SPRUCE BUDWORM AND ENERGY METABOLISM?

Thakor R. Patel

Biochemistry Department
Memorial University of Newfoundland
St. John's, Newfoundland A1A 3R3

ABSTRACT

The utilization of stored lipids (fat) for energy metabolism appears to be a fundamental process for many biological systems especially during the early stages of their development. The participation of the glyoxylate cycle (GOC) together with other metabolic sequences like gluconeogenesis and beta oxidation are necessary for the conversion of lipids to carbohydrates. This report describes the distribution of the GOC amongst biological systems and examines its importance to the development and existence of biological cells under different physiological conditions. The report also describes some of our work which focuses on the role of the GOC in non-parasitic (Caenorhabditis elegans) and parasitic (Ascaris lumbricoides) nematodes. It was possible to demonstrate the in vivo and in vitro inhibition of isocitrate lyase (ICL), a key enzyme of the GOC operating in these nematodes by itaconate. Finally this report explores the possibility of using target specific inhibitor like itaconate to control spruce budworm (Choristoneura fumiferana), populations.

Introduction

The spruce budworm is widely distributed in North America including Canada. The organism has been declared as an insect pest of national concern. It causes extensive defoliation and mortality to a wide variety of coniferous tree species including balsam fir (Abies balsamea), white spruce (Picea glauca), red spruce (Picea rubens), and black spruce (Picea mariana). Outbreaks of spruce budworm in Canada have been reported as early as 1700's (Blais, 1965). Timber losses in recent outbreaks when converted into dollar values amount to millions of dollars. Although the life cycle and the population dynamics of the spruce budworms have been explained no information is available on the biochemistry and physiology of the embryonating eggs and the developing larvae. The energy metabolism at the different developmental stages of the spruce budworm has not been examined. The examination of the energy metabolism of the embryonating eggs and the developing larvae of spruce budworm may result in information which may be used to come up with target specific inhibitors directed against the key enzymes of the GOC.

The Glyoxylate Cycle And Its Distribution In The Nature.

Kornberg and Krebs (1957) first revealed the evidence for the function of the GOC in replenishment of the tricarboxylic acid (TCA) cycle intermediates depleted during the glyconeogenesis and the amino acid biosynthesis. Its operation has been shown to be essential in cells which depend on acetate, ethanol, lipids, hydrocarbons and C_1 compounds for its carbon and energy needs (Cioni et al, 1981). Although the GOC was first described in bacteria utilizing acetate or ethanol as sole carbon and energy sources this pathway has a large representation in nature being operative under various nutritional and physiological phases of the life of many organisms.

The glyoxylate pathway can be described as a cyclic set of reactions starting from isocitrate and returning to it. This cycle (Fig. 1) allows the transformation of two acetate (as acetyl-CoA) into one of succinate. Five enzymes that participate in this catalysis include isocitrate lyase (ICL), malate synthase (MS), malate dehydrogenase (MDH), citrate synthase (CS) and acomitase (ASE). Reactions 1 and 2 (Fig. 1) immediately reveal the anaplerotic significance of the two enzymes (ICL and MS) as they produce two TCA cycle intermediates (succinate and malate) starting from one TCA cycle six-carbon compound (isocitrate) and a molecule of the substrate of the cycle (acetyl-coA). The glyoxylate cycle thus replenishes intermediates of the TCA cycle and conserves carbon that would otherwise be completely oxidized and lost to biosynthetic pathways. This replenishing function of the GOC has been termed 'anaplerotic'.

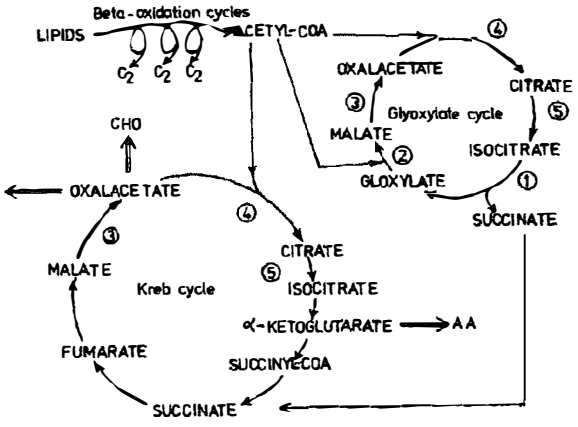

Fig. 1. Relationships between Krebs cycle, beta-oxidation and glyoxylate cycle. (AA = amino acid for biosynthesis; CHO = carbohydrates; C_2 = two carbon units, acetyl CoA).

