Section A6.2 Metabolism

Annex Point IIA6.2

Official use only

1 REFERENCE

1.1	**Reference**	Sterenborg, I. (2007) Lactic acid as biocidal active substance, Statement to address requirements of Directive 98/8/EC ENVIRON Report, report nr. PU-LBD-20070039. Not GPL, Unpublished
1.2	**Data protection**	Yes
1.2.1	Data owner	Purac Biochem BV
1.2.2	Companies with letter of access	No
1.2.3	Criteria for data protection	Data on existing or new [a.s. / b.p.] to [maintain or vary a.s. Annex I/IA entry / vary conditions of a b.p.'s authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No, review report
2.2	**GLP**	No, review report
2.3	**Deviations**	Not applicable, review report

3 MATERIALS AND METHODS

Not applicable, review report

4 RESULTS AND DISCUSSION

Not applicable, review report

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Not applicable, review report
5.2	**Results and discussion**	The role of lactic acid in metabolism has kept researchers occupied for a long time. Many years, lactic acid was considered a dead-end waste product of the glycolysis, the conversion of glucose into pyruvate (producing a relatively small amount of ATP), in the absence of oxygen. The formation of lactic acid was thought to occur following exercise, as a result of muscle anoxia, and was thought to be the major cause of muscle fatigue (Gladden, 2004). Pyruvate serves as the starting point in the citric acid (or Krebs) cycle under aerobic conditions. It was thought that under anaerobic conditions, lactic acid is formed from pyruvate. The formation of lactic acid from glucose supplies the cell with limited amounts of energy. The main region for the formation of lactic acid in humans was believed to be the muscle, as it was hypothesized that, when muscles require energy for a short duration, for example during exercise, the citric acid cycle

cannot be entered due to the lack of oxygen. This means that pyruvate, which is formed during glycolysis, is further broken down to lactic acid. To prevent lactic acidosis, it was thought that lactic acid diffuses into the blood, is taken up by the liver, and converted back into pyruvate by the enzyme lactate dehydrogenase. Subsequently, gluceogenesis would convert pyruvate to glucose, which could be released into the blood to be used again for energy by the red blood cells and muscles. This reaction is energy consuming, as the gluceogenesis in the liver requires ATP (Holten, 1971, Stryer, 1995). This cycle is also known as the Cori cycle, and is depicted in Figure 1~~Figure 1~~~~Figure 1~~.

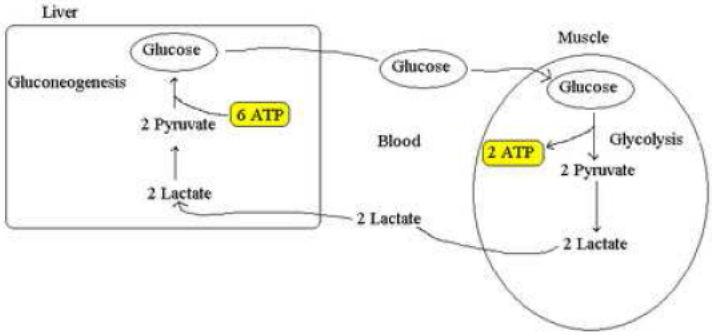

Figure 1: The Cori cycle

Based on this cycle, it has long been thought that during physical exercise, the working muscle needs energy more rapidly than oxidative metabolism can supply, which means the anaerobic conversion of pyruvate to lactic acid will occur. Consequently, lactic acid and pyruvic acid are released into the blood circulation, which was believed to result in lactic acidosis and oxygen debt (Holten, 1971).

Recently, the role of lactic acid in metabolism is reconsidered, and L-lactate is considered as a functional metabolite and mammalian fuel. Recent studies show that during hard exercise, glycolysis in muscles involves the formation of L-lactate regardless of the state of oxygenation. Furthermore, several studies support a carrier-mediated process for lactic acid transport in and out of skeletal muscle (Philp *et al.*, 2005). The concept "lactate-shuttle", which was introduced by Brooks (1998, 1999), basically means that lactate is able to transfer from its site of production (cytosol) to neighbouring cells and other organs, as well as intracellular, where its oxidation or continued metabolism can occur. The discovery of an entire family of monocarboxylate transport (MCT) proteins, which facilitate lactate transport into and out of the cell, supports this concept (Philp *et al.*, 2005).

