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Biochemical Aspects of Host-Parasite Relationships in Plant Parasitic Nematodes

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Increase in enzyme activities of oxidoreductases and lyases have been reported to occur in nematode-infected plant tissues. Amongst lyases, levels of phenylalanine ammonia lyase and tyrosine ammonia lyase have been shown to increase in resistant plant over infected one. Possible mechanism of increase in enzyme activities and their significance in nematode-plant relationships is discussed. Levels of some organic and inorganic substances like carbohydrates, amino acids, proteins, lipids, organic acids, nucleic acids, nucleotides and minerals have also been reported to increase in infected plants. Mineral absorption was more in the infected plant tissues. Phenolics are associated with the resistance of plant to nematode infection. The more necrotic a tissue is, the more phenolics are supposed to be produced by the host which ultimately result in the extermination of the invading parasite. Nematode infection also disbalances the levels of plant growth regulators, which results in varied response of plant to the infection.

Key Words: Nematode-plant relationships, Oxidoreductases, Lyases, Plant growth regulators, Biochemistry of infection

Introduction

In an earlier paper roles of hydrolases have been discussed in nematode-plant relationship (Roy 1980). In addition to hydrolases, levels of other enzymes are also changed in plants due to nematode infection such as oxidoreductases and lyases. Enzymes of transferase, racemase and ligase groups have not been studied in nematode-infected plants, and hence would not be dealt with here. As compared to hydrolases, which have been studied most extensively, little information is available on oxidoreductases and lyases.

In addition to enzymes, changes occur in some other biochemical constituents of plants after onset of nematode infection, such as increase or decrease in levels of carbohydrates,

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amino acids, lipids, organic acids, nucleic acids, phenolics, minerals and growth regulating substances. Although no direct study has been made to show that changes in levels of these biochemical constituents in nematode infected plants accrue as a result of changes in enzyme levels but information available till date suggest that such changes occur due to changes in various enzyme levels, however, the correlation between the two may not be as easy as would have been sought. Detailed discussion on this aspect is given in section III.

I. Oxidoreductases

A. Enzymes of intermediary metabolism: Enzymes of intermediary metabolism which have been determined biochemically in phytophagous, mycophagous and free-living nematodes are mentioned in table 1. In nematode infected plants substantial increase in activities of isocitrate dehydrogenase, malate dehydrogenase and succinate dehydrogenase has been reported in syncytia of soybean infected with *Meloidogyne incognita acrita* (Endo & Veech 1969). Increase in levels of former two enzymes was also observed in syncytia of soybean plant infected with *Heterodera glycines* (Endo & Veech 1970). Glucose-6-phosphate dehydrogenase was localized in tomato roots infected with *M. incognita acrita* (DeMott 1965) and soybean roots infected with *H. glycines* (Endo & Veech 1970), and 6-phosphogluconate dehydrogenase in soybean roots infected with *H. glycines* (Endo & Veech 1970). NADP-alcohol dehydrogenase has been cytochemically localized in soybean roots infected with *H. glycines* (Endo & Veech 1970). Krusberg (1960), however, failed to detect the activities of alcohol dehydrogenase and glucose dehydrogenase in homogenates of *Ditylenchus triformis.*

B. Enzymes of energy metabolism: Enzymes of erergy metabolism determined in parasitic and free-living nematodes, both biochemically and cytochemically, have been listed in table 2. The enzymes which have been localized cytochemically in nematode infected plants are NADH- and NADPH-dependent diaphorases in soybean roots infected with *M. incognita acrita* (Endo & Veech 1969), and NAD-dependent diaphorase in soybean roots infected with *H. glycines* (Endo & Veech 1970).

C. Enzymes for oxidation of harmful metabolites: Table 3 shows the enzymes of this group which have been studied in nematodes. Earlier workers failed to detect the peroxidase activity in some nematodes like *Meloidogyne javanica* (Bird & Rogers 1965), *D. triformis* and *D. dipsaci* (Hussey & Krusberg 1971). However, later workers have demonstrated the presence of this enzyme in some nematodes (table 3). Cytochemically, the enzyme has been detected in stylet exudates of *M. incognita* (Hussey & Sasser 1973).