The ICL has been found in bacteria (Woolfson

and Krulwich, 1972; Ozaki and Shiio, 1968; McFadden and Howe, 1962, 1963; Sariaslani et al, 1961; Chell et al, 1978;), algae (John and Syrett, 1967; Haigh and Beevers, 1964a; 1964b; Dumham and Thurston, 1978; Collins and Merrett, 1975), pteridophytes (Gemmrich, 1979), gymnospermes (Firenzuoli et al, 1968; Vani et al, 1973), angiosperms (Gientka-Rychter and Cherry, 1968; Kagawa et al, 1973; Ihle and Dure, 1972; Theimer and Rosnitschek, 1978; Khan et al, 1977; Vani et al, 1979; Lango et al, 1975; Canvin and Beevers, 1961; Ford et al, 1976; Tester, 1976), fungi (Maxwell et al, 1975; O'Sullivan and Casselton, 1973; Flavell and Woodward, 1970; Reisener and Jager, 1967; Polakis and Bartley, 1965; Mendgen, 1973), protozoa (Hogg and Kornberg, 1963; Muller, Hogg and DeDure, 1968; Lovel et al, 1974), and nematodes and trematodes (Barrett et al, 1970, 1971; Colonna and McFadden, 1975; Rothstein and Mayoh, 1964, 1965, Prichard and Schofield, 1979; Patel and McFadden, 1977). The presence of the ICL has also been reported in the prepupae and pupae of the Prodenia eridania (Carpenter and Jaworski, 1962). It must be pointed out that the GOC has never been detected in animals more highly evolved than worms such as nematodes and trematodes.

The Physiological Role Of The GOC

In higher plants the GOC occurs in germinating seedlings of numerous plants where it is required for the conversion of stored lipids into carbohydrates which then form the source of energy during germination. In germinating spores of fern a similar lipid to carbohydrate transformation occurs. If one examines the levels of ICL and MS in the germinating fatty seeds a typical pattern is observed (Fig. 2). The increases in ICL and MS follow a simultaneous decrease in the total lipids in these seeds. The enzymes of the GOC are compartmentalized in single membrane, subcellular particles called 'glyoxysomes'. It must be noted here that glyoxysomes, unlike mitochondria, have a single bounding membrane.

In algae, the GOC participates in the utilization of acetate for growth when photosynthesis is not operative and carbohydrates are not available. The enzymes of the GOC have also been reported in the trematode, Fasciola hepatica (Prichard and Schofield, 1979), which is a mammalian liver fluke. In the case of Fasciola hepatica dults which live in the bile duct, it has been suggested that the GOC enzymes are involved in the production of glycogen to be incorporated into eggs which are very rich in glycogen (Horstmann, 1962; Wilson, 1967).

Although the data suggests that ICL and MS are scattered through out metazoan phyla, almost nothing is known about the function of this cycle in developing larvae and/or adult worms. Indeed only in the case of A. lumbricoides and C. elegans has the function of the GOC been clearly established (Rothstein and Mayoh 1965; Patel and McFadden, 1977, 1978, 1978b, 1978c). It has been shown that extensive synthesis of the disaccharide, trehalose, occurs in the developing embryos of A. lumbricoides at the expense of fat and that this conversion correlates with the appearance and disappearance of ICL and MS (Barrett et al, 1970). Quite recently Rubin and Trelease (1976) have found that the disaccharide resynthesis after embryonation accompanies the disappearance of triglyceride droplets found largely in the posterior half of the larvae. Dense microbody-like granules, restricted to the lipid-body region, are considered as possible subcellular sites for the enzymatic conversion of lipids to carbohydrates (Rubin and Trelease, 1977a, 1977b).

