52 and 60 min of perfusion when it reached the steady state and were expressed as mean ± S.E.M.

4. Discussion

In the present study, the glucose efflux by the perfused liver of walking catfish was found to be about 1.25 μmol g^{-1} liver per min in well fed fish during iso-osmotic perfusion condition, which is comparable with glucose efflux by the perfused rat liver [14]. Moderately higher levels of glycogen (411 ± 37 μmol glucosyl U g^{-1} tissue, $n = 10$) were found in the liver of this fish under well fed conditions. These are possibly an indication that carbohydrate metabolism plays an important role in walking catfish for proper energy supply at least under these environmental conditions, as suggested for various groups of fishes in a recent review by Moon and Foster [18].

Although fish like mammals, possess various volume regulatory mechanisms ([9,10,22]), nevertheless cells remain in a partly swollen or shrunken state during aniso-osmotic conditions as in erythrocytes of various teleosts [29]. In the present study, although the cell volume changes were not measured directly in the perfused liver, the percentage water content changed during the period of aniso-osmotic exposure (Fig. 4) indicating that the liver cells remained partly swollen or shrunken during aniso-osmotic conditions. A little change in hydration status of hepatocytes during aniso-

Table 2
Effect of aniso-osmotic exposure on the activities of GPase and GSase (U g^{-1} wet wt) in the perfused liver of *C. batrachus*

Enzymes		Conditions		
		Iso-osmotic	Hypo-osmotic	Hyper-osmotic
Total GPase ($a+b$)		3.83 ± 0.48	3.81 ± 0.51	4.04 ± 0.38
GPase a (active)		1.45 ± 0.21	0.75 ± 0.12 (-48 ± 4.2)	2.29 ± 0.32 ($+58 \pm 5.1$)
	P		<0.01	<0.01
% GPase a		37.8 ± 4.5	19.7 ± 3.7	56.7 ± 6.1
Total GSase ($a+b$)		0.74 ± 0.11	0.85 ± 0.15	0.69 ± 0.08
GSase a (active)		0.41 ± 0.09	0.67 ± 0.12 ($+63 \pm 5.4$)	0.21 ± 0.06 (-49 ± 4.2)
	P		<0.01	<0.05
% GSase a		55.4 ± 5.7	78.8 ± 7.6	30.5 ± 4.6
GPase a/GSase a ratio		3.54	1.12	10.9

Values are expressed as mean $\pm$ S.E.M. ($n = 4$).
One unit of enzyme activity was expressed as 1 μmol of NADP reduced in case of GPase and 1 μmol of NADH oxidised in case of GSase per min at 30°C.
The enzyme activities obtained in liver after perfusion with iso-osmotic medium for 40 min were taken as control values and were set to 100% for calculation of the percentage change of activity during aniso-osmotic exposure.
The percentage increase (+) or decrease (−) of activity compared to control are given in parentheses.

osmotic exposure (Table 1Fig. 4) was sufficient to cause significant changes in carbohydrate metabolism in this walking catfish. In addition to carbohydrate metabolism, possibly other metabolic pathways are also effected by the cell volume changes which needs to be investigated.

The decrease in glucose efflux, reported here in the perfused liver of our fish during hypo-osmotic conditions, is mainly due to a decrease in glycogenolysis, since this change of glucose efflux was accompanied by a significant ($P < 0.01$) decrease in the activity of GPase a enzyme. It was further accompanied by the decreases in both lactate and pyruvate effluxes from the perfused liver, indicating that some lactate and pyruvate were taken up by the perfused liver. Although in the present study we did not make an attempt to study the metabolic fate of lactate and pyruvate taken up by the perfused liver during hypo-osmotic condition, it may be possible that these three carbon precursors are converted to glucose, and ultimately stored as glycogen, since GSase a activity in the perfused liver after 20 min of hypo-osmotic exposure was also stimulated significantly ($P < 0.01$). Similar flux of three carbon precursors such as lactate and alanine to six carbon end products has also been reported when the isolated hepatocytes of hagfish, sea raven, and American eel were exposed to insulin [6,7], which could also be correlated with the cell swelling, since insulin is known to cause the swelling of hepatocytes by cellular hydration in teleosts [9] and mammals [12]. Conversely, the reverse is true, i.e. stimulation of glycogenolysis and inhibition of glycogenesis, during hyper-osmotic exposure as evidenced with our present study (Tables 1 and 2Figs. 1–3). It should be noted further that anisotonicity only changed the lactate and pyruvate efflux from the perfused liver without effecting the lactate/pyruvate ratio during hypo- and hyper-osmotic exposure, thereby suggesting that the anisotonicity only caused the changes of metabolic activity without effecting the acid-base status and/or the redox status within the cell. Similar effects of hydration status on glucose efflux and also on the GPase a activity was found in brown bullhead catfish (*Ictalurus nebulosus*) and in a marine scorpaenid fish (*Sebastes caurinus*) liver (Hallgreen, Busby and Mommsen, unpublished observations). Therefore, it appears that the effect of hydration status on glucose metabolism is a widespread phenomenon occuring in various groups of fish.

