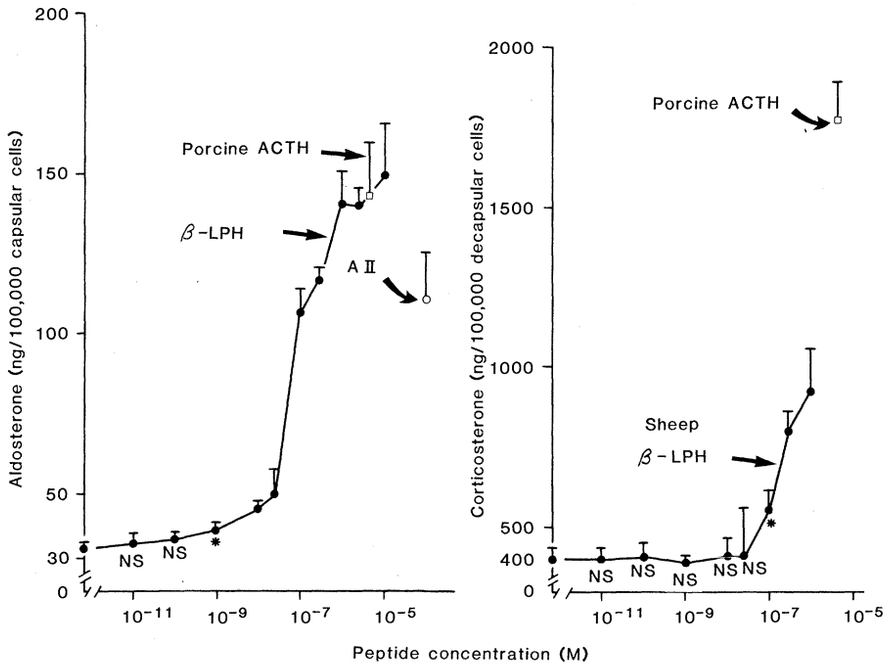

Fig. 2. Aldosterone and corticosterone production by β-LPH. Each point represents the mean ± standard error of eight experiments (four experiments with human β-LPH and four experiments with sheep β-LPH); *NS*, not significantly different from the control value; asterisk, significantly different from the control value ($P < .02$). All other points are significantly different from the control value ($P < .01$).

obtained with β-LPH (144.9 ± 15.5 ng per 100,000 capsular cells) is greater than that obtained with AII (110.8 ± 16.1 ng per 100,000 capsular cells; $P < .01$) and the same as that obtained with ACTH (142.7 ± 17.3 ng per 100,000 capsular cells). However, the concentration of β-LPH (about $5 \times 10^{-8} M$) required to induce half-maximum aldosterone production is approximately 100 times higher than that required for AII (*5*). The corticosterone response to β-LPH is less sensitive than the aldosterone response, with no significant increases at concentrations less than $10^{-7} M$. In contrast, the sensitivities of the aldosterone and corticosterone responses to ACTH are approximately equal (*5, 12*). This difference in the sensitivities of the aldosterone and corticosterone responses suggests that β-LPH and ACTH have different modes of action. Furthermore, there was no detectable ACTH in the β-LPH preparations when millimolar concentrations were assayed by an ACTH radioimmunoassay that could detect 1 pmole of ACTH. Also, we have found that doses of sheep β-LPH that maximally stimulated production of aldosterone did not significantly increase production of adenosine 3′,5′-monophosphate (cyclic AMP) by the adrenal cells. In contrast, maximally stimulating doses of synthetic human ACTH caused a 4.5-fold increase in cyclic AMP production. In other experiments, various amounts (10^{-8} to $10^{-4} M$) of Met^5-enkephalin (Met, methionine) and of α- and β-endorphin did not stimulate aldosterone and corticosterone production. Several lines of evidence suggest that unidentified factor or factors may contribute to aldosterone regulation under some conditions. In the rat, a non-ACTH pituitary factor is essential for the aldosterone response during sodium restriction (*1*). The clinical syndrome of primary aldosteronism associated with bilateral adrenal hyperplasia suggests the presence of an unknown stimulator. Recently, a glycoprotein extracted from human urine has been found to stimulate aldosterone production by capsular cells in vitro and to produce hypertension and hyperaldosteronism when it is administered to rats (*13*). Our results show that β-LPH has a potent aldosterone-stimulating effect. These data raise the question whether β-LPH and possibly other pituitary factor is essential for the aldosterone response during sodium restriction in some types of primary aldosteronism.