Increased peroxidase activity has been demonstrated cytochemically in galled tissues of several plants infected with *M. incognita acrita* (Myuge 1956b), cabbage roots infected with *Pratylenchus penetrans* (Acedo & Rohde 1971), soybean roots infected with *H. glycines* (Endo & Veech 1970), Wando peas infected with *D. dipsaci* (Hussey & Krusberg 1970), and bur marigold and grapevine infected with *Longidorus africanus* (Epstein 1972). Catalase has been localized in *L. africanus*-infected bur marigold and grapevine (Epstein 1972), and *D. dipsaci*-infected Wando peas (Hussey & Krusberg 1970).

Monophenol mono-oxigenase (commonly known as tyrosinase, phenolase, catechol oxidase, polyphenol oxidase and monophenol oxidase) has been cytochemically demonstrated in cysts of *Heterodera rostochiensis* and *H. schachtii* (Ellenby 1946), egg sac and outermost layer of the cuticle of *Meloidogyne hapla* and *M. javanica* (Bird 1958), and gelatinous matrix of *M. javanica* (Bird &

Table 1. *Oxidoreductive enzymes of intermediary metabolism in plant parasitic and free-living nematodes*

Enzyme	Nematode	Reference
Phosphoglyceraldehyde dehydrogenase	*Panagrellus redivivus*	Morikawa 1965
	M. javanica, M. incognita, M. hapla and *M. arenaria*	Dickson et al. 1971
Lactate dehydrogenase	*D. triformis* and *D. dipsaci*	Krusberg 1960, Dickson et al. 1971
	A. avenae, M. javanica and *M. hapla*	Dickson et al. 1971
	M. arenaria and *M. incognita*	Dickson et al. 1971 Hussey et al. 1972
	P. redivivus and *T. aceti*	Barrett et al. 1971
2-Glycerophosphate dehydrogenase	*M. incognita* and *M. arenaria*	Dickson et al. 1971 Hussey et al. 1972
	M. javanica and *M. hapla*	Dickson et al. 1971
NADP-dependent isocitrate dehydrogenase	*D. dipsaci* and *D. triformis*	Krusberg 1960
	P. redivivus and *T. aceti*	Barrett et al. 1971
Succinate dehydrogenase	*P. redivivus*	Read 1961, Barrett et al. 1971
	T. aceti	Barrett et al. 1971
Malate dehydrogenase	*D. triformis* and *D. dipsaci*	Krusberg 1960, Dickson et al. 1971
	T. aceti	Elis and Read 1961, Barrett et al. 1971
	P. redivivus	Barrett et al. 1971
	A. avenae	Dickson et al. 1971
	M. incognita and *M. arenaria*	Dickson et al. 1971, Hussey et al. 1972
	M. javanica, M. hapla and *H. glycines*	Dickson et al. 1971
Glucose-6-phosphate dehydrogenase	*D. triformis*	Krusberg 1960, Dickson et al. 1971
	D. dipsaci	Krusberg 1960
	A. avenae, M. hapla and *M. javanica*	Dickson et al. 1971
	M. incognita	Dickson et al. 1971, Hussey et al. 1972
	M. arenaria	Hussey et al. 1972
6-Phosphogluconate dehydrogenase	*D. dipsaci* and *D. triformis*	Krusberg 1960
	M. incognita and *M. arenaria*	Hussey et al. 1972

Rogers 1965). Ellenby and Smith (1967) associated the tanning process of the cyst walls of *H. rostochiensis, H. schachtii* and *Aphelenchus avenae* with the presence of this enzyme. The enzyme has also been localized in Wando peas infected with *D. dipsaci* (Hussey & Krusberg 1970) but not in bur marigold and grapevine infected with *L. africanus* (Epstein 1972).

Results on oxidoreductive enzymes, so far accumulated, reflect that activities of most of the enzymes studied are increased in infected plant tissues. The hyperactivity of enzymes indicates that the infected plant cells are

Table 2 *Oxidoreductive enzymes of energy metabolism in plant parasitic and free-living nematodes*

Enzyme	Nematode	Reference
Diaphorase	*D. triformis* and *D. dipsaci*	Krusberg 1960
NADH-dependent cytochrome *c* reductase	*D. triformis* and *D. dipsaci*	Krusberg 1960
Cytochrome oxidase (a_3)	*Panagrellus silusiae*	Pennoit-de-Cooman 1950
	D. triformis	Krusberg 1960
	Pratylenchus scribneri, *C. briggsae* and *P. redivivus*	Deubert and Zuckerman 1968*

