Biochemical Studies on the Liver Fluke, *Fasciola hepatica*, L.

W. Thorsell

Department of Chemistry, Royal Veterinary College, Stockholm, Sweden

The liver fluke lives in the bile ducts, an environment poor in oxygen. Flukes, however, are not strictly anaerobic as is shown in Table 1.

Table 1. Time of survival, *in vitro*, of liver fluke. Various environments.

Environment	Time of survival
N_2	> 1 h
Air	> »
O_2	> »
Coal-gas, CO*	> 1 h
CO_2	< »
H_2S	< 10 min
Air + KCN, 0.01 M	> 1 h
Air + H_2O_2, 0.01 M	< 10 min

* *In vivo* experiments are reported by E. Lienert. *(Private Communication).*

In order to find out whether there is a qualitative difference between parasite and host in the aerobic phase of the metabolism a survey of TCA-cycle acids present and some of the oxidation steps was performed. Some of the results obtained, *e.g.*, with paper chromatography [1] are shown in Table 2.

With the aid of a fraction from the liver fluke, Keilin-Hartree-particles [2], it was possible to show the occurrence of heat-labile systems which in the presence of succinic acid, DPNH and TPNH reduced cytochrome c [3]. DPNH and possibly TPNH were also oxidized by air in the presence of the mentioned fraction. Preliminary isolation experiments for cytochrome c [4,5] gave results which implies that if cytochrome c is present it occurs in concentrations less than 0.3 mg/50 g liver fluke.

1. Nordmann, R., Gauchery, O., du Ruisseau, J. P., Thomas, Y. and Nordmann, J. *Bull. Soc Chim Biol.* **36** (1954) 1641.
2. Colowick, S. P. and Kaplan, N. O. *Methods in Enzymology*. Vol. 1 (1955) 725.
3. Colowick, S. P. and Kaplan, N. O. *Methods in Enzymology*. Vol. 2 (1955) 688.
4. Colowick, S. P. and Kaplan, N. O. *Methods in Enzymology*. Vol. 2 (1955) 750.
5. Alexander, P. and Block, R. J. *Separation and isolation of proteins*. Vol. 1 (1960) 36.

A Test for the Disulphide Reducing Capacity of Red Blood Cells

H. C. Börresen* and L. Eldjarn

Institute of Clinical Biochemistry, Rikshospitalet, University of Oslo, Norway

Human erythrocytes reduce some disulphides [1]. The rate of appearance of the corresponding thiol is the resultant of its spontaneous oxidation and the activity of the enzyme systems which participate in the reduction, *i.e.* the glutathione reductase system and the TPNH generating steps of the pentose phosphate shunt. Hexokinase may be rate deter-

* Fellow of the Norwegian Cancer Society.

Table 2. Occurrence of some TCA-cycle acids in the liver fluke.

Citric acid	Positive finding.
Aconitic acid	Negative finding, or less than 100 μg/50 g liver fluke.
Isocitric acid	Not separated from citric acid.
α-Ketoglutaric acid	Experiment not finished.
Succinic acid	Positive finding. Large amounts.
Fumaric acid	Positive finding.
Malic acid	Positive finding.
Oxalacetic acid	Experiment not finished.

mining with glucose as substrate, whereas with inosine the thiol production could be dependent on nucleoside phosphorylase, ribose phosphate isomerase, transketolase or transaldolase. A decreased activity of glucose 6-phosphate dehydrogenase, glutathione reductase or a lack of glutathione, which are all known to be connected with hemolytic states or abnormal drug sensitivities [2-4], may express themselves as a lowered rate of thiol appearance.

The following test system for disulphide reducing capacity has been developed to minimize the role of spontaneous oxidation, to sustain optimal disulphide concentration, and to permit accurate assay of the amount of thiol present. 2 ml of heparinized blood (or a somewhat concentrated suspension of erythrocytes) is incubated aerobically at 37°C with a mixture of cystamine (RSSR, 8.34 mM) and oxidized glutathione (GSSG, 2.5 mM). Most of the extracellular disulphide then at equilibrium occurs as the mixed disulphide, GSSR [5] which does not penetrate the cell membrane. In this system RSSR does not attain toxic concentrations despite the large amount of ultimately reducible disulphide. Furthermore this "buffer system" keeps [RSSR] relatively constant. The amount of thiol produced is assayed by an accurate and rapid modification of the amperometric titration at the rotating platinum electrode, the $AgNO_3$ being added to the titration vessel before the blood sample [6]. This counteracts spontaneous oxidation during the titration (30 sec). With saponin present in the titration vessel, the total amount of rapidly reacting —SH groups in the hemolysed blood is measured, whereas in the absence of saponin the extracellular and the diffusible thiols are assayed selectively.

Details of the method and data from its application to the red cells of several mammalian species as well as to abnormal human erythrocytes will be presented.

1. Eldjarn, L., Bremer, J. and Börresen, H. C. *Biochem. J.* **82** (1962) 192.
2. Carson, P. E. *Federation Proc.* **19** (1960) 995.
3. Carson, P. E., Brewer, G. J. and Ickes, C. E. *J. Lab. Clin. Med.* **58** (1961) 804.
4. Oort, M., Loos, J. A. and Prins, H. K. *Vox Sanguinis* **6** (1961) 370.
5. Eldjarn, L. and Pihl, A. *J. Am. Chem. Soc.* **79** (1957) 4589.
6. Börresen, H. C. *Anal. Chem. (Submitted for publication).*

Yeast Carboxylase and Cell Permeability

Erkki Oura and Heikki Suomalainen

Research Laboratories of the State Alcohol Monopoly (Alko), Helsinki, Finland

Yeast carboxylase decarboxylates, besides pyruvic acid, other α-keto acids at least up to acids with chains of 8 carbon atoms [1]. The longer the carbon chain of the acid is, the slower is the decarboxylation. Also α-ketoglutaric acid and phenylpyruvic acid are split by carboxylase.

Yeast carboxylase is located inside the cell; thus the keto acid molecule must penetrate the cell wall and plasma membrane in order to enter the intact cell and is then almost immediately decarboxylated. However, the plasma membrane limits the permeation so effectively that the keto acid only slowly enters the cell from the medium.

Hence, the speed of the liberation of carbon dioxide measures the slowest stage of the reaction, *i.e.* the penetration of keto acid into the yeast cell [2]. In this way the ability of the yeast to decarboxylate α-keto acids offers a useful and easily determinable way to follow the penetration of some organic acids into intact yeast cells, by different yeasts and in different conditions [3]. Determinations have been made by which it has been possible to prove that α-keto acids with a longer carbon chain, *i.e.* with a greater lipid solubility, penetrate into the cell more rapidly. The fact that a dissociated keto acid ion cannot penetrate into the cell at anaerobic conditions, clearly proves that the lipid solubility is a presumption for this permeation. However, in the same conditions, keto acid esters are decarboxylated quite rapidly, although the liberation of carbon dioxide presupposes hydrolysis of the esters before decarboxylation and the hydrolysis evidently is the slowest stage of the reaction series. This esterase activity is also located inside the cell, contrary to earlier reported opinions.

The decarboxylase activity and cell permeability of baker's yeast clearly differs from that of brewer's yeast, the latter having a higher decarboxylase activity and cell permeability. The decarboxylase activity of baker's yeast varies markedly with the growth conditions, correlating with the changes in the thiamine content of the plasma fraction of the cells [4]. In the fermentation solution of yeasts with a weaker decarboxylase activity the con-