DEPARTAMENTO DE BIOQUÍMICA E BIOFÍSICA
Diretor: Prof. Dr. Metry Bacila

# BIOCHEMICAL ASPECTS OF THE CARBOHYDRATE METABOLISM IN BIOMPHALARIA GLABRATA

## (ASPECTOS BIOQUÍMICOS DO METABOLISMO DE CARBOIDRATOS EM **BIOMPHALARIA GLABRATA**)

METRY BACILA
Prof. Catedrático

## INTRODUCTION

**Biomphalaria glabrata** is a dulciaquicola gastropode, transmitter of the **Schistosoma mansoni.** As a pulmonated snail, respiration is based on the utilization of atmospheric oxygen. It also possesses a very active cutaneous respiration. From the ecological point of view, **B. glabrata** is extensively found in large territories, mainly in shallow waters and in slow moving currents. It is an eunionic, eurobiotic and stenohyaline snail, a herbivorous which can also live on a trophic level as a saprophite (1).

By being the intermediate of one of the most frequent and most serious human parasites in the tropical and semitropical areas of the world, **B. glabrata** has been extensively studied from several points of view, due to the epidemiological interest aroused by the knowledge of its behaviour either in its natural habitat as well as when kept in captivity or when raised in axenic conditions. Most important are also the implications of the behaviour of **B. glabrata** in the hostparasite relationship, with the transmissibility to man of the infestant forms of **Schistosoma mansoni**, a fact which might imply fundamental problems pertinent to the biochemical and immunological aspects of the disease.

In recent years, research on the biochemistry of Planorbidae and especially on **B. glabrata** has been emphasized, mainly in this country (for a list of references, see MAGALHÃES NETTO and MAGALHÃES (2). Most of the recent research work carried out have been communicated in three Simposia (3-5) held at the University of Paraná (1961 and 1966) and at the University of Recife (1963) as well as in several meetings of the Sociedade Brasileira para o Progresso da Ciência.

With a grant in aid from Projeto FUNTEC N.º 31, Banco Nacional do Desenvolvimento Econômico, and from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

A considerable part of the research work carried out on the biochemistry of **B. glabrata** has been directed to the carbohydrate composition of the snail, their origin, and meaning, the enzymes involved in the pathway of carbohydrate metabolism, and the involvement and significance of several energy yielding compounds, with the aim of knowing the common and the anaplerotic mechanisms of the animal and its metabolic regulations.

Recent work carried out in this laboratory has been directed to the problem of the biochemistry of the snail pigmentation (ARAGÃO and BACILA (6) and on the purification, kinetic studies and meaning of hemolymph acid and alkaline phosphatases (GIACOMETTI and BACILA (7).

## THE SOURCE OF SNAILS AND GENERAL EXPERIMENTAL TECHNIQUES

Most of the specimens used in the experiments were obtained from their natural habitat (the Northeastern region of the State of Paraná, Brazil, and the Dique, Salvador, Bahia, Brazil) and kept in captivity in special prepared aquaria sufficiently aerated.

The animals were fed with lettuce. The soft parts of the snail were obtained by breaking the shell after pressing the animal between two microscope slides. After a convenient washing of the whole body, the foot muscle, the albumen gland, the digestive glands and the ovotestis were carefully dissected. For the enzymatic work, the dissected parts were immediately frozen and kept at 25°, and used subsenquently. For the work on mitochondria, isolated foot muscle, digestive gland, and ovotestis were immediately homogenized according to the method of VOSS, CAMPELLO and BACILA (8) and separated by differential centrifugation. Anaerobic glycolysis (9) was also performed by the conventional Warburg technique in Ringer-biocarbonate buffer ($N_2$ : $CO_2$, 95 : 5%). For the determination of fructose-1,6-diphosphate aldolase and transaldolase levels (10) foot muscle and digestive gland from 55 animals were obtained and homogenized in two volumes of 0.04-0.01 M triethanolamine-EDTA, pH 7,6 buffer. The homogenate was spun down 10 min at 12,000 rpm and the supernatant used as a source of enzyme.

For the carbohydrate studies several techniques were used. For the hemolymph, the circulating blood was collected by cardiac puncture and kept in sodium fluoride at - 25° during the collection step.

For preparative purposes, the hemolymph was collected in ethanol (80% v/v). Carbohydrates were then measured either in protein-free filtrates or in acid hydrolysates ($H_2SO_4$ and 6N HCl). Several specific tchniques were also used by DUARTE (28) and his coworkers for the study of neutral carbohydrates, polysaccharides, amino-sugars and mucopolysaccharides.

Extraction and purification steps were also used in the study of several carbohydrases from which **B. glabrata** is an excellent source. These carbohydrases are very significant for the circulation of carbohydrates in the snail.

## ISOLATED TENTACLES

By showing that the tentacles isolated from **B. glabrata** are capable of an unidirectional mobility in water which can be measured within statistic limits of certainty, BACILA and MEDINA (11) used this biological system to study the effect of metabolic inhibitors and moluscocides on the mobility normal values.

The isolated antennae were obtained by cutting them as near as possible to the base of their insertion (Fig. 1) with a pair of ophtalmological scissors and kept either in distilled or tap water.

**Fig. 1 — The technique used for obtaining the isolated tentacles from B. glabrata. The cutting of the tentacles is carried out as near as possible to the basis of their insertion with a pair of ophtalmological scissors and kept either in distilled or in tapwater.**

Measurements of the unidirectional mobility of the isolated tentacles were carried out in a special "track" consisting of the graduated capillaries of a standard Warburg manometer (Fig. 2). The double capillaries were sometimes used independently, one for measuring the mobility in normal conditions, the other for measuring the effect of one of the compounds used in these experiments.