*Demonstrated cytochemically

Table 3 *Oxidoreductive enzymes to oxidize harmful metabolites in plant parasitic and free-living nematodes*

Enzyme	Nematode	Reference
Peroxidase	*A. avenae*	Evans 1971
	M. incognita	Hussey et al. 1972, Hussey and Sasser 1973*
	M. arenaria	Hussey et al. 1972
Catalase	*M. incognita* and *M. arenaria*	Hussey et al. 1972
	T. aceti	Aueron and Rothstein 1974
Monophenol mono-oxigenase	*H. rostochiensis* and *H. schachtii*	Ellenby 1946*
	M. hapla	Bird 1958*
	M. javanica	Bird 1958*, Bird and Rogers 1965*
Dihydroxyphenylalanine (DOPA)-oxidase	*D. dipsaci* and *D. triformis*	Hussey and Krusberg 1971

*Demonstrated cytochemically

metabolically more active. This hyperactivity may result to the accelerated ageing of the plant in general and cells/tissues in particular. The resultant effect of this may be retarded growth or early death of the plant and subsequent elimination of the parasite. Although as one of the factors of the host-parasite relationships, parasite never wishes its host to die but it is beyond the capability of the parasite to prevent the secondary infection in the plant host. This secondary infection may be responsible for the death of the host. Even in the absence of secondary infection plants may die due to heavy infection of the nematode itself.

Most of the studies have been performed on plants infected with *Meloidogyne* spp. which induce the formation of giant cells by dissolution of cell walls around its vicinity. Increase in oxidoreductive enzymes in pathologic cells may thus be contributed to cumulative activity of enzymes by merging of

several cells into one and/or increased synthesis of enzymes by pathologic cells. It has been shown that enzymes do not increase in soybean resistant to *M. incognita acrita*, however, in rare cases, where syncytia were induced in some resistant cultivars enhanced enzyme activity was noticed (Veech & Endo 1970). This suggests that increase in enzyme level is associated with the formation of giant cells or syncytia but it is difficult to ascertain that whether this increase is a result of cell-merging or the increased synthetic activity. The latter prospect can be verified by investigating if such changes do occur in plants infected with ectoparasitic nematodes which usually do not induce the formation of giant cells.

Giant cells or any sort of modified pathologic cells are the only source of nutrition for sedentary endoparasitic nematodes and thus such cells ought to be more active to remain alive. If the concept that nematode induces the levels of enzyme synthesis by infected cells is accepted then there ought to be continuous stimulation from the nematode for the synthetic processes to undergo. In fact, interdependence of syncytia and *Meloidogyne* development has been demonstrated by Bird (1974) suggesting that the nematode stimulates the metabolism of plant cells.

The role of peroxidase in plant physiology or metabolism is not clearly understood. Van Fleet (1961) has reported that activity of peroxidase increases in wound meristems. Since peroxidase and catalase oxidize many mono- and di-phenols and aromatic amines to quinones in presence of hydrogen peroxide, it is suggested that hydrogen peroxide, which is a toxic end product of aerobic dehydrogenases and oxidases is removed by peroxidase or catalase. The increase in peroxidase activity has been found to confine mostly to the cortical cells adjacent to necrotic epidermal cells (Hussey & Krusberg 1970) suggesting that host produces the enzyme at the site of infection to combat the toxic effect of cellular response to nematode infection. A tremendous increase in bound phenolics has been reported in citrus cultivars resistant to *Radopholus similis* and decrease in susceptible cultivars (Feldman & Hanks 1968). These phenolics are oxidized by phenolase which on subsequent polymerization turn brown. This discolouration process has been shown in a variety of plant tissues (Farkas & Kirlay 1962, Uritani 1963). Wallace (1961a,b) observed such browning reaction in Chrysenthemum leaves infected with foliar nematode, *Aphelenchoides ritzemabosi*.

II Lyases

Lyases which have been studied in plant and soil nematodes are listed in table 4. The first report of the isocitrate lyase activity in a multicellular organism was made by Rothstein and Mayoh (1964) in a free-living nematode, *Caenorhabditis briggsae*. Later the enzyme was reported in several other nematode species (table 4). Gershon and Gershon (1970) showed that the specific activity of isocitrate lyase decreased as *Turbatrix aceti* aged.