Häussinger et al. [12] suggested that carbohydrate metabolism in rat is critically dependent on the hepatocellular hydration state. Similarly, when the liver of walking catfish was perfused with a range of aniso-osmotic media, the glucose efflux varied with the change of water content of the perfused liver, and there exists a direct correlation between these two (Fig. 4). Although in the present study we did not measure the changes in activities of GPase a and GSase a with a range of aniso-osmotic conditions (except in -80 and $+80$ mOsmol l^{-1}), it appears that the phosphorylation status of both enzymes vary at different hydration states of liver cells as suggested in catfish *(I. nebulosus)* (Hallgreen, Busty and Mommsen, unpublished observations), thereby changing the glucose efflux and glucose metabolism during aniso-osmotic conditions. Further, it is evident from the present study that the alterations of GPase a and GSase a activities in the perfused liver for a short term exposure to aniso-osmotic condition were mainly due to changes of phosphorylation status of these two enzymes as suggested in rat hepatocytes [12,17], since the total activity of both enzymes remained unchanged. But, it can not be overruled the possibility of regulation of these enzymes at a transcrip-

tional level, which probably needs a long term exposure to aniso-osmotic stress. There could also be some other factors which might regulate these enzymes, such as the changes of intracellular chloride concentration during aniso-osmotic conditions as reported in rat hepatocytes (for review, [12]). However, to get a conclusive result, one should study in detail the possible mechanism(s) of regulation in this walking catfish.

A critical question addressed in our study whether the changes in NaCl concentrations, varied to adjust the osmolarity of the medium, would have any effect on the glucose efflux by the perfused liver. In fact, alterations of NaCl concentrations could have some effect on the membrane potential as reported in rat hepatocytes [11]. Therefore, in another set of experiments, when the anisotonicity was maintained with mannitol instead of NaCl (without changing the NaCl concentration all through the experiment), the same effects on the glucose, lactate and pyruvate effluxes were observed (Table 1; graphs not shown). Hyper-osmotic stress probably demands more on requirement of energy, thereby releasing more of glucose from the endogenous glycogen to serve as an oxidative substrate in extrahepatic tissues. The reverse is possibly true during hypo-osmotic stress. We, therefore, hypothetize from the present study that the alteration of hydration status of liver cells is mainly responsible for the changes of GPase *a* and GSase *a* activities by changing their phosphorylation status as one of the metabolic effects of anisotonicity, thereby causing the changes of glucose efflux and glucose metabolism. This is probably necessary in this catfish to meet the energy demand, and also for maintenance of glucose homeostasis during osmotic stress.

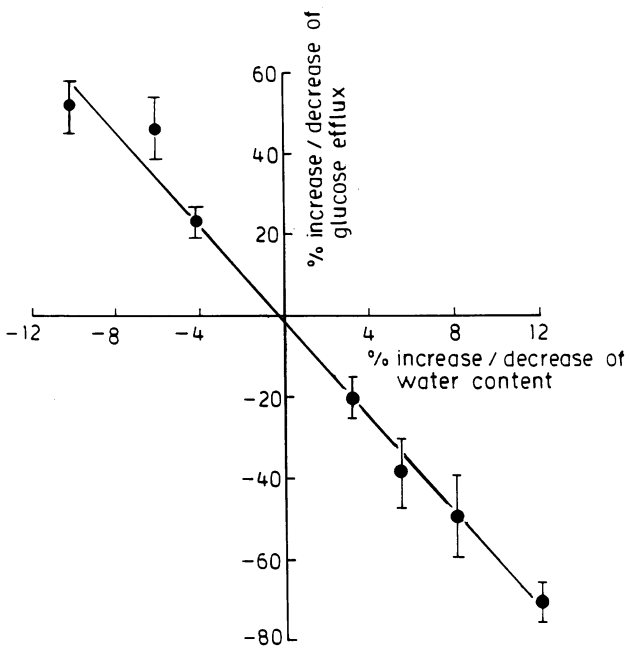

Fig. 4. Relationship between glucose efflux and hydration status of the perfused liver of *C. batrachus* during iso- and aniso-osmotic exposure. Livers were perfused with the standard medium as mentioned in Section 2 containing lactate (1.2 mM) and pyruvate (0.3 mM). The anisotonicity of the perfusion medium was altered by adjusting the NaCl concentration into the standard medium. The steady state of glucose efflux obtained between 32 and 40 min of perfusion with iso-osmotic medium was taken as control value and was set to 100% in each set of experiments. The data on aniso-osmotic induced changes of glucose efflux was taken between 52 and 60 min of perfusion when it reached the steady state and calculated the percentage change of glucose efflux from the control value. The percentage of water content in perfused livers were determined in separate set of experiments. The percentage of water content in liver perfused with iso-osmotic medium was found to be $74 \pm 1.5\%$ ($n = 10$), which was taken as control and was set to 100%. For determination of water content in perfused liver during aniso-osmotic conditions, livers were not perfused back with iso-osmotic medium. The aniso-osmotic induced changes of water content in perfused livers were calculated from the control value and were plotted against the percentage changes of glucose efflux.

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