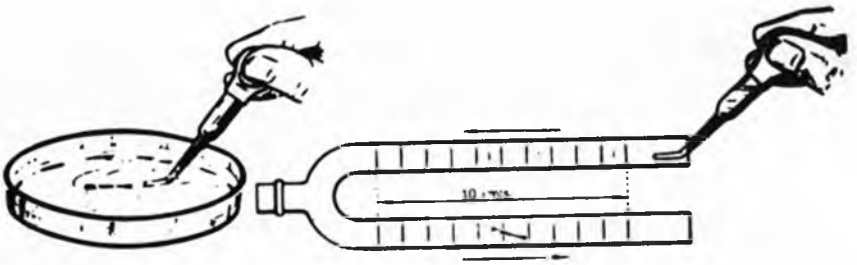

Fig. 2 — The technique for measuring the unidirectional mobility of the isolated tentacle. The graduated capillaries of a standard Warburg manometer were filled with the proper medium previously to the introduction of the tentacle into the capillary tubes. The tentacles were first sucked up into a small pipette and then introduced into the capillary tubes. The mobility was then measured as the time taken by the isolated tentacle to move through a fixed distance of 100 mm.

Normal values for the mobility of the isolated tentacles were established for a total of 99 tentacles and are shown in Table I.

**TABLE I**

**Normal values for the isolated tentacles ***

| Mobility ($t_1$) (sec.) | Mobility ($t_2$) (sec.) | Velocity ($V_1$) (mm sec $^{-1}$) | Velocity ($V_2$) (mm sec $^{-2}$) | Wet Weight (mg) | Dry Weight (mg) |
|---|---|---|---|---|---|
| 61.961 | 62.840 | 0.174 | 0.173 | 0.248 | 0.046 |

* All values show the average of 99 determinations. Mobility was measured in water. Velocity/mg dry weight = 3,8 mm. $sec^{-1}$

Mobility of isolated tentacles of **B. glabrata** is affected by metabolic inhibitors. Malonic acid, as an inhibitor of the Krebs-Johnson cycle, sodium azide as an inhibitor of the respiratory chain, and

2, 4-dinitrophenol as an uncoupler of oxidation and phosphorylation were used. At concentrations of sodium azide ranging from $10^{-6}$ to $10^{-2}$ M, there was complete inhibition of the tentacle mobility (Table II).

**TABLE II**

**Inhibition of tentacle mobility by metabolic inhibitors**

| Inhibitors (M) | Velocity (mm. $sec^{-1}$) | Inhibition (Percent) |
|---|---|---|
| None | 0.174 | — |
| Glucose | | |
| $10^{-2}$ M | 0.175 | — |
| Na Azide | | |
| $10^{-2}$ to $10^{-6}$ M | 0.000 | 100 |
| $10^{-7}$ M | 0.142 | 19 |
| $10^{-8}$ M | 0.157 | 14,6 |
| Malonate | | |
| $10^{-1}$ to $10^{-3}$ M | 0.000 | 100 |
| $10^{-4}$ M | 0.089 | 48,8 |
| $10^{-6}$ M | 0.180 | — |
| 2,4-DNP | | |
| $10^{-2}$ to $10^{-3}$ M | 0.000 | 100 |
| $10^{-4}$ M | 0.076 | 56,4 |
| $10^{-5}$ M | 0.182 | — |

Mobility of the isolated tentacles is sensitive to compounds with pharmacological activities toward definite sites of action (Table III). The isolated tentacles are very sensitive to prostigmine bromide, an inhibitor of cholinesterase, but less sensitive to the action of iproniazide, a monoaminoxidase inhibitor. Chlorobutanol, an anesthetic agent, can also inhibit mobility of the antennae. This can be explained in terms of the findings of CAMPELLO et al. (12), showing that chlorobutanol, besides inhibiting respiration of rat tissue slices, also acts on the normal properties of the isolated mitochondria.

**TABLE III**

**The effect of drugs with pharmacological properties on the mobility of isolated tentacles**

| Compound (M) | Pharmacological effect | Velocity (mm. $sec^{-1}$) | Percent inhibition |
|---|---|---|---|
| None | — | 0.174 | — |
| Prostigmine Bromide | Inhibitor of cholinesterase | | |
| $10^{-2}$ | | 0.066 | 62 |
| $10^{-3}$ | | 0.092 | 47 |
| $10^{-4}$ | | 0.111 | 36.2 |
| $10^{-5}$ | | 0.143 | 23.5 |
| Iproniazide | Inhibitor of mono-aminoxidase | | |
| $10^{-2}$ to $10^{-3}$ | | 0.000 | 100 |
| $10^{-4}$ | | 0.142 | 19.5 |
| $10^{-6}$ | | 0.147 | 15.5 |
| Hyamine | Compound with surface action | | |
| $10^{-1}$ to $10^{-9}$ | | 0.000 | 100 |
| $10^{-10}$ | | 0.114 | 34.5 |
| $10^{-12}$ | | 0.123 | 29.4 |
| $10^{-13}$ | | 0.145 | 16.6 |
| Chlorobutanol | Anesthetic | | |
| $10^{-1}$ to $10^{-2}$ | | 0.000 | 100 |
| $10^{-3}$ | | 0.099 | 43 |
| $10^{-5}$ | | 0.102 | 41.4 |
| $10^{-7}$ | | 0.186 | — |
| Cyanamide | | | |
| $10^{-3}$ | | 0.111 | 36.2 |
| 10-4 | | 0.164 | 5.6 |
| $10^{-2}$ | | 0.135 | 22.4 |
| $10^{-1}$ | | 0.157 | 9.8 |

The mobility of the isolated antennae is abolished by extremely low concentrations of hyamine, a quaternary compound of ammonium with high surface action. The molluscocide properties of hyamine have been studied by VALLEJO-FREIRE et al. (13). At concentrations ranging from $10^{-9}$ to $10^{-1}$ M, hyamine completely abolishes the mobility of the antennae. With lower concentrations ($10^{-13}$ to $10^{-10}$ M), mobility can be fully measured during the first 10 cm; after this, it slows down in such a way that $t_s$ (see Table I) can not be measured.