It has been shown that aldolase activity becomes more intense in the early stage of disease in the syncytia of susceptible soybean plants infected with *H. glycines* (Endo & Veech 1970). Giebel (1973) reported increase in the activity of phenylalanine ammonia lyase and tyrosine ammonia lyase in potato plants infected with *H. rostochiensis* but still more increase was observed in resistant plants. The increase in the activity of the two lyases in resistant potato plants over susceptible ones has been correlated with increase of phenolics in nematode resistant plants (Webster 1975). The correlation appears sound and these enzymes may contribute to the development of resistance in plants against nematode infection. However, on the other hand, about 50% reduction of phenylalanine ammonia lyase activity has been

Table 4 *Lyases in plant parasitic and free-living nematodes*

Enzyme	Nematode	Reference
Isocitrate lyase	*P. redivivus* and *Rhabditis anomala*	Rothstein and Mayoh 1965
	C. briggsae	Rothstein and Mayoh 1964
	Caenorhabditis elegans	Colonna and Mc Fadden 1975
	T. aceti	Rothstein and Mayoh 1965, Gershon and Gershon 1970, Reiss and Rothstein 1974
Citrate synthase	*D. triformis*, *D. dipsaci* and *Partylenchus zeae*	Colonna and Mc Fadden 1975
	P. redivivus	Barrett et al. 1971
	T. aceti	Elis and Read 1961
		Barrett et al. 1971
Fumarate hydratase	*D. triformis*	Krusberg 1960
	P. redivivus	Barrett et al. 1971
	T. aceti	Barrett et al. 1971, Aueron and Rothstein 1974
Malate synthase	*P. redivivus*, *C. briggsae*, *R. anomala*, and *T. aceti*	Rothstein and Mayoh 1966
	C. elegans	Colonna and Mc Fadden 1975
Enolase Phospho-enolpyruvate carboxikinase	*D. triformis* and *D. dipsaci*	Krusberg 1960
	P. redivivus and *T. aceti*	Barrett et al. 1971
Aldolase	*T. aceti*	Zeelon et al. 1973 Reznick and Gershon 1977
Phenylalanine ammonia lyase	*P. penetrans* and *Tylenchus agricola*	Zuckerman et al. 1966

reported in galled roots of carrot infected with *M. hapla*, as compared to healthy roots but the enzyme activity was enhanced in the secondary xylem and phloem of the storage roots of the infected plants (Chylinska & Knypl 1975). These workers proposed that the decrease in enzyme activity is associated with reduced lignin biosynthesis, and thus facilitating cell enlargement and cortical hypertrophy. The enzyme seems to play an important role in the pathogenesis of nematodes and needs to be worked out extensively.

III Changes in Biochemical Constituents:

A. Carbohydrates: There are few reports on the determination of carbohydrates in nematode-infected plants. Little more than 50% reduction of free sugars was reported in galled tomato roots infected with *M. incognita incognita* and *M. incognita acrita* over healthy ones (Owens & Specht 1966). Free sugars identified were ribose, fructose, glucose and sucrose. Trehalose and mannitol/sorbitol were found only in galled roots. On the other hand, glucose was not found in root exudates of galled tomato roots infected with *M. incognita* but increase in fructose, sucrose and polysaccharide by 133–836 percent was reported over healthy ones (Wang & Bergeson 1974). Interestingly both the studies were done on tomato plants infected with *Meloidogyne* spp. This variation in sugar contents is probably due to varied culture

methods. Owens and Specht (1966) used sand culture with infection period of 60 days, whereas Wang and Bergeson (1974) used filter paper culture with infection period of two weeks. When silica sand was used by latter workers for the same period of infection, the increase in sugars was only 133 per cent and when the infection period was increased with monoxenic culture conditions, a decrease in sugars with increase in infection was observed.

Recently, Van Gundy et al. (1977) reported that during the first 14 days following infection of tomato plants by *M. incognita*, carbohydrates were the major constituents in root exudates. Similarly four times more glucose was reported in giant cells and syncytia of soybean roots infected with *Meloidogyne* and *Heterodera* over uninfected ones (Gommers period & Dropkin 1977).