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Ethanol: Novel End Product of Vertebrate Anaerobic Metabolism

Abstract. *During periods of short-term experimental anoxia, the goldfish produces metabolic carbon dioxide and does not accumulate lactate to the extent expected. The goldfish has evolved a novel pathway of vertebrate anaerobic metabolism in which glucose carbon is metabolized to ethanol and excreted.*

Most vertebrates can tolerate only very short excursions into anoxic environments. The common goldfish, however, is remarkably resistant to total anoxia. At low temperatures, during winter (when resistance is maximum), the goldfish can survive several days in the complete absence of oxygen (*1, 2*). Although the metabolic basis for this capability is unknown, it does not seem to reside in classical vertebrate glycolysis for two reasons. First, metabolic CO_2 is known to be an anaerobic end product resulting, at least in part, from the catabolism of glucose (*3, 4*). Second, lactate does not accumulate to the extent predicted either on the basis of glycogen depletion or on the duration of anoxia (*1, 4*). In fact, less than half the glycogen utilized during anoxia can be accounted for in terms of lactate accumulation (*4*). Nor does lactate appear to be excreted. Prosser *et al.* (*5*) looked for but were unable to detect lactate in the water of hypoxic fish. They did however find an excretory product which they were unable to identify but which they postulated was the result of glucose fermentation.

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We hypothesized that the unidentified excretory product might be the result of further lactate catabolism. In order to test this hypothesis, we first looked at changes in metabolite concentrations in goldfish and their surrounding water after an extended period of anoxia. Goldfish were poisoned with 99.5 percent carbon monoxide for 3 hours before the start of the experiment. We did this to block the oxygen transport system and to inhibit cytochrome oxidase so that any residual oxygen stores (such as in the swim bladder) would be essentially unavailable for oxidative metabolism. We then placed the fish in individual chambers which had been previously gassed with high purity nitrogen, and kept them under nitrogen for 12 hours. At the end of this period the fish were freeze-clamped in aluminum tongs cooled with liquid nitrogen, and perchloric acid extracts (*6*) were made of individual whole fish. Hydrochloric acid (to a final concentration of 0.5 percent) was also added to the water in the experimental chamber. We found (Table 1) that lactate and ethanol were the only significant end products and that they accumulated in the tissues in approximately a 1:1 ratio (*7*). Roughly 60 percent of the total ethanol produced during 12 hours of anoxia is excreted and lactate accumulation amounts to about half the total ethanol produced. Table 2 shows the results of a similar experiment in which perchloric acid extracts were made from individual tissues. The ratio of the accumulation of lactate to ethanol is approximately 2 in the red muscle and liver and about 1 in the white muscle after 24 hours of anoxia.

These results suggested that lactate was being both produced and metabolized and that its ultimate fate was excretion as ethanol. To test this we injected (intraperitoneally) anoxic goldfish with sodium lactate uniformly labeled with ^{14}C and looked for the appearance of a radioactive excretory product. We found an acid stable ^{14}C-labeled end product in the water; this product could be easily concentrated by repeated freezing and thawing of the water sample, decanting off the liquid just as the sample started to thaw. We analyzed this on a preparative gas chromatograph (*8*) and found that 88 to 93 percent of the ^{14}C injected onto the column could be collected with the ethanol peak.