Unlike the other compounds tested, the inhibitory mechanism of action of cyanamide on the mobility of isolated tentacles cannot be explained yet in terms of a direct biochemical lesion.

Phenothiazines and related compounds have been shown to act as inhibitors of the normal properties of isolated heart sarcosomes besides showing an effect on the membrane structure. Two compounds — Prochlorpromazine (Tementil metasulfonate), and Thioxanthone — were used in this experiment, both of them showing a very pronounced effect as inhibitors of the mobility of the isolated tentacles (Fig. 3). Inhibition was also observed with Stibinol.

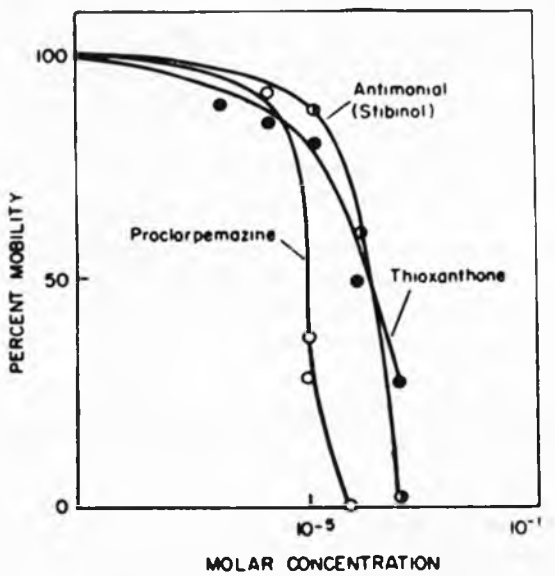


**Fig. 3 — The effect of Stibinol, prochlorpromazine and thioxantone on the mobility of isolated tentacles. Mobility was measured as described in Fig. 1 and 2, and the 100% mobility was taken from the normal values showed on Table 1. Inhibition was calculed as percent mobility.**

## ENZYMATIC ACTIVITY IN **B. glabrata**

Foot muscle and digestive gland have been used as the sources for the study of enzymes connected with the carbohydrate metabolism in **B. glabrata.** It has been found that the snail possesses a variety of polysaccharases and disaccharases particularly important for nutritional purposes. Furthermore, it also possesses an extraordinary equipment of phosphatases. Several enzymes linked to the glycolytic pathway and to the glucose-6-phosphate "shunt" have also been found. Very significant for the regulatory mechanisms in **B. glabrata** were the findings of high contents of α-glycerophosphate dehydrogenase and "malic" enzyme, as well as the fact that lactic acid dehydrogenase form **B. glabrata** is a facultative enzyme in regard to $NADPH_2$ and $NADH_2$.

1. Carbohydrases.

Amylase activity from the digestive gland of **B. glabrata** was described by VIEIRA (14) and by MAGALHÃES NETTO and ALMEIDA (15). This enzyme, an α-amylase, has been purified by VIEIRA and LADEIRA (16) as an enzyme-glycogen complex. Cellulase, found as a component of the digestive gland of the snail, has been purified and several of its properties studied by DUARTE, FOKAMA, and BACILA (17, 18). The optimum pH was shown to be 4.8. The specificity of this cellulase was studied with carboxymethyl cellulose, aminoethylcellulose, DEAE-cellulose, cellulose powder, and cellulose phosphate. Most significant was the fact that by column chromatography of one of the purified fractions, in Sephadex G 75, preliminary results on the obtention of a cellulase fraction free from cellobiase was obtained.

The presence of invertase in the stomach of **B. glabrata** has been described by MAGALHÃES NETTO (19). More extensive work on the purification of carbohydrases by DUARTE, OLIVEIRA, and FOKAMA (20) showed of hydrolytic activity for a variety of substrates: cellobiose, lactose, maltose, gentiobiose, melibiose, turanose, sucrose, trehalose, as well as for trisaccharides, among them, raffinose, panose, and melizitose. Hydrolysis of stachyose has also been found.

LADEIRA and VIEIRA (21) have been reported cellulase activity in the digestive gland of **B. glabrata** raised in axenic conditions, while SCHWAB, BRANDÃO, VIANNA and BACILA (22) failed to demonstrate any cellulolitic activity in microorganisms isolated from the digestive gland of the snail.

2. Phosphatases.

Phosphatase activity in **B. glabrata** was studied by ZANCAN and BACILA (23) in the partially purified preparations from the digestive gland (Table IV). Crude extracts have been precipitated by salting out with ammonium sulfate at different concentrations followed by the assay of enzyme activity. Similar experiments were carried out with foot muscle extracts (ZANCAN and BACILA, unpublished data) showing also phosphatasic activity for all the substrates tried with the digestive gland except α-glycerophosphate. The specific activity of the enzymes from the foot muscle were lower than the enzymes from the digestive gland. Very important is the finding of fructose-1,6-diphosphate phosphatase activity in both extracts because of the implication of this enzyme in the fructose-diphosphate metabolism.

**TABLE IV**

**Phosphatase activity in BIOMPHALARIA GLABRATA in crude extracts of the digestive gland**

| SUBSTRATE | SPECIFIC ACTIVITY |
|---|---|
| Glucose-1-phosphate | 71.3 |
| Glucose-6-phosphate | 35.49 |
| Fructose-1-phosphate | 20.37 |
| Fructose-1,6-diphosphate | 27.37 |
| Fructose-6-phosphate | 40.4 |
| 6-phosphogluconate | 40.48 |
| $\beta$-glycerophosphate | 47.6 |
| $\alpha$-glycerophosphate | 53.82 |

Extensive work on the hemolymph phosphatases is being carried out by GIACOMETTI and BACILA (7). Acid and alkaline phosphatases are present in hemolymph in high levels of activity. Both have been partially purified from the supernatant of hemolymph spun down 30 minutes in a Spinco LB65 preparative ultracentrifuge at 60,000 rpm.

A spectrophotometric method using p-nitrophenylphosphate as the working substrate was established, and kinetic data were thus obtained in a Gilford recording spectrophotometer. Michaelis-Menten constants, optimum pH, as well as the effect of several ions on the enzymatic activity of both enzymes were studied. Berylum was found to act as a powerful inhibitor for alkaline phosphatase, thus allowing correct determination of the pH curves for both phosphatases.

## ENZYMES OF CARBOHYDRATE METABOLISM

Preliminary data on the enzymes of carbohydrate metabolism of **B. glabrata** were obtained by ZANCAN and BACILA (24) (as are shown in Table V), in crude preparations from the foot muscle. Three enzymes related to the carbohydrate metabolism were also studied: $\alpha$-glycerophosphate dehydrogenase, lactic dehydrogenase, and the "malic" enzyme. It is significant that the ratio between $\alpha$-glycerophosphate dehydrogenase and lactic dehydrogenase activities is 11.2 to 1.0.

**TABLE V**

**Enzymes of the carbohydrate metabolism from the foot muscle of the snail B. GLABRATA**

| ENZYMES | SPECIFIC ACTIVITY |
|---|---|
| Phosphoglucomutase | 1.5 |
| Phosphoglucoisomerase | 2.5 |
| Aldolase | 0.45 |
| Pyruvatekinase | 0.27 |
| Glucose-6-phosphate dehydrogenase | 5.0 |
| 6-phosphogluconate dehydrogenase | 4.5 |
| $\alpha$-glycerophosphate dehydrogenase | 150.0 |
| Lactic dehydrogenase | 13.3 |
| "Malic" enzyme | 6.8 |

Recently, CRIVELLARO and BACILA (25) have further purified fructose-1,6-phosphate aldolase and also purified transaldolase from the foot muscle and the digestive gland of **B. glabrata** (Table VI).

## TABLE VI

**Fructose-1,6-diphosphate aldolase and transaldolase in the foot muscle and digestive gland from BIOMPHALARIA GLABRATA**

| FOOT MUSCLE | | | | DIGESTIVE GLAND | | | |
|---|---|---|---|---|---|---|---|
| Fraction | Protein (mg/ml) | Units (per ml) | Specific activity | Fraction | Protein (mg/ml) | Units (per ml) | Specific activity |
| ALDOLASE | | | | ALDOLASE | | | |
| 1. Crude extract | 3.74 | 6.825 | 1.825 | 1. Crude extract | 2.838 | 2.46 | 0.866 |
| 2. Precipitate 0.60 sat. Ammonium sulfate | 3.96 | 16.0 | 4.04 | — — — | — — — | — — — | — — — |
| TRANSALDOLASE | | | | TRANSALDOLASE | | | |
| 1. Crude extract | 3.74 | 1.0 | 0.267 | 1. Crude extract | 2.838 | 0.78 | 0.274 |
| 2. Supernatant from 0.60 sat. Am. sulf. | 0.87 | 0.325 | 0.37 | — — — | — — — | — — — | — — — |

SYSTEM: 55 foot muscles and digestive glands were homogenized with 11 ml of TEA-EDTA (0.04-0.01 M), pH 7,6 buffer. The whole homogenate was let to sit (0°) for min. and then centrifuged 20 min. at 15,000 rpm. For the ammonium sulfate precipitation, 8.3 ml of the extract containing 8,125 units of aldolase and 1,7 units of transaldolase was precipitated with 1.41 g of ammonium sulfate (0.30 sat.), let sit 10 min. at 0° and centrifuged at 12,000 rpm for 10 min. The sediment was dissolved in 1.0 ml of TEA-EDTA (0.04-0.01 M), pH 7.6 buffer. The supernatant was brought first to 0.45 and then to 0.60 sat. with ammonium sulfate, then centrifuged and dissolved in the same way. Aldolase was measured according to RACKER (**J. Biol. Chem. 167,843 (1947)**), and transaldolase according to ROWLEY, TCHOLA and HORECKER, (**Arch. Biochem. Biophys.**, 1964).

## ANAEROBIC GLYCOLYSIS

The aspects of anaerobic glycolysis were studied by ACCIOLY (9) after measuring the total content of organic acids (0.0001 meq/mg dry weight) in **B. glabrata.** Measurements of anaerobic glycolysis of the foot muscle of the snail showed high endogenous values and a ratio of $CO_2$ : $O_2$ equivalent to 2 : 1. Chromatographic analysis of the organic acids formed showed malic, fumaric, and lactic acids among other products produced as a consequence of the anaerobic glycolysis. These results suggested a possible pathway for the formation of organic acids.

## RESPIRATORY CHAIN AND RESPIRATION

Studies on the respiratory chain components of the mitochondria from **B. glabrata,** as well as electronmicrographs of mitochondrial preparations from the several parts of the snail body were carried out by VOSS, BRANDAO, CARDOSO, and BACILA (26).

Differential spectroscopy has been carried out with a suspension of mitochondrial fractions from the digestive gland, showing typical absorption bands at 597, 560, 548, 440, and 416 nm. The relative concentrations for the cytochromes were 0.44 for cyt. **b,** and 0.54 for cyt. **c,** taking the total content of cyt. **a** as equal to 1.0. For this assay, the suspension of mitochondria was enzymatically reduced in the presence of succinate.

Isocitric dehydrogenase activity in the foot muscle of **B. glabrata** was first assayed by ZANCAN and BACILA (24).

## PATHWAYS OF CARBOHYDRATE METABOLISM

Most of the significant enzymes of the glycolytic pathway have been identified in **B. glabrata** (24, 25). From the Warburg-Dickens-Lipmann pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were first demonstrated by ZANCAN and BACILA (24), and transaldolase by CRIVELLARO and BACILA (25).

Figure 4 shows the main pathways of carbohydrate metabolism in **B. glabrata.** Glycogen has been detected in the foot mucous gland, in the digestive gland, and in the ovotestis of the snail by MOLFI (27), using histochemical methods. Glucose-6-phosphate plays a central role in the regulatory mechanisms of both glycolytic and pentose phosphate pathways. The ratio between glucose-6-phosphate dehydrogenase and phosphoglucomutase is 3.3 to 1.0 and with

phosphoglucoisomerase, 2.0 to 1.0. Glucose-6-phosphate dehydrogenase and phosphoglucomutase is 3.3 to 1.0 and with phosphoglucoisomerase, 2.0 to 1.0. Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase show a ratio of activity of about 1.0: 1.0. The enzymatic mechanism for glucuronate formation has not been studied yet.

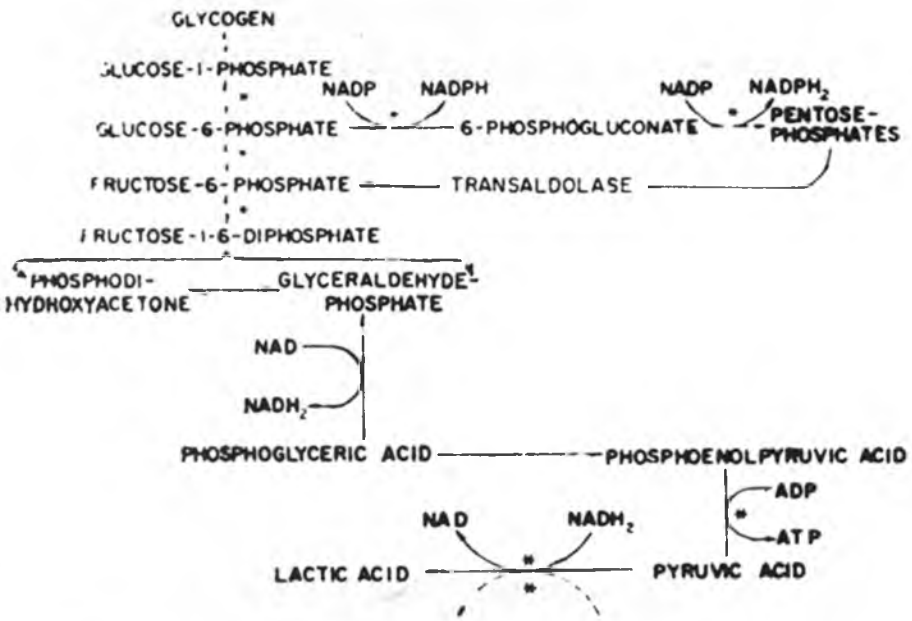


Fig. 4 — Main pathways of carbohydrate metabolism in B. glabrata. All the enzyme activities already identified are marked with *.

## METABOLISM OF PYRUVIC ACID

Pyruvate kinase, lactic dehydrogenase and "malic" enzyme activities have been shown by ZANCAN and BACILA (24) in **B. glabrata.** Thus, pyruvate is formed through the catalytic activity of pyruvatekinase. However, pyruvate can be metabolized in three different ways (Fig. 5), that is, through the respiratory pathways, through lactic dehydrogenase in **B. glabrata** (24) is a facultative NADH or NADPH dependent enzyme, a property with implications for the "malic" enzyme on the regulation of pyruvate metabolism. The ratio between lactic dehydrogenase and "malic" enzyme in the foot muscle of the snail is about 2:1.

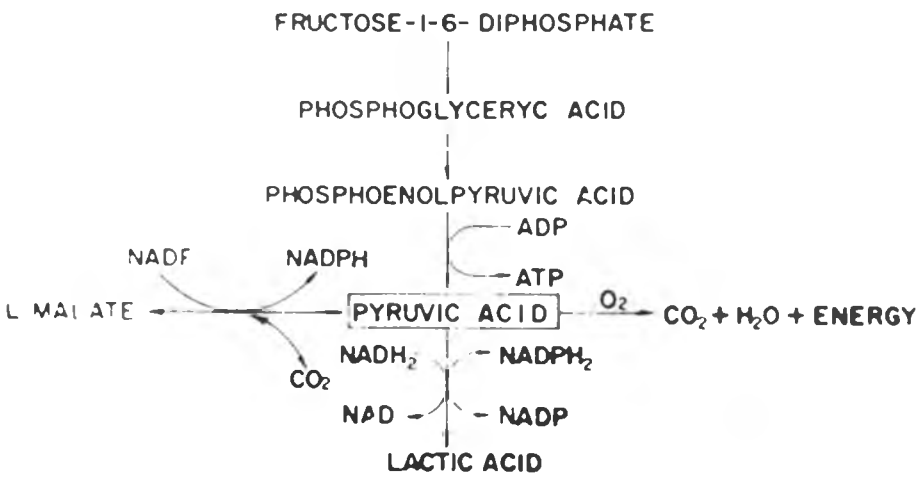


Fig. 5 — Pathways of pyruvate metabolism in B. glabrata.

## REGULATION OF NAD/NADH AND NADP/NADPH LEVELS

The regulation of NAD/NADH and NADP/NADPH levels in **B. glabrata** shows several peculiarities (Fig. 6).

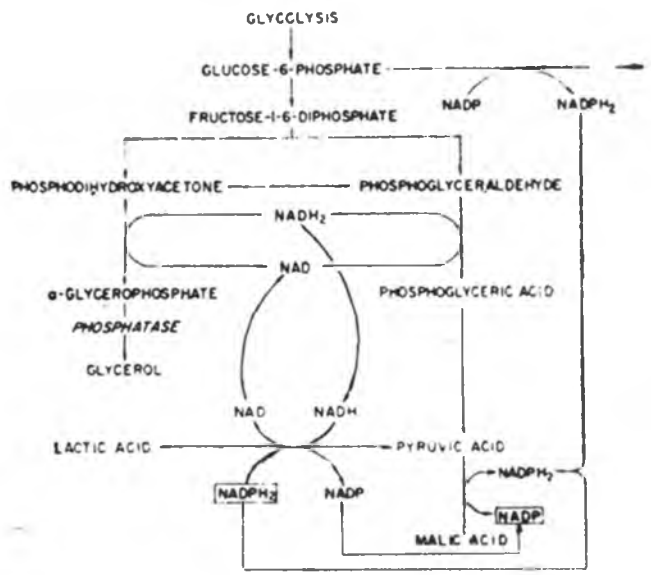


Fig. 6 — Regulation of NAD/NADH and NADP/NADPH levels in B. glabrata.

The hexosemonophosphate "shunt" plays the important role of producing most of the NADPH needed for the biosynthetic metabolism of the snail. Another interaction between the glycolytic pathway and the "shunt" is derived from the fact that reduction of pyruvate to lactic acid is carried out by a lactic dehydrogenase which is both NADH and NADPH dependent. Since "malic" enzyme is also present, it is possible to postulate that part of the malic acid is formed when the equilibrium of the lactic dehydrogenase is displaced to the side of pyruvate. This step, as well as the one that leads to malate biosynthesis contribute to the reoxidation of NADPH, thus maintaining the ratio between NADP/NADPH on the physiological levels. Both mechanisms, that is, the reversal of the lactic acid dehydrogenase activity as well as the reversal of "malic" enzyme action, may be very important for the biosynthetic pathways of glycogen formation in the snail.

Reoxidation of NADH, fundamental for the maintainance of the glycolytic metabolism, is carried out only in part by the lactic dehydrogenase, as is commonly found in vertebrates, as well as in yeast for the case of alcohol dehydrogenase. **B. glabrata** possesses an ext remely active α-glycerophosphate dehydrogenase (Table V). Since the ratio between this enzyme and lactic dehydrogenase is 11.2 to 1.0, it is correct to postulate that the main mechanism of NADH reoxidation is through the reduction of phosphodihydroxyacetone to α-glycerophosphate by the catalytic action of α-glycerophosphate dehydrogenase.

Such mechanism can also explain the formation of glycerol in the snail, since a phosphatase activity for α-glycerophosphate has also been found in the digestive gland of **B. glabrata** (23).

## METABOLIC INTERRELATIONS BETWEEN THE TRICARBOXILIC ACID CYCLE AND THE GLYCOLYTIC AND HEXOSEMONOPHOSPHATE "SHUNT" PATHWAYS

"Malic" enzyme activity offers a possibility for an anaplerotic mechanism in **B. glabrata.** Furthermore, it establishes a metabolic link between the tricarboxylic acid cycle and the hexosemonophosphate "shunt" and the glycolytic pathway (Fig. 7). For the TCA cycle, isocitric dehydrogenase in **B. glabrata** has been measured by ZANCAN and BACILA (24) and succinic oxidase activity by VOSS, BRANDÃO, VIANNA and BACILA (26).

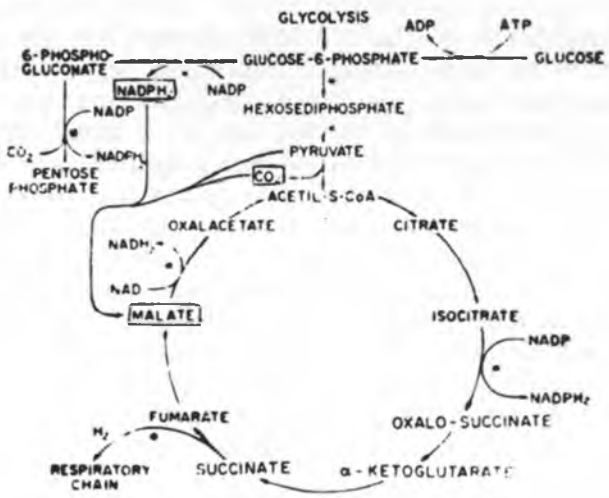


**Fig. 7 — The anaplerotic mechanism involving the "malic" enzyme, the TCA cycle, the glycolytic pathway and the hexosemonophosphate "shunt" in B. glabrata.**

Thus, in a working TCA cycle, malate can be either produced by the cycle itself or can be originated from pyruvate by the catalytic action of the "malic" enzyme. Glucose-6-phosphate plays an important role by being the substrate of the "zwischenferment" and thus contributing for the production of NADPH, essential for the biosynthesis of malate by the "malic" enzyme. Malate itself can then contribute to the cell respiratory mechanisms, by being oxidized to oxaloacetate through the regular activity of malic dehydrogenase.

## THE CIRCULATION OF CARBOHYDRATES IN **B. glabrata**

A variety of carbohydrates have been reported in **B. glabrata.** Among them, two neutral polysaccharides — glycogen and galactogen — have been studied extensively by DUARTE et al (28). A number of mucopolysacharides and glycoproteins have also being detected. Distribution of 2-amino-2-deoxihexopyranosis has been studied extensively by DUARTE et al (28). A number of mucopolysacharides and glycoproteins have also being detected. Distribution of 2-amino-2-deoxihexopyranosis has been studied by DMYTRACZENKO, CARDOSO and DUARTE (29) in acid hydrolysates of **B. glabrata.** No free hexosamine has been found. Total carbohydrate in the snail body amounts 12-14 g per cent; 10 to 12 per cent are neutral carbohydrates; 1.3 to 1.4 per cent are aminosugars, acid sugars, and methylpentose.

Most important for the studies on the circulation of carbohydrates in **B. glabrata** are the findings of carbohydrates in the circulating blood of the snail. Free glucose in the hemolymph of **B. glabrata** has been detected by VIEIRA and GOLDBERG (30). A variety of free sugars, however, have been detected by MOTTA, CARDOSO, and DUARTE (31) in the hemolymph. Besides glucose, a number of oligosaccharides have been found, probably maltose and gentiobiose, and two trisaccharides, one containing only glucose and one containing glucose and galactose.

The digestive gland of **B. glabrata** contains a variety of digestive enzymes for carbohydrates. On the other hand, the phosphatasic activity in the digestive gland is very high, and splits almost every sugar phosphate known (Table IV). Figure 8 shows the phosphatasic activity in the digestive gland is very high, and splits almost every sugar phosphate known (Table IV). Figure 8 shows the phosphatasic activity found in the digestive gland of **B. glabrata**, in connection with the glycogenolysis and the glycolytic pathway. Thus, such activity might be of a very high importance for

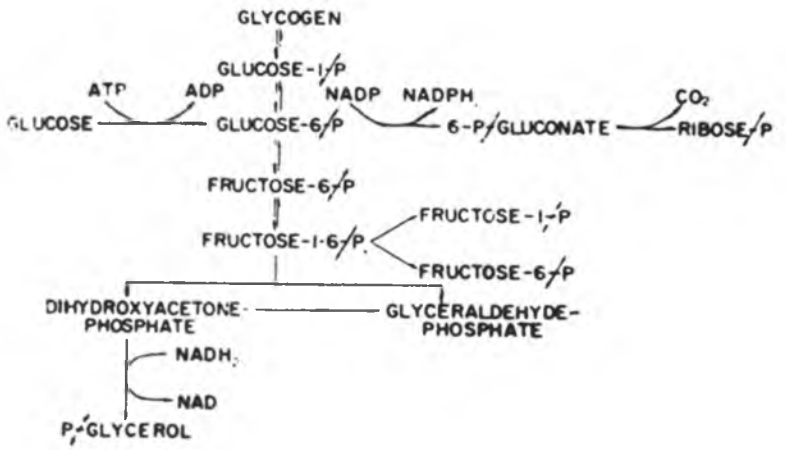


Fig. 8 — Phosphatasic activity in the digestive gland of the B. glabrata.

the physiological role of the digestive gland. The free circulating glucose in **B. glabrata** might arise from a glucose-6-phosphatase activity, in spite of the fact that the specificity of this enzyme has not yet been established for the snail preparations.

As for the total circulation of carbohydrates in **B. glabrata,** Figure 9 gives a general picture from the nutritional and the biochemical point of views.

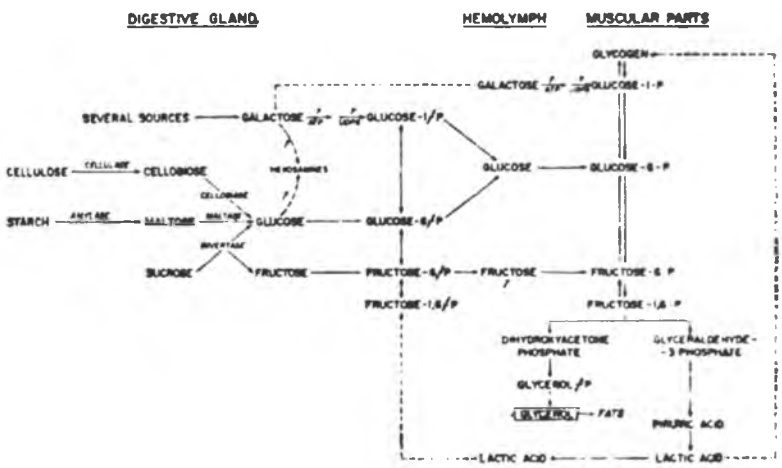


Fig. 9 — The circulation of carbohydrates in B. glabrata.

The circulation of carbohydrates can be considered as occupying three different sites: the digestive gland, the hemolymph and the muscular parts. In the digestive glands, cellulose and starch give rise to the disaccharides cellobiose and maltose. Sucrose can be originated from natural sources. Those disaccharides are split into glucose and fructose by the catalytic action of cellobiose, maltase, and invertase. It can be postulated that galactose can be obtained from several sources, but no direct evidence for this fact has yet been established. The free sugars thus formed may go through intermediate phosphorylating steps in the digestive gland for glycogenesis purposes. The probability of this step has been confirmed by CRIVELLARO and BACILA (25) after partial purification of fructose-1,6-diphosphate aldolase from the digestive gland of the snail. The free sugars may also give rise to hexosamines, a biochemical mechanism not yet studied in the snail.

The high phosphatasic activity of the digestive gland might account for the free glucose found in the hemolymph. Unless new facts contribute to what is known, it seems that the glucose is the only free sugar found in the hemolymph which is important from the nutritional and energetic point of views. So far, it has to be admitted that glucogenesis and glucogenolysis might occur in the digestive gland (hepatopancreas) thus accounting for the

metabolic interconversion of hexoses. A mechanism for the galactose-1-phosphate-glucose-1-phosphate interconversion is possible but has not yet been identified.

For the muscle parts, the glycolytic pathway is already quite known. However, the mechanism of NADH, reoxidation is peculiar to **B. glabrata** since the muscle displays a very high activity of α-glycerophosphate dehydrogeenase, which accounts for such metabolic step with much higher efficiency than the lactic dehydrogenase mechanism. Furthermore, lactic acid might contribute to the resynthesis of glycogen in the digestive gland in a similar way as the Cori cycle known for vertebrates. Two other peculiarities distinguish the carbohydrate metabolic pathways in the muscular parts of the **B. glabrata** from the common pathways that being the NADH of NADPH dependence of lactic acid dehydrogenase, and the presence of "malic" enzyme.


## RESUMO

**Biomphalaria glabrata** apresenta grande capacidade para metabolizar carboidratos, além de mecanismo ativo de biossíntese de carboidratos. Sabe-se que o conteúdo total de carboidratos que oscila entre 12-14% no pêso total do organismo varia de acôrdo com condições específicas nas quais vive o molusco, por exemplo, durante períodos de jejum ou em condições de vida fora d'água.

Mecanismo anaplerótico especial pode ser associado com os princípais padrões biossintéticos do metabolismo de carboidratos, isto é, a operação da enzima "málica". Essa última pode ser associada aos mecanismos respiratórios através do ciclo do TCA ou com a via significativa para a reoxidação do NADH formado durante a glipiruvato.

**B. glabrata** é também muito peculiar no que se refere à regulação de níveis de NADP/NADPH e NAD/NADH, êsse último sendo regulado pela desidrogenase de α-glicerofosfato de pé muscular, enquanto que a atividade de desidrogenase láctica é menos significativa para a reoxidação do NADH formado durane a glicólise.

Além disso, foi ainda verificado que os tentáculos isolados de **B. glabrata** são capazes de mobilidade unidirecional na água. Tal mobilidade pode ser medida e usada como reagente biológico sensível para o estudo do efeito biológico de inibidores metabólicos e de moluscocidas e uma variedade de compostos quimoterapêuticos usados no tratamento da esquisostomose mansônica.

A antena isolada é também muito sensível à ação de vários agentes farmacológicos.

## REFERENCES


1. BARBOSA, F. S. — História natural dos planorbídeos transmissores de **Schistosoma mansoni** no nordeste do Brasil. Simpósio sôbre bioquímica de planorbídeos, Curitiba. Imprensa da Universidade do Paraná, 1962, pág. 11.
2. MAGALHÃES NETO, B. & MAGALHÃES, M. R. — Catálogo bibliográfico de bioquímica de planorbídeos. In Simpósio sôbre bioquímica de planorbídeos, 2.ª, Recife, 1963, pág. 124.
3. BACILA, M. ed. — Simpósio sôbre bioquímica de planorbídeos. Curitiba, 1963.
4. LINS, M. ed. — Simpósio sôbre bioquímica de planorbídeos, 2.ª, Recife, 1963.
5. CAMPELLO, A. P. ed. — Simpósio sôbre bioquímica de planorbídeos, 3.º, Curitiba, 1966.
6. ARAGÃO, G. A. & BACILA, M. — Unpublished data.
7. GIACOMETTI, D. & BACILA, M. — Unpublished data.
8. VOSS, D. O.; CAMPELLO, A. P. & BACILA, M. — **Biochem. Biophys. Res. Commun.**, New York **4**: 48, 1961.
9. ACCIOLY, L. G. A. — Alguns aspectos da glicólise anaeróbica de planorbídeos. In BACILA, M. ed. Simpósio sôbre bioquímica de planorbídeos. Curitiba, Imprensa da Universidade do Paraná, 1961, pág. 116.
10. BACILA, M. & CRIVELLARO, O. — Unplished data.
11. BACILA, M. & MEDINA, H. — The effect of metabolic inhibitors and molluscocides on the mobility of the isolated tentacles from **Biomphalaria glabrata. Anais Acad. bras. Ciênc. Rio de Janeiro, 42**: 141, 1970.
12. CAMPELLO, A. P.; VIANNA, C. H. M.; BRANDÃO, D.; VOSS, D O. & BACILA, M. — **Biochem. Pharmac.** London, 13: 211, 1964.
13. VALLEJO-FREIRE, A.; RIBEIRO, O. F. & RIBEIRO, I. F. **Science, New York, 119**: 470, 1954.
14. VIEIRA, E. C. — Reunião Anual da Sociedade Brasileira para o Progresso da Ciência, 14ª, Curitiba, 1962. Abstract.
15. MAGALHAES NETTO, B. & ALMEIDA, A. M. — **Pub. Avuls. Inst. Aggeu Magalhães**, Recife, 2: 115, 1963; **ibid.**, 3: 131, 1954.
16. VIEIRA, E. C. & LADEIRA, B. A. — **Comp. Biochem. Physiol 14: 281**, 1965.
17. DUARTE, J. H.; FOKAMA, G. & BACILA, M. — Simpósio sôbre bioquímica de planorbídeos, 2.º, Recife, 1963, pág. 29.
18. DUARTE, J. H.: FOKAMA, G. & BACILA, M. — International Congress of biochemistry, 7.th., New York, 1964. Abstract.
19. MAGALHÃES NETTO, B. — **Pub. avuls. Inst. Aggeu Magalhães, 2**: 1, 1953.
20. DUARTE, J. H.; OLIVEIRA, E. B. & FOKAMA, G. — Simpósio sôbre bioquímica de planorbídeos, 2.º, Recife, 1963. p. 43.

21. LADEIRA, B. A. & VIEIRA, E. C. — Simpósio sôbre bioquímica de planorbídeos, 3.ª, Curitiba, 1966, p. 47.

22. SCHWAB, A.; BRANDÃO, D.; VIANNA, C. H. M. & BACILA, M. — Simpósio sôbre bioquímica de planorbídeos, 2.º, Recife, 1963. p. 25.

23. ZANCAN, G. T. & BACILA, M. — Simpósio sôbre bioquímica de planorbídeos, Curitiba, 1961, p. 115.

24. ZANCAN, G. T. & BACILA, M. — Simpósio sôbre bioquímica de planorbídeos, Curitiba, 1961, p. 117.

25. CRIVELLARO, O. & BACILA, M. — Unpublished data.

26. VOSS, D. O.; BRANDÃO, D.; CARDOSO, T. J. T. & BACILA, M. — Simpósio de bioquímica de planorbídeos, 2.º, Recife, 1963, p. 45.

27. MOLFI, A. — Simpósio sôbre bioquímica de planorbídeos, 3.º, Curiiba, 1966, p. 99.

28. DUARTE, J. H.; DMYTRACZENKO, A. & FOKAMA, G. — Polissacarídeos de **Australorbis glabratus**. Simpósio sôbre bioquímica de planorbídeos, 2.º, Curitiba, 1966, p. 33.

29. DMYTRACZENKO, A.; CARDOSO, T. J. T. & DUARTE, J. H. — Simpósio sôbre bioquímica de planorbídeos, 2.º, Recife, 1963, p. 39.

30. VIEIRA, E. C. & GOLDBERG, D. — Reunião Anual para o Progresso da Ciência, 4, Curitiba,1962. Abstract.

31. MOTTA, E. S; CARDOSO, T. J. T. & DUARTE, J. H. — Simpósio sôbre bioquímica de planorbídeos, 2.º, Recife, 1963, p. 23.