The lactate shuttle results in the distribution of lactic acid to other cells, where it is directly oxidised, re-converted back to pyruvate or glucose, allowing the process of glycolysis to restart and ATP provision maintained. The processes involved in the lactate shuttle are depicted in Figure 2 (taken from Philp *et al*, 2005).

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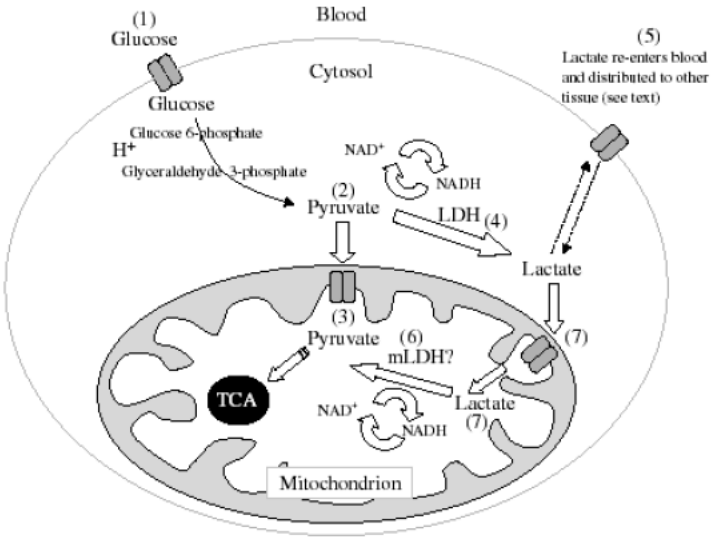

Figure 2: The processes involved in the lactate shuttle hypothesis (Brooks, 1986). The pathway proposes that (1) glucose enters the cell, where it is sequentially broken down to pyruvate (2). Pyruvate enters the mitochondrion, allowing respiration to continue in the tricarboxylic acid (TCA) cycle (3). Lactate is subsequently formed via the lactate dehydrogenase (LDH) reaction (4) and is then exported from the cytosolic compartment via monocarboxylate transporter (MCT) transport (5), where it is redistributed to a variety of functional sites. Note the suggested presence of mitochondrial lactate dehydrogenase (mLDH) (6), which forms the construct of the intracellular shuttle system (7). (taken from Philp *et al*., 2005)

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In light of the newly discovered role of lactic acid in metabolism, its role in muscle fatigue is questioned. Several investigations indicate that the classical theory of exercise-induced fatigue due to anaerobic conversion of pyruvate into lactic acid is superseded; an increase of inorganic phosphate produced during contraction is suggested as the major cause of muscle fatigue. Lactic acid may even delay the onset of fatigue by maintaining the excitability of muscles during extremely intensive exercise. However, the exact mechanism remains unclear (Philp *et al*., 2005).

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The effect of artificially elevated lactate concentrations was investigated in human subjects in a study by Miller et al. (2002). The authors concluded that when lactate is provided by intravenous infusion, blood glucose is spared and glucose production decreased. The processes underlying these effects are depicted in Figure 3 (taken from Philp *et al*., 2005).