A search for the enzyme alcohol dehydrogenase revealed that the enzyme is mainly localized in the red and white muscle tissues in the goldfish. Maximal activities at 15°C in the red muscle are 90.8 ± 9.1 units per gram (wet weight) and in the white muscle 29.2 ± 7.0 units per gram, wet weight (N = 4) (*9*). These data indicate that two different catabolic pathways are operating concurrently in the anoxic goldfish, namely, (i) classical vertebrate glycolysis in tissues lacking alcohol dehydrogenase such as the heart and brain, and (ii) alcoholic fermentation in both the red and white muscle. Are they independent? Two pieces of evidence (*10*) suggest a concerted metabolic strategy in which lactate produced in the glycolytic tissues is metabolized to ethanol in muscle. First, the rate of production of [^{14}C]ethanol from [^{14}C]lactate is three to four times greater than that from [^{14}C]glucose. Thus blood lactate appears to be a better substrate for alcoholic fermentation than blood glucose. Second, the greatest increase in whole body lactate concentration occurs during the first 12 hours of anoxia; thereafter lactate increases very slowly. For example, after 72 hours of anoxia, we measured whole body lactate concentrations of 7.43 ± 0.56 μmole/g, wet weight (N = 8), only 1.3 times the value reported in Table 1 for 12 hours of anoxia. Since lactate is not excreted, and since there is no reason to suspect a drastic reduction in glycolytic rate as a function of the duration of anoxia, the most likely fate of lactate is conversion to ethanol.

What advantages does this metabolic strategy confer to the goldfish over classical vertebrate anaerobic glycolysis? Perhaps most significant is the fact that lactate never reaches high levels. Goldfish, and teleosts in general, have a rather poor bicarbonate buffer system. This is a direct consequence of having to breathe in an aquatic environment where CO_2 is about 30 times more soluble than is O_2 (*11*). In order to extract the necessary oxygen a very large volume of water (acting as a CO_2 sink) must be passed over the respiratory surface, making it impossible for fish to maintain high HCO_3^- levels. If goldfish relied solely on anaerobic glycolysis, they might quickly become acidotic, the result of lactic acid accumulation, and their tolerance to anoxia would be greatly reduced. By producing a neutral end product the problem of metabolic acidosis is avoided. Also, because ethanol is freely diffusible, it is easily removed across the gills and never reaches toxic levels in the fish. The only disadvantage of the strategy is that it is wasteful of carbon, but this may be a relatively minor consideration for a vertebrate without oxygen.

Table 1. Metabolite changes in whole goldfish and surrounding water after 12 hours of anoxia at 4°C. All values are means ± standard errors (S.E.); N = 4.

Metabolites (μmole/g, wet weight)		
Tissue lactate	Tissue ethanol	Ethanol excreted
	Control	
0.18 ± 0.06	0	0
	Anoxic	
5.81 ± 0.64	4.58 ± 0.31	6.63 ± 1.18

Table 2. Metabolite changes in red muscle, white muscle, and liver of goldfish after 24 hours of anoxia at 4°C; N = 4. All values are means ± S.E.; N.D., not detectable.

Tissue	Changes (μmole/g, wet weight)	
	Lactate	Ethanol
	Control	
Red muscle	0.84 ± 0.09	N.D.
White muscle	0.59 ± 0.04	N.D.
Liver	0.88 ± 0.24	N.D.
	Anoxic	
Red muscle	5.92 ± 0.51	2.94 ± 0.12
White muscle	3.74 ± 0.85	3.72 ± 0.36
Liver	6.19 ± 0.89	2.99 ± 0.18

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8. We used a Varian Aerograph A90-P3 gas chromatograph with a Porapak Q column for this determination: column, 120°C; carrier gas, He at 30 ml/min. Ethanol elutes in about 12 minutes.
9. Alcohol dehydrogenase was assayed under the following conditions: 100 m*M* potassium phosphate buffer, *p*H 7.0, 8.9 m*M* acetaldehyde, 1 m*M* glutathione (reduced form), 0.2 m*M* NADH. The fish used for these assays were "winter" acclimated. Values are reported as means ± S.E. One unit of enzyme activity is defined as that amount of enzyme which catalyzes the formation of 1 μmole of ethanol per minute under the above assay conditions.
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