Wang and Bergeson (1974) believed that absence of glucose in infected xylem sap is due to rapid turnover rate as a result of increased metabolic activities. They also suggested that glucose is utilized in polysaccharide synthesis. The general observation was that the amount of total sugars in xylem sap of infected plants tended to increase with time after inoculation, and it was inoculum-level dependent. At higher inoculum level sugars decreased with time, whereas at lower inoculum level, increase in sugar concentration was noticed. Recently, Maheshwari and Chaturvedi (1979) reported that *M. javanica* infection on *Solanum melongena* increased the PAS-positive carbohydrates in root galls.

Since the increase in both free sugars and polysaccharide synthesis has been shown to occur in roots of infected plants, the possible source of this increase may be their translocation from the aboveground parts of the plant. Secretion of highly hydrated hexose polysaccharide in corn roots has been shown to mediate through Golgi apparatus (Moore & Jones 1967). Increase in Golgi apparatus was shown in galled tomato roots (Bird 1961), and it was proposed that the increase in polysaccharide accounts for increase in Golgi apparatus (Wang & Bergeson 1974). However, this proposition has to be considered with caution because it is not necessary that Golgi apparatus of healthy and diseased tissues would behave alike. It has already been pointed out that the infection of plants by nematodes bring about changes in the metabolism of cells (Roy 1979, 1980) which would obviously change the behaviour of various cell organelles.

Increase in phosphorylated sugars like glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate and fructose-1, 6-diphosphate by 35, 347,7 and 90 per cent, respectively (Owens & Specht 1966), suggests an increase in phosphorylation. In giant cells and syncytia of *Meloidogyne* and *Heterodera*-infected soybean roots, glucose-6-phosphate concentration was comparable to that of actively growing root tip tissues (Gommers & Dropkin 1977). Increase in these phosphorylated sugars may occur by transformation of starch and glucose or other hexoses involving some enzyme regulated reactions (figure 1). Few of these enzymes like hexokinase and

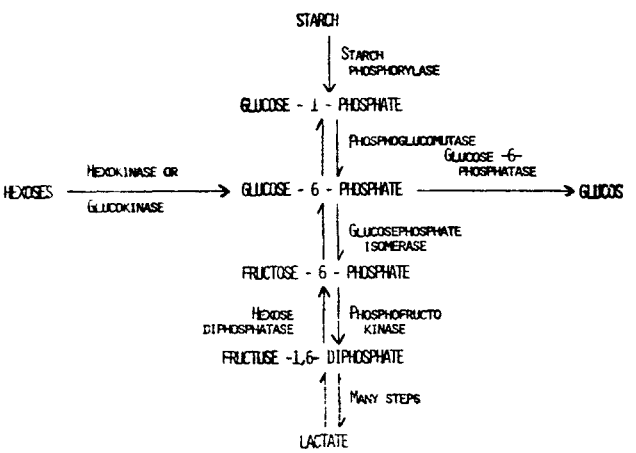

Figure 1 A part of glycolytic pathway showing the conversion of starch and hexoses to various phosphorylated sugars.

phosphoglucomutase have been studied in nematodes (Krusberg 1960) but none of them has been studied in infected plant tissues. Also, dephosphorylation of phosphorylated sugars may be attributed to the increase in free sugar concentration. The latter view is based on the fact of increased activities of phosphatases in galled roots (Roy 1979). If the phosphorylation and dephosphorylation processes undergo simultaneously, there should be an equilibrium between the phosphorylated and dephosphorylated sugars unless of course one process is slower than the other or some variations in the experimental conditions take place.

B. Amino acids and proteins: In almost all the studies a tremendous increase in free and bound amino acids have been observed in nematode infected plant tissues. It has been reported that free amino acids and amides in the roots of grapefruit seedlings infected with *R. similis* increased by 17–316% over the amount found in uninfected seedlings, however, their percentage composition in both the groups of seedlings was essentially the same (Hanks & Feldman 1963). In a similar type of experiment, using same system, 2–5 times more free amino acids was found in infected roots and this level returned to normal during recovery of the plant (Feldman & Hanks 1964). These authors also reported 39–58% increase of protein bound amino acids in infected seedlings, whereas roots from "recovered" seedlings bore slightly less bound amino acids than from corresponding healthy roots. The important observation was the accumulation of high concentration of arginine and tyrosine in declined roots. Leaves of grapefruit seedlings infected with *R. similis* for eight and 12 months showed 18 and 80 percent increase, respectively, in free amino acid content but the bound amino acids increased by only 13% after 12 months infection (Hanks & Feldman 1966). Leaves of infected plants also showed very high proportion of arginine which along with other free amino acids returned to normal level in "recovered" plants.

Owens and Specht (1966) reported 100–720% increase in amino acid contents in galled tomato roots infected with *M. incognita incognita* and *M. incognita acrita* over uninfected roots. On the contrary, a decrease of 52–56% in amino acid contents was reported in exudates of *M. incognita*-infected tomato roots (Wang & Bergeson 1974). Cytochemically an increase in syncytia size and number with a parallel increase in amino acids (5–14%) and total proteins (3–20 folds) was detected depending on the age and the metabolic state of the tissue (Owens & Specht 1966). An elevated protein level was also observed in giant cells of okra infected with *M. incognita acrita* (Littrell 1966). Total nitrogen as well as free and bound amino acids were found to increase with successive harvest in *D. dipsaci*-infected alfalfa and pea tissues than uninfected ones (Howell & Krusberg 1966).

The galled roots of bur marigold infected with *L. africanus* were reported to contain 70% more cell wall protein and 184% more free amino acids over uninfected ones (Epstein & Cohn 1971). Proline concentration registered an increase by 1900% while aspartic acid concentration decreased by 56% in galled roots. Observations by the same workers on infected grapevine showed that protein concentration decreased in protoplasmic and cell wall fractions but the amount of free amino acids remained unchanged. Significant increase of aspartic acid, glutamic acid and glutamine was also reported in roots of sugar beet infected with *H. schachtii* (Doney et al. 1971). Free amino acids were shown to be more concentrated on giant cells than in syncytia of soybean roots infected with *Meloidogyne* and *Heterodera* spp. and that their quantity was slightly higher in giant cells of garden balsam as compared to control (Gommers & Dropkin

1977). Recently, Poehling et al. (1980) reported considerable changes in levels of some amino acids in root tip galls and modified cells of *Ficus carica* infected with *Xiphinema index*. Among the most pronounced were increase of glutamic acid and decrease of glycine.

The possible factors for increase in amino acid levels in infected plant tissues may be (i) increased rates of synthesis in diseased tissues, (ii) increased rates of translocation towards diseased tissues, (iii) decreased rates of translocation from diseased tissues, (iv) decreased rates of degradation, and (v) deposition by nematodes. In case of decrease of amino acids in infected tissues, any of the above steps might work in reverse direction. It may, however, be mentioned that the changes in total amino acids in infected plants is disease related as evidenced by the fact that the levels returned to normal in "recovered" plants or seedlings.

Increased rates of synthesis of amino acids can be supported by the evidence of overall increase in metabolic activities of diseased tissues (Roy 1979, 1980). Increased rates of translocation towards diseased tissues deserve some reservation because the increase in amino acids has been observed both in roots and leaves of same seedlings. Decreased rates of translocation from diseased tissues also does not hold any support if amino acid contents of healthy and infected plants are compared. Because even if decreased translocation is to occur, one should observe reduced levels of amino acids unless there is very high rate of synthesis. This indirectly supports the first factor. Overall increase in metabolism of infected tissues would not cause decrease in degradation and, thus, the fourth factor also holds no merit. The fifth factor merits some consideration. Nematodes contain both free and bound amino acids (Krusberg 1971). Since nematodes have been shown to secrete several enzymes (Roy 1980) and other inorganic and organic substances, it can be assumed that they secrete amino acids also into the cells they feed upon. Aist and Riggs (1969) reported that larval and especially adult *H. glycines* gave off large quantities of alanine, glutamic acid, aspartic acid, glycine and proline. Therefore, out of five proposed possible factors, only first and fifth hold merit to be responsible for increase in amino acid levels in pathologic cells.

Owens and Rubinstein (1966) showed that labelled carbon from ^{14}C-glucose was incorporated into amino acids of excised roots. Therefore, if it is assumed that carbohydrates are converted to amino acids, then increase in carbohydrates should not occur, which is not true. As reported in case of carbohydrates, variations in levels of amino acids in leaves observed by different workers may be due to variations in inoculum level and length of infection. It has been concluded that change in levels of amino acids is not involved with the galling in plants, as galling initiates long before significant accumulation of amino acids occur in galls. Thus the change appears to be secondary but certainly associated with the nematode-plant relationship because the change in amino acid levels in infected plants returned to normal in "recovered" ones. It is yet to be determined that what is the significance of the increased levels of amino acids and proteins in infected tissues and what happens to those constituents when plant recovers from infection in the sense that where do they go to return to normal level in recorvered plants.

C. Lipids: Occurrence of some class of lipids, viz., monor-, di- and tri-glycerides, sterols and other fatty acids in healthy as well as *Meloidogyne*-infected tomato roots has been reported. An increase of about 150 percent was observed in galls and none of the lipid fractions had any growth promoting effects on seedling roots (Owens & Specht 1966). Histochemically, abundance of lipids in giant cells of root-knot susceptible soybean has been demonstrated over resistant plants

(Sheetz & Crittenden 1966). Nothing is known about the origin of the increase in lipids in infected plant tissues. It may be due to: (i) increased rates of synthesis, (ii) conversion of carbohydrates to lipids, (iii) transportation of lipids from other parts of the plant, and (iv) breakdown of cell wall lipids.

Recently, Myuge and Estley (1978) proposed that infection of tomato and radish plants by *M. hapla* and *M. incognita* is associated with physiological changes characteristic of ageing. They showed the oxidation of cell membrane lipids and suggested that cell membranes subjected to such oxidation lost some of their capacity for water retention. They also showed that treating plants with lidocain hydrochloride, an inhibitor of lipid oxidation, retarded above-ground symptoms of root-knot nematode infection and ageing. Unfortunately not much work has been done on this aspect, and more information is needed to establish the role of lipids or fatty acids in nematode-plant relationships.

D. Organic acids: Similar to carbohydrates, there are contradictory results on the organic acids in tomato roots infected with *Meloidogyne* spp. Owens and Specht (1966) reported 67% increase of total organic acids in galled roots, whereas Wang and Bergeson (1974) reported a decrease by 56% in root exudates. Krusberg (1961) did not find any change in free organic acids in alfalfa tissue infected with *D. dipsaci* and *A. ritzemabosi* but Hanks and Feldman (1968a) reported approximately 41% less organic acids in *R. similis*-infected grapefruit seedlings. However, leaves from declined plants contained about 16% more organic acids than from uninfected ones. Owens and Specht (1966) reported that succinate, α-keto-glutarate and glycoxylate decreased in *Meloidogyne*-infected tomato roots, whereas malate, citrate, fumarate and oxaloacetate increased substantially. There was no change in pyruvate concentration.

The variation in levels of organic acids of galled tomato roots infected with *Meloidogyne* spp. (Owens & Specht 1966) and those in roots and leaves of grapefruit seedlings infected with *R. similis* (Hanks & Feldman 1968a) may be primarily due to the nematode-host combinations. Again, no correlation can be made between the changes in levels of some enzymes and products of their reactions. Whereas most of the enzymes studied have revealed enhanced levels in infected tissues, much variations have been noticed in some of their reaction products in relation to enzyme activities. This aspect also requires more attention of workers.

E. Nucleotides and nucleic acids: Merging of cells is known to occur in the formation of syncytia where fusion of nuclei has been demonstrated (Bird 1972). With the evidence of nuclear fusion an increase in nucleic acid contents is expected to occur. Owens and Specht (1966) found components of free nucleotides like CMP, CDP, CTP, AMP, ADP, ATP, UMP, UDP, UTP, UDPG, GMP, GDP, and GTP which were usually present in both healthy and galled tomato roots. They observed an increase of all the nucleotides in syncytia over normal roots, however, the presence of IMP and ITP was doubtful. Gommers and Dropkin (1977) reported that both giant cells and syncytia of soybean roots infected with *Meloidogyne* and *Heterodera* contained concentration of ATP comparable to that of actively growing root tip tissues, a situation similar to that observed in case of carbohydrates by these workers. This suggests that although pathologic cells and growing root tip tissues are functionally not similar but metabolically both appear to have some similarity so far as hyperactivity is concerned.

Bird (1961) reported increased levels of nucleic acids in *Meloidogyne*-induced giant cells using specific stains for DNA and RNA. He, further, reinforced his past observation by showing that increase in DNA content in

giant cells was nematode growth dependent (Bird 1972), and proposed that stylet secretion contains histone like protein which influences DNA synthesis and enzyme production (Bird 1969, 1972). Increase in total RNA was reported in giant cells of okra infected with *M. incognita acrita* (Littrell 1966).

Enhanced rate of nucleic acid synthesis has been shown in root-knot syncytia using labeled thymidine and uridine (Rubinstein & Owens 1964, Owens & Rubinstein 1966). Endo (1971) reported that although syncytial nuclei were relatively quiscent in terms of DNA synthesis, but ^{3}H-thymidine was incorporated in syncytial nuclei of cortical and vascular regions of soybean roots infected with *H. glycines*. Active site of DNA synthesis was shown to be the nuclei of developing syncytium situated at its leading edge. It has also been reported that DNA and RNA levels were greater in *L. africanus*-infected bur marigold than in uninfected tissues, and suggested that the nematode induced the increased synthesis (Epstein 1974). Polyploidy has been shown in tomato roots infected with *M. javanica* which may be responsible for increased DNA and RNA contents.

In light of the situation as it exists, the increased rate of nucleic acid synthesis may occur either as a result of secretion of histone-like protein as suggested by Bird (1972) or due to some other stimulant(s) secreted by nematodes whose nature is not yet known. Since increased levels of nucleic acids have been reported in plants infected with both endo- and ecto-parasites, it appears that nematode secretion contains some stimulant(s) which may enhance the nucleic acid synthesis. Hypertrophy and hyperplasia are the resultant features of nematode infection which occur during gall formation, and it appears quite likely that the increased nucleic acid contents contribute to these features.

F. Phenolics: Determination of aromatic compounds in citrus cultivars infected with *R. similis* has been made by Feldman and Hanks (1968). They reported that the net effect of infection was a 27–300% increase in the bound phenolics in the roots of tolerant cultivars, and 16–34% decrease in susceptible ones. No significant change was observed in the total bound phenolics of leaves of both susceptible and tolerant cultivars, though individual bound phenolics was observed to have increased. There was no consistent pattern of increase or decrease of total or individual free phenolics in roots of any of the cultivars, whereas in leaves total free phenolics increased by 2–7% in tolerant cultivars while the value remained unchanged in susceptible ones.

Merely on the basis of one report no conclusion can be drawn on the role of phenolics in nematode-plant association, however, their involvement to some extent in the susceptibility or resistance of a plant to the parasite infection seems probable.

G. Inorganic substances: Increase in Na, K, P and N and decrease in Ca has been reported in galls of pepper plants infected with *M. incognita acrita* and *M. hapla* (Shafiee 1962) and tomato roots infected with *M. incognita incognita* and *M. incognita acrita* (Owens & Specht 1966). Hunter (1958) did not observe any significant difference in Fe content in the roots and tops of tomato roots infected with *M. incognita acrita.* Higher concentrations of Ca, Mg, Na, K, Fe, Mn and Cu were found in tomato roots infected with different species of *Meloidogyne* (Owens & Specht 1966, Dasgupta & Deb 1972, Van Gundy et al. 1977). Oleifa and Elgindi (1962) reported that infection of tomato roots with *M. javanica* induced ^{32}P absorption, whereas other workers reported decrease in absorption in tomato roots infected with *M. incognita incognita* (Dropkin 1956, Hunter 1958) and *M. javanica* or *M. arenaria* (Dasgupta & Deb 1972). In an

another study Jenkins and Malek (1966) reported that decrease in Na, Ca and P and increase in K in roots of *Vicia villosa* was more when infected with *M. hapla*, an endoparasite, rather than by *Criconemoides curvatum*, *Scutellonema brachyurum* and *Trichodorus christiae*, the ectoparasitic nematodes.

It is not known whether increase in inorganic substances results from their mobilization towards diseased tissues or decreased rate of translocation from diseased tissues. Nevertheless, whatever little variations in some inorganic substances were observed by different workers might be due to factors like span of infection, age of plant, level of inoculum or other varied experimental conditions. Incidentally, increase in N and P coincides with increase in amino acids and nucleic acids, and it is quite likely that some of these inorganic substances are incorporated into various organic compