Cette identité morphologique permet, à notre avis, de rapporter à une Entomophthorale le cas de parasitisme fongique que nous observons chez *P. sergenti*. Mais nous partageons l'opinion émise par Rioux *et al.* (1966) selon laquelle en l'absence d'information sur les stades conidies il serait imprudent d'attribuer un nom de genre et d'espèce à l'un ou l'autre de ces organismes.

Il convient pourtant de signaler que notre materiel tout comme celui de Rioux *et al.* (1966) ressemble à celui décrit par Leão et Pedroso (1965) sous le nom de *Coelomomyces ciferrii*. Dans les trois cas, les spores de résistance sont sphériques, comme chez les *Entomophthora*, alors que les sporanges de *Coelomomyces* sont toujours plus ou moins ovales. Et même lorsque ces derniers sont à majorité ovoïdes (comme c'est le cas chez *C. anophelesicus* Iyengar, 1935), aucun ne montre un contour parfaitement circulaire. De plus, la paroi externe du sporange de *Coelomomyces*, épaisse, soit lisse soit ornementée, est piquetée de façon caractéristique et offre une structure totalement différente de la paroi des spores durables décrites ici, ou chez Rioux *et al.* (1966). Les photographies illustrant l'article de Leão et Pedroso (1965) montrent des spores à paroi identique à celle que nous décrivons chez *P. sergenti* mais de taille supérieure (35 à 40 μm).

De même la constance de la taille des spores observées dans les trois cas que nous citons s'oppose à la grande variation des dimensions des sporanges de *Coelomomyces*.

De plus, les spores présentées par les auteurs brésiliens contiennent des images d'une paire de noyaux, parfois également observées dans notre matériel (fig. 3 et 4). De telles structures n'ont jamais été observées dans les sporanges de résistance de *Coelomomyces*, à l'intérieur desquels se produit la formation de zoospores, celles-ci pouvant éventuellement être libérées à la déhiscence à travers une fente préformée. Dans le case des spores de résistance des Entomophthorales, MacLeod (1963) a suggéré la fusion possible des noyaux de chaque paire au moment de la germination lorsque le tube germinal est expulsé par une fente formée à la rupture de la paroi renflée de la spore.

Tous ces éléments nous incitent à penser que le cas de parasitisme rapporté à *Coelomomyces ciferrii* par Leão et Pedroso (1965) doit, en fait, être considéré comme un cas de parasitisme par Entomophthorale.

Ce qui reviendrait à recenser quatre cas de parasitisme par Entomophthorale chez des phlébotomes, compte tenu de *Entomophthora (= Empusa) papatasii* à Malte en l'absence, il est vrai, de description caractéristique par l'auteur (Marett 1915).

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Activities of enzymes in cardiac energy metabolism

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The activities of enzymes of fat and carbohydrate energy metabolism were determined in hearts from a number of species. Activities of citrate synthase and 3-hydroxyacyl CoA dehydrogenase were lower in hearts from ectothermic animals than rats or pigeons. However, activities of hexokinase and phosphofructokinase in the ectothermic hearts were equal to or greater than the activities in the endothermic hearts. It thus appears that the hearts from the ectothermic species have a greater dependence upon a glucose-based metabolism than hearts from endothermic animals.

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L'activité des enzymes du métabolisme énergétique des graisses et des hydrates de carbone dans le cœur ont été déterminées chez un certain nombre d'espèces. L'activité de la citrate-synthétase et celle de la 3-hydroxyacyl CoA déshydrogénase sont plus faibles dans les cœurs d'animaux ectothermes que chez les rats ou les pigeons. Cependant, l'activité de l'hexokinase et celle de la phosphofructokinase dans le cœur d'animaux ectothermes sont égales ou plus grandes que l'activité des mêmes enzymes dans le cœur d'animaux endothermes. Il semble que, chez les espèces ectothermes, le cœur dépende plus d'un métabolisme à base de glucose que chez les espèces endothermes.

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Introduction

Cardiac tissue requires a continuous supply of ATP in order to meet the demands of constant contractile activity. The organization of metabolic pathways resulting in ATP generation are well understood only in the mammalian heart. In this tissue, under conditions of high work and adequate oxygen delivery, fatty acids are the predominant fuel of metabolism; however, during periods of oxygen limitation carbohydrate breakdown is activated with the resultant production of lactic acid (Neely and Morgan 1974). This relationship is advantageous since fatty acid degradation is energetically efficient only when oxygen delivery is adequate, whereas carbohydrate metabolism can result in ATP production under both aerobic and anaerobic conditions. As an initial step in elucidating the metabolic fuels of hearts from nonmammalian species the activities of a number of enzymes of energy metabolism were determined. This approach allows close approximations of *in vivo* activities when enzymes catalysing reactions far displaced from equilibrium in the cell are considered and identifies qualitative differences amongst tissues when the activities of enzymes which catalyse near-equilibrium reactions in the cell are determined (Newsholme and Start 1973). The enzymes selected in this study were citrate synthase, 3-hydroxyacyl CoA dehydrogenase, hexokinase, phosphofructokinase, and lactate dehydrogenase. Citrate synthase activity is an index of citric acid cycle activity regardless of the source of acetyl CoA for entry into the cycle; 3-hydroxyacyl CoA dehydrogenase is involved exclusively with fatty acid degradation, whereas, hexokinase, phosphofructokinase, and lactate dehydrogenase are all associated with carbohydrate metabolism. The findings of this study suggest that the hearts of ectothermic vertebrates have a greater dependence upon glucose-based metabolism for ATP production than the hearts of endotherms.

Materials and methods

Animals

Pigeons (*Columba livia*) were livetrapped in Sackville, N.B. Rats (Wistar strain) were purchased from Canadian Breeding Farm, St. Constant, P.Q., and turtles (*Pseudemys* sp.) and frogs (*Rana pipiens*) were purchased from Boreal Laboratories, Mississauga, Ont. Brook trout (*Salvelinus fontinalis*) were obtained from the Fish Culture Station, Saint John, N.B., and eels (*Anguilla rostrata*) were captured in Morice Lake, N.B. Lobsters (*Homarus americanus*) and crabs (*Cancer irroratus*) were obtained from commercial fishermen. All vertebrates were killed by decapitation prior to dissection.

Extraction procedures

Freshly dissected tissue was homogenized manually in a ground glass homogenizer with 5–10 volumes of extraction medium. For the assay of hexokinase (EC 2.7.1.1) and lactate dehydrogenase (EC 1.1.1.27) the extraction medium consisted of 50 m*M* triethanolamine, 1 m*M* EDTA, 5 m*M* $MgCl_2$, and 30 m*M* mercaptoethanol at pH 7.4 (Zammit and Newsholme 1976). The extraction medium for phosphofructokinase (EC 2.7.1.11) contained 70 m*M* Tris, 1 m*M* EDTA, 5 m*M* $MgSO_4$ at pH 8.2 (Zammit and Newsholme 1976). The extraction medium for citrate synthase (EC 4.1.3.7) contained 25 m*M* Tris and 1 m*M* EDTA at pH 7.4 (Alp et al. 1976). The extraction medium for 3-hydroxyacyl CoA dehydrogenase (EC 1.1.1.36) was a modification of that of Beenakkers et al. (1967) containing 25 m*M* Tris and 2 m*M* EDTA at pH 7.5.

Assays

In most cases, enzyme activity was monitored at 340 nm by following the oxidation or reduction of pyridine nucleotides. Citrate synthase activity was followed at 412 nm with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) as the optically active substance utilizing an extinction coefficient of 13.6. All assays were performed in duplicate at 25°C. Data are expressed as micromoles per minute per gram wet weight.

Conditions for assays

Citrate synthase—Tris (50 m*M*), 0.2 m*M* DTNB, 0.1 m*M* acetyl CoA, 0.5 m*M* oxaloacetate (omitted for control), pH 8.1 (Alp et al. 1976).

3-Hydroxyacyl-CoA dehydrogenase—Triethanolamine (100 m*M*), 5 m*M* EDTA, 0.45 m*M* NADH, 0.1 m*M* acetoacetyl CoA (omitted for control), pH 7.0 (Beenakkers et al. 1967).

Hexokinase—Tris (75 m*M*), 7.5 m*M* $MgCl_2$, 0.8 m*M* EDTA, 1.5 m*M* KCl, 4 m*M* mercaptoethanol, 0.4 m*M* $NADP^+$, 2.5 m*M* ATP, 1 m*M* glucose (omitted for control), 10 m*M* creatine phosphate, excess creatine phosphokinase, excess glucose 6-phosphate dehydrogenase, pH 7.3 (Zammit and Newsholme 1976).

Phosphofructokinase—Tris (50 m*M*), 6 m*M* $MgCl_2$, 250 m*M* KCl, 1 m*M* ATP, 2 m*M* AMP, 0.17 m*M* NADH, 1 m*M* KCN, 3 m*M* fructose-6-phosphate (omitted for control), excess glycerol 3-phosphate dehydrogenase, excess aldolase, excess triose phosphate isomerase, pH 8.2 (Zammit and Newsholme 1976).

Lactate dehydrogenase—Tris (50 m*M*), 0.17 m*M* NADH, 1 m*M* KCN, 0.3 m*M* pyruvate (omitted for control) (Zammit and Newsholme 1976).

TABLE 1. Heart enzyme activities

Animal	Citrate synthase	3-Hydroxyacyl CoA dehydrogenase	Hexokinase	Phosphofructokinase	Lactate dehydrogenase
Pigeon	125 (3) 112–137	39.0 (2) 38.0–40.0	4.4 (3) 4.3–4.5	6.6 (3) 6.1–7.0	152 (3) 131–168
Rat	93.2 (3) 90.0–98.7	54.5 (2) 54.0–55.0	6.3 (3) 6.1–6.6	10.9 (5) 9.3–13.5	277 (3) 237–310
Turtle	29.5 (3) 28.0–32.0	5.0 (2) 5.0	12.3 (3) 11.1–13.0	14.5 (3) 14.0–15.3	340 (3) 321–359
Frog	36.0 (3) 35.0–37.0	10.4 (3) 10.2–10.5	9.8 (3) 9.3–10.3	12.3 (3) 11.3–13.4	65 (2) 63–66
Trout	34.0 (4) 31.0–36.5	6.5 (2) 6.2–6.9	10.6 (4) 9.6–13.1	26.7 (3) 22.5–29.3	144 (3) 135–152
Eel	23.1 (3) 20.4–25.0	12.8 (3) 12.3–13.4	17.2 (3) 16.4–17.6	21.7 (3) 18.0–25.5	454 (4) 408–538
Crab	6.3 (3) 6.2–6.3	13.7 (2) 12.6–14.8	4.2 (3) 3.9–4.5	6.3 (3) 6.1–6.7	5.0 (3) 4.8–5.1
Lobster	17.0 (2) 14.4–19.6	14.1 (2) 14.0–14.2	5.9 (2) 5.4–6.4	4.4 (3) 4.3–4.4	13.8 (3) 11.8–16.1

NOTE: All values are expressed as micomoles per minute per gram fresh weight. Assays were conducted at 25°C. The number of determinations is in parentheses and the range is given below each average.

Results and discussion

The activities of myocardial citrate synthase and 3-hydroxyacyl CoA dehydrogenase fall into two distinct groups. Activities in the hearts from the endotherms are 5- to 10-fold greater than activities in the hearts from the remaining animals (Table 1). The activities of hexokinase, however, exhibit a different pattern. The hearts from ectothermic vertebrates possess 1.5- to 4-fold greater activities than the hearts from the crustaceans, pigeons, or rats. The pattern for phosphofructokinase is similar to that for hexokinase although the demarcation is less precise. It is clear though that the fish species have the highest activities of heart phosphofructokinase. Lactate dehydrogenase activities are much lower in crustacean hearts than in any of the vertebrate hearts analyzed.

The activities of enzymes reported here are similar to other published values. Specific examples of this consistency are evident in the activities of citrate synthase from pigeon, rat, trout, and frog hearts (Alp et al. 1976), 3-hydroxyacyl CoA dehydrogenase from pigeon and rat hearts (Beenakkers et al. 1967; Bass et al. 1969), and the enzymes of carbohydrate metabolism from rat and trout hearts (Bass et al. 1969; Crabtree and Newsholme 1972; Bass et al. 1973).

The high citrate synthase activities in pigeon and rat hearts relative to the remainder of the species are not surprising in light of the high energetic demands placed upon the endothermic heart. The rat and pigeon myocardia also exhibit high activities of 3-hydroxyacyl CoA dehydrogenase, a feature which is in keeping with known high rates of fatty acid catabolism *in vivo*. The hearts of ectothermic animals have lower activities of both of these enzymes suggesting lower rates of aerobic energy production consistent with lower rates of energy demand. In contrast with citrate synthase and 3-hydroxyacyl CoA dehydrogenase, the activities of heart hexokinase and phosphofructokinase in the ectotherms are as high as or higher than in the endotherms. The latter enzymes are associated with the degradation of either blood-borne and (or) glycogen-derived glucose. It therefore appears that the hearts of ectothermic vertebrates utilize six-carbon carbohydrates to a higher extent than do the hearts of the mammals and birds examined. The hearts of the latter species though have a greater dependence upon fatty acid metabolism. The high levels of lactate dehydrogenase in all but the crustacean hearts suggest that all these organs may have lactate-catabolizing capacities; however, the relative contribution of lactate oxidation remains unsettled.

Given that the activity of citrate synthase reflects maximal relative energetic demands, the hexokinase and phosphofructokinase data are interpreted to mean that the hearts of the ectothermic animals are better able to meet energetic demands under anaerobic conditions. This is not to imply that the total anaerobic ATP production capability is higher in the ectothermic hearts but only that anaerobic ATP production can more nearly match that of aerobic ATP production.

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