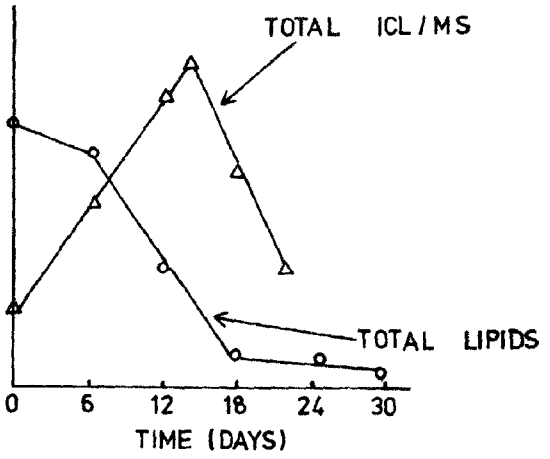

Fig. 2. Levels of MS and ICL (-△-) and total lipid (-O-) content during germination of seeds.

Materials And Methods

Materials. Succinic acid, itaconic acid, dithiothreiotol (DTT), glutathione (GSH), DL-isocitrate, morpholinopropanesulfonic acid (MOPS), beta-mercaptoethanol, Tris-HCl, and ethylene diaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co. Sephadex G-200 came from Pharmacia Fine Chemicals. All inorganic chemicals were commercially available and were of analytical grade.

Organisms And Growth Conditions.

Caenorhabditis elegans were kindly supplied by Dr. R.L. Russel, University of Pittsburgh, Pittsburgh, U.S.A. Escherichia coli K8-5m (CGSC strain No. 4868) is a mutant which fails to grow on acetate as a sole source of carbon and lacks isocitrate lyase when grown on glucose. The method for cultivation of C. elegans under monoxenic growth conditions has been reported earlier (Colonna and McFadden 1975; Patel and McFadden 1976).

Freshly recovered female Ascaris lumbricoides containing eggs were purchased from Nebraska Scientific Co., Omaha, Nebraska, U.S.A., shortly after preservation in 2% formalin. Eggs, obtained by removing the reproductive tracts from female worms, were embryonated for 22 days and treated with hypochlorite by the method described by Colonna and McFadden (1975) prior to breakage.

Preparation Of Crude Enzyme Extracts.

Crude extracts of C. elegans and A. lumbricoides were prepared by the methods of Patel and McFadden (1977).

Enzyme Assays

Isocitrate lyase was assayed at pH of 7.7 according to Roche et al (1971) with the inclusion of 0.2M phenylmethylsulfonyl fluoride (PMSF) in the assay buffer. Other enzymes of the GOC namely, malate synthase, citrate synthase, fumarase and catalase were also examined but will not be discussed here.

Protein concentrations in crude extracts were determined by the method of Lowry et al (1951).

Results

When washed eggs of A. lumbricoides were suspended in a solution of 0.1 N sulfuric acid and incubated on a shaker at 30°C ICL activity began to appear in the embryonating eggs on the eleventh day. It rearched a peak on day 18-20 and began to decline gradually thereafter. In the case of C. elegans the situation is slightly different. This non-parasitic nematode is self-fertilizing (hermaphroditic) worm living in soil where it feeds on bacteria. A fully grown worm measures about 1 to 1.5 mm in length. In a laboratory it can be cultivated just like bacteria using either axenic or monoxenic growth conditions. A single worm takes about 48 hrs from an egg stage to mature into a fully grown adult worm. During this short period it undergoes four molts representing the four larval stages, namely L_1 through L_4 (Fig. 3). Methods to obtain synchronous cultures of C. elegans have been described in our earlier report (Patel and McFadden, 1978).

The levels of ICL in the embryonating eggs and the larvae derived from them were monitored. The data in Figure 4 shows the specific activity of ICL was minimum in the zero-time sample representing unhatched embryonating eggs. The enzyme acitivity in the 48 and 60 hr samples was undectable. These samples contained young adult worms of uniform length bearing no eggs. These data suggest that ICL may have an important role in the biochemistry of the embryonating eggs and the young developing larvae, and that the adult organisms may not require the enzyme for its survival.

Itaconic acid (methylene succinic acid) was used as a target specific inhibitor to test in vivo and in vitro inhibition of ICL of A. lumbricoides. Figure 5 shows that itaconate inhibits the growth of C. elegans. At a 60 mM concentration the inhibitor caused 50% reduction in the growth of C. elegans in a random monoxenic culture of C. elegans. However, its effect on the growth of C. elegans in a random axenic culture was much pronounced. The results in Table 1 shows that 5, 30 and 60 mM itaconate caused 70, 93 and 99% inhibitions, respectively.