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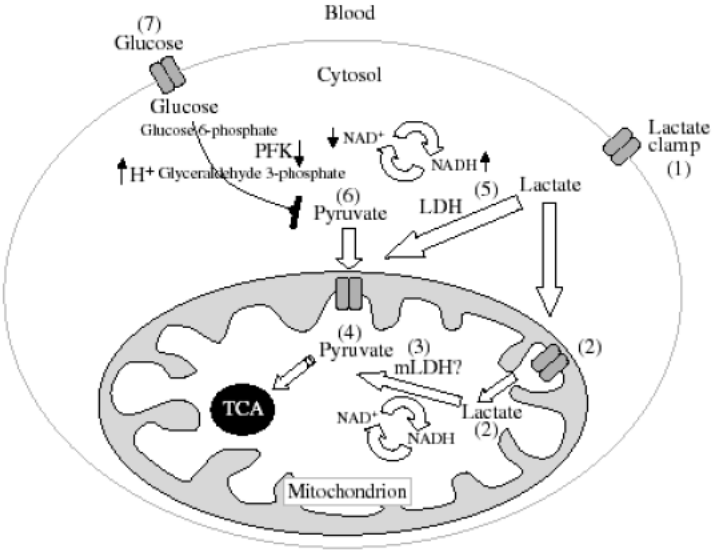

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Figure 3: The effect of artificially elevated lactate concentrations (lactate clamp) on metabolic processes. Increased circulatory lactate concentrations (1) result in lactate entering the cytosol, where it then enters the mitochondrion via MCT1 (2). Within the mitochondrion, lactate is converted to pyruvate via mLDH (3), which then progresses into the tricarboxylic acid (TCA) cycle (4). However, artificially raised cytosolic lactate concentrations (5) lead to suppression in glycolysis. Therefore, a resulting increase in H+ and NADH occurs, and acidosis inhibits phosphofructokinase (PFK) activity (6). This suppression finally results in reduced glycolytic activation and a reduction, or sparing, of glycogenolysis. (taken from Philp *et al.*, 2005)

After the discovery of the lactate shuttle, the exact role of lactic acid *in vivo* remains still to be investigated. It is suggested that lactic acid plays a role as metabolic signal at the whole-organism level, due to its ability to be transported and regulate the cellular redox state in cells. Several investigations indicate that lactate plays a role in wound-healing, which may be associated with the signalling function (Philp *et al.*, 2005, Gladden, 2004).

5.3 Conclusion

It may be concluded that, based on the discovery of the lactate shuttle, lactic acid can no longer be considered as a "dead-end" waste product, but should instead be seen as a central player in cellular, regional, and whole body metabolism.

5.3.1 Reliability 1

5.3.2 Deficiencies Not applicable, review report

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2008/05/21

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Materials and Methods	Not applicable (see remarks).
Results and discussion	Applicant's version adopted with minor correction: Figures 2 and 3: NADH is not consumed during conversion of lactate to pyruvate by mLDH as the direction of the arrows in figures 2 and 3 suggest, but is generated from NAD^+. Philp et al. (2005) concluded that "elevated lactate helps reduce glucose usage and glycogenolysis, minimising depletion of these stores as escalating acidosis reduces PKC (phophofructokinase) function." Furthermore, gluconeogenesis was increased under artificially elevated lactate levels during moderate exercise.
Conclusion	Other conclusions: Lactate is an integral part of normal mammalian metabolism. Physiological plasma levels in man range between 1 mM at rest and 10 mM during exercise. Monocarboxylate transport proteins facilitate the distribution of lactate between organs, cells and subcellular organelles. Cytosolic and mitochondrial lactate dehydrogenases convert lactate into pyruvate, producing NADH. Pyruvate can be transformed to oxaloacetate, which is utilised for gluconeogenesis via production of phosphoenolpyruvate by decarboxylation and phosphorylation. Alternatively, metabolites of pyruvate (oxaloacetate and acetyl-CoA) are consumed in the tricarboxylic (citric) acid cycle generating NADH and ATP, or pyruvate may be transaminated to the amino acid L-alanine. Increased cellular levels of lactate influence pathways of cellular metabolism, resulting in reduction of glycolysis and glycogenolysis (reducing the generation of pyruvate from other sources such as glucose) and in enhancement of gluconeogenesis.
Reliability	2
Acceptability	Acceptable with restrictions (see remarks)
Remarks	Review, no original data

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	