TIME (HOURS)		
0	HATCH, 600 SOMATIC CELLS, FURTHER GROWTH BY ENLARGEMENT OF THESE CELLS.	
6-11	FIRST LARVAL MOLT	L1 STATE
18	SECOND LARVAL MOLT	L2 STATE
26	THIRD LARVAL MOLT	L3 STATE
36	FOURTH LARVAL MOLT	L4 STATE ADULT
45-46	EGG LAYING BEGINS	
	4 EGGS PER HOUR INITIALLY	
	9 EGGS PER HOUR AFTER 92 HOUR.	
	EGG LAYING ENDS AFTER 120 HOUR.	

Fig. 3. Developmental stages in the life of Caenorhabditis elegans. (from Hirsch et al. 1976).

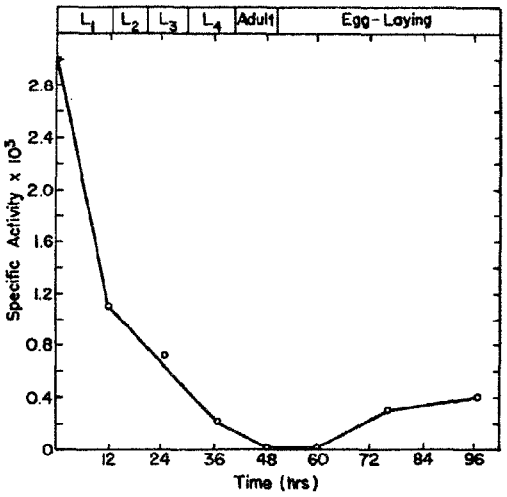

Fig. 4. Levels in ICL during synchronous growth of C. elegans larvae.

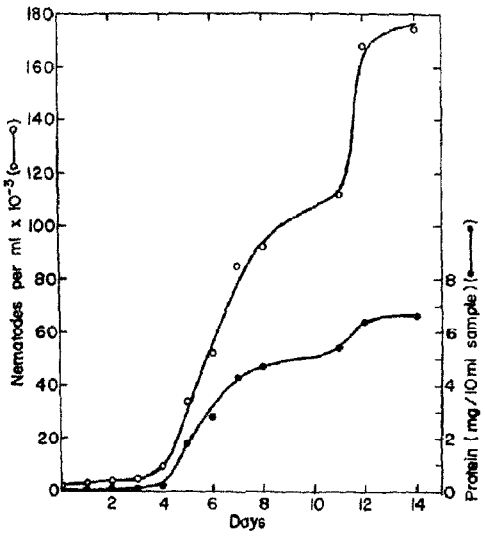

Fig. 5. Growth inhibition of C. elegans by itaconate (60 mM) in monoxenic cultures.

Table 2. Effect of itaconate on the growth of C. elegans.[a]

Days	Worms/ml x 10^{-3}				
	Itaconate				
	0.0 mM	5 mM	15 mM	30 mM	60 mM
0	0.40	0.4	0.40	0.40	0.40
2	0.60	0.46	0.26	0.20	0.20
5	2.80	2.06	1.60	0.60	0.20
6	8.60	2.52	1.87	0.60	0.20
8	12.00	7.00	3.40	2.40	0.20
10	41.00	12.56	--	2.80	0.27

[a]Each of the 500-ml Erlenmeyer flasks contained 60 ml of axenic medium with the indicated concentrations of itaconate. The flasks were inoculated with 0.5 ml of a random population of C. elegans, and incubated on a shaker in a Psychroterm drawn from each flask at the given times to determine the worm population as described in MATERIALS AND METHODS.

Table 1. Growth of C. elegans in the presence and absence of metabolic inhibitors[a].

Day	Worms/ml x 10^{-3}						
	Control	Itaconate			Oxalate	Maleate	Succinate
		10 mM	30 mM	60 mM	10 mM	10 mM	10 mM
1	3.8	3.8	3.8	3.8	3.8	3.8	3.8
3	9.1	7.2	6.0	3.2	8.0	8.8	8.9
5	64.7	32.1	13.8	6.3	51.5	32.0	67.9
7	162.0	106.2	22.5	9.2	146.9	139.0	175.0

[a]Each 250-ml flask contained 15 ml of axenic medium plus one of the above filter-sterilized compounds. Worm counts were made by direct microscopic examination as described. All the flasks were shaken at 120 rpm and 23°C in a Psychroterm incubator.

In order to test the specificity of itaconate inhibition of growth of C. elegans other compounds shown in Table 2 were tested. Itaconate in a10, 30 and 60mM concentrations resulted in 34, 86 and 94% inhibitions, respectively. In contrast, oxalate and maleate caused negligible inhibition of the growth of C. elegans while succinate showed a stimulatory effect. The effect of itaconate upon the growth of synchronously growing monoxenic culture of C. elegans was maximum. In this case the inhibition is almost 100% (Fig. 6). This is not unexpected since itaconate may inhibit ICL in embryos and L_1 larvae of C. elegans. This result compliments nicely the earlier observation in which it was shown that ICL level is higher during embryogenesis and L_1 larvae stage after eggs were allowed to hatch in a monoxenic medium (Fig. 4).

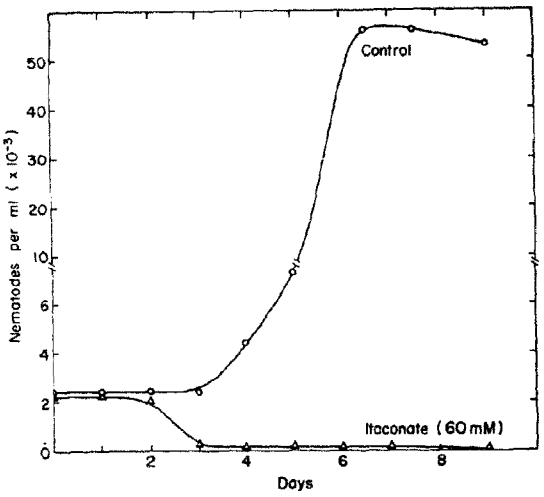

Fig. 6. Effect of itaconate (60mM) on the synchronous growth of C. elegans in a monoxenic culture.

Increasing concentrations of itaconate caused higher in vitro inhibition of ICL in crude extracts of C. elegans (Fig. 7). More than 80% inhibition was observed with 10 nanomolar concentrations of itaconate. A similar pattern of inhibition was also observed with ICL in crude extracts of A. lumbricoides. Kinetic studies using the enzyme from both the organisms (C. elegans and A. lumbricoides) indicate itaconate is a non-competitive inhibitor of ICL. The apparent inhibition constants were 19 micromolar for C.

elegans enzyme and 7.3 micromolar for A. lumbricoides enzyme.

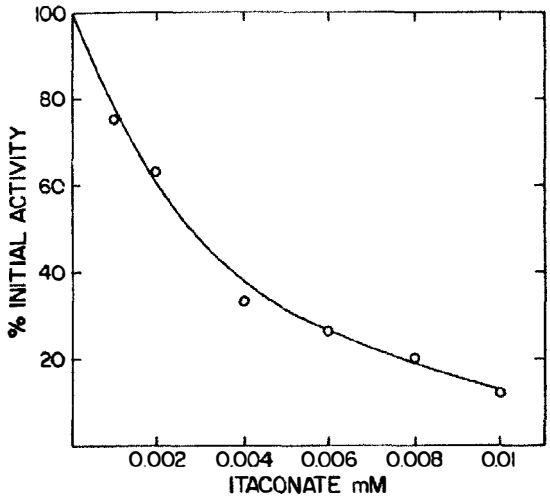

Fig. 7. In vitro inhibition of ICL of C. elegans by itaconate.

Discussion

The above results indicate that GOC cycle plays an important role in the biochemistry of embryonating eggs and the young developing larvae of C. elegans and A. lumbricoides. In the case of C. elegans it was possible to demonstrate that itaconate acts as a specific inhibitor of ICL in vivo as well as in vitro. The inhibitor caused drastic reduction in the population of the nematodes in monoxenic and axenic cultures.

An important question is raised here. Is it possible to employ target specific inhibitor like itaconate or other such compounds to blockade the GOC and hence prevent the growth of an organism? The answer is yes. The experiments described above suggest that itaconate may be used as a target specific inhibitor to prevent the growth of nematodes like C. elegans.

The work in McFadden's laboratory showed that ICL in crude extracts of Ps. indigofera and N. crassa were inhibited by itaconate, an analogue of succinic acid. The enzymes of the bacterial and the fungal sources have been purified to homogeneity. The inhibition studies using the purified enzymes indicated that itaconate is a competitive inhibitor with respect to succinate in the condensation (or reverse) reaction and is uncompetitive with respect to glyoxylate. In the forward (cleavage) reaction the itaconate acted as a non-competitive inhibitor. In the present study itaconate was found to be non-competitive inhibitor when ICL from C. elegans and A. lumbricoides were tested.

Recently Bruce McFaddens group at Washington State University, Pullman, have shown that itaconate can bring about in vivo inhibition of Pseudomonas indigofera growing on acetate. The results obtained with cultures of C. elegans compliment their finding. The inhibition of isocitrate lyase by itaconate in the case of Tetrahymena pyriformis in vitro and in vivo has been reported by Dang and Cook (1981).

If one examines the life cycle of the spruce budworm one finds some interesting features which raise important practical questions. In Newfoundland in late July or early August the moth lays eggs which embryonate and hatch into first instar larvae in about ten days. These larvae do not feed but are very active, moving on branches or hanging by their silky threads. They soon begin to look for a place in preparation for wintering over. They prepare hybernacula in which the second instar larvae winter over. These larvae hibernate until the arrival of the spring in May or early June. The second instar larvar become active and undergo molting to complete the remaining developmental stages to produce sixth instar larvae. The pupation occurs in July and August. The young moth appears and begins to lay eggs shortly thereafter.

There is some similarity in the early developmental stages in the life of C. elegans and the spruce budworm. Perhaps they resemble in theirtheir biochemistry and physiology during their early stages. If this is true then it may be possible to use itaconate as a target specific inhibitor to prevent the progress in the development of the larval stages of the spruce budworm. Before such practical approach is adopted it is necessary initially to do some fundamental research to investigate the biochemistry and physiology of the embryonating eggs and developing larvae of spruce budworm.

Literature Cited

Barrett, J.; Ward, C.W., Fairbairn, D. The glyoxylate cycle and the conversion of triglycerides to carbohydrates in developing eggs of Ascaris lumbricoides. Comp. Biochem. Physiol. 35: 577-585; 1970.

Blais, J.R. Spruce budworm parasite investigations in the lower St. Lawrence and Gaspe regions of Quebec. Can. Ent. 92: 384-396; 1965.

Canvin, D.T.; Beevers, H. Sucrose synthesis from acetate in the germinating castor bean: Kinetics and Pathway. J. Biol. Chem. 236: 988-995; 1961.

Carpenter, E.D.; Jaworski, E.G. The presence of isocitrate lyase activity in the southern armyworm Prodenia eridania. Biochem. Biophys. Acta. 58: 369-371; 1962.

Chell, R.M.; Sundaram, T.K.; Wilkinson, A.E. Isolation and characterization of isocitrate

lyase from a thermophilic Bacillus sp. Biochem. J. 173: 165-177; 1978.

Cioni, M.; Pinzauti, G.; Vanni, P. Comparative biochemistry of the glyoxylate cycle. Comp. Biochem. Physiol. 70B: 1-26; 1981.

Collins, N.; Merret, M.J. Microbody-marker enzymes during transition from phototrophic to organotrophic growth in Englena. Pl. Physiol. 55: 1018-1022, 1975.

Colonna, W.J.; McFadden, B.A. Isocitrate lyase from parasitic and free-living nematodes. Arch. Biochem. Biophys. 170: 608-619; 1975.

Dang, A.Q.; Cook, D.E. Itaconic acid: An inhibitor of isocitrate lyase in Tetrahymena pyriformis in vitro and in vivo. Biochem. Biophys. Acta. 678: 316-321; 1981.

Dixon, H.; Kornberg, H.L.; Lund, P. Purification and properties of malate synthetase. Biochem. Biophys. Acta 41: 217-233; 1961.

Dunham, S.M.; Thurston, C.F. Control of isocitrate lyase synthesis in Chlorella fusca var. vacuolata. Biochem. J. 176-185; 1978.

Firenzuoli, A.M.; Vanni, P.; Mastronuzzi, E.; Zanobin, A.; Baccari, V. Enzymes of glyoxylate cycle in conifers. Pl. Physiol. 43: 1125-1128; 1968.

Flavell, R.B.; Woodward, D.O. The concurrent regulation of metabolically related enzymes. The Krebs cycle and glyoxylate shunt enzymes in Neunspora. Eur. J. Biochem. 17: 284-291; 1970.

Ford, M.J.; Slack, P.; Black, M.; Chapman, J.M. A reexamination of the reputed control of cotyledonary metabolism by the axis. Planta. 132: 205-208; 1976.

Gemmrich, A.R. Isocitrate lyase in germinating spores of the fern Anemia phyllitidis. Phytochemistry 18: 1143-1146; 1979.

Gientka-Rychter, A.; Cherry, J.H. De novo synthesis of isocitratase in peanut (Arachis hypogaea L.) cotyledons. Pl. Physiol. 43: 653-659; 1968.

Haigh, W.G.; Beevers, H. The occurrence and assay of isocitrate lyase in algae. Arch. Biochem. Biophys. 107: 147-151; 1964a.

Haigh, W.G.; Beevers, H. The glyoxylate cycle in Polytomella caeca. Arch. Biochem. Biophys. 107: 152-157; 1964b.

Hirsh, D.; Oppenheim, D.; Klass, M. Development of the reproductive system of Caenorhabditis elegans. Developmental Biol. 49: 200-219; 1976.

Hogg, J.F.; Kornberg, H.L. The metabolism of C_2-compounds in microorganisms (9). Role of the glyoxylate cycle in protozoal glyconeogenesis. Biochem. J. 86: 462-468; 1963.

Hortsmann, H.J. Sauerstoff verbrauch and glykogengehalt der eier von Fasciola hepatica wahrend der entwicklung der miracidin Z. Parasit. 21: 437-443; 1962.

Ihle, J.N.; Dure, L.S. The developmental biochemistry of cottonseed embryogenesis and germination (III) Regulation of the biosynthesis of enzymes utilized in germination. J. Biol. Chem. 247: 5048-5055; 1972.

John, P.C.L.; Syrett, P.J. The purification and properties of isocitrate lyase from Chlorella. Biochem. J. 105: 409-416; 1967.

Kagawa, T.; McGregor, D.I.; Beevers, H. Development of enzymes in the cotyledons of watermelon seedlings. Pl. Physiol. 51: 66-71; 1973.

Khan, P.R.; Saleemuddin, M.; Siddiqi, M.; McFadden, B.A. Purification and properties of isocitrate lyase from Flax seedlings. Arch. Biochem. Biophys. 183: 13-23; 1977.

Kornberg, H.L.; Krebs, H.A. Synthesis of cell constituents from C_2 units by a modified tricarboxylic acid cycle. Nature (London). 179: 988-991; 1957.

Longo, G.P.; Bernasconi, E.; Longo, C.P. Solubilization of enzymes from glyoxysomes of maize sutellum Pl. Physiol. 55: 1115-1119; 1975.

Lord, J.M.; McFadden, B.A.; Kornberg, H. Changes in microbody-marker enzymes during growth of Tetrahymena pyriformis. E. Proc. R. Soc. Lond. B. 185: 19-31; 1974.

Lowry, D.H.; Rosenbrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265-275; 1951.

Maxwell, D.P.; Maxwell, M.D.; Hanssler, G.; Armentrout, V.N.; Murray, G.M.; Hoch, H.C. Microbodies and glyoxylate cycle enzymes activities in filamentous fungi. Planta. 124: 109-123; 1975.

McFadden, B.A.; Howes, W. Isocitrate lyase in Hydrogenomonas faciles. J. biol. chem. 237: 1410-1412; 1962.

McFadden, B.A.; Howes, W. Crystallization and some properties of isocitrate lyase from Pseudomonas indigofera. J. biol. chem. 238: 1737-1742; 1963.

Mendgen, K. Microbodies (glyoxysomes) in infection structures of Uromyces phaseoli Protoplasma. 78: 477-482; 1973.

Muller, M.; Hogg, J.F.; DeDuve, C. Distribution of tricarboxylic acid cycle enzymes and glyoxylate cycle enzymes between mitochrondria and peroxisomes in Tetrahymena pyriformis. J. biol. chem. 243: 5385-5395; 1968.

O'Sullivan, J.; Casselton, P.J. The subcellular localization of glyoxylate cycle enzymes in Coprinus lagopus (sensu Buller). J. gen. Microbiol. 75: 333-337; 1973.

Ozaki, H.; Shiio, I. Regulation of the TCA and glyocylate cycles in Brevibacterium flavum (I) Inhibition of isocitrate lyase and isocitrate dehydrogenase by organic acids related to the TCA and glyoxylate cycles. J. Biochem. 64: 355-363; 1968.

Patel, T.R.; McFadden, B.A. A simple spectrophotometric method for measurement of nematode populations. Anal. Biochem. 70: 447-453; 1976.

Patel, T.R.; McFadden, B.A. Particulate isocitrate lyase and malate synthesae in Caenorhabditis elegans. Arch. Biochem. Biochem. Biophys. 183: 24-30; 1977.

Patel, T.R.; McFadden, B.A. Fragmentation of isocitrate lyase in crude extracts. Exp. Parasitology. 44: 72-81; 1978a.

Patel, T.R.; McFadden, B.A. Inhibition of isocitrate lyase by itaconage. Exp. Parasitology. 44: 262-268; 1978b.

Patel, T.R.; McFadden, B.A. Axenic and synchronous cultures of Caenorhabditis elegans. Nematologica. 24: 51-62; 1978c.

Polakis, E.S.; Bartley, W. Changes in the enzyme activities of Saccharomyces cerevisiae during aerobic growth on different carbon sources. Biochem. J. 97: 284-297; 1965.

Prichard, P.K.; Schofield, P.J. The glyoxylate cycle, fructose-1, 6-diphosphatase and glyconeogensis in Fasciola hepatica. Comp. Biochem. Physiol. 29: 581-590; 1969.

Reisener, H.J.; Jager, K. Wintersuchungen iibex die guantitative bedetung eininger stoffwechsezwedge in den uredospores von Puccinia graminis var tritici. Planta. 72: 265-283; 1967.

Roche, T.E.; McFadden, B.A.; Williams, J.O. Modification of active site of isocitrate lyase from Pseudomonas indigofera. Archs Biochem. Biophys. 147: 192-200; 1971.

Rothstein, M., Mayoh, H. Nematode biochemistry (IV) on isocitrate lyase in Caenorhabditis briggsae. Archs. Biochem. Biophys. 108: 134-142; 1964.

Rothstein, M.; Mayoh, H. Nematode biochemistry (VII) Presence of isocitrate lyase in Panagrellus redivus, Turbatrix aceti, and Rhadbitis anomala. Comp. Biochem. Physiol. 16: 361-365; 1965.

Rubin, H.W.; Trelase, R.N. Ultrastructure of developing Ascaris larvae undergoing lipid to carbohydrate interconversion. J. Parasitology 61: 577-588; 1975.

Rubin, H.W.; Trelase, R.N. Subcellular localization of glyoxylate cycle enzymes in Ascaris suum larvae. J. Cell. Biol. 70: 374-383; 1976.

Sariaslani, F.S.; Westwood, A.W.; Higgins, I.J. Control of isocitrate lyase in Nocardia salmonicolor J. gen. Microbiol. 91: 315-324; 1975.

Tester, C.F. Control of the formation of isocitrate lyase in soybean cotyledons. Pl. Sci. lett. 6: 325-333; 1976.

Theimer, R.R.; Rosnitschek, I. Development and intracellular localization of lipase activity in rapeseed (Brassica napus L.) Cotyledons. Planta. 139: 249-256; 1978.

Vanni, P.; Mastronuzzi, E.; Vincenzini, M.T.; Firenzuoli, A.M. The glyoxylate cycle in germinating Gingko biloba seeds. Can. J. Biochem. 51: 961-963; 1973.

Vanni, P.; Vincenzini, M.T.; Nerozzi, F. Sinha, S.P. Studiès on isocitrate lyase from Lupinus cotyledons. Can. J. Biochem. 57: 1131-1137; 1979.

Wilson, R.A. A physiological study of the development of the egg of Fasciola hepatica L., the common liver fluke. Comp. Biochem. Physiol. 21: 307-312; 1967.

Woolfson, P.J., Krulwich, T.A. Inhibition of isocitrate lyase: the basis for inhibition of growth of two Arthrobacter species by pyruvate. J. Bact. 112: 356-364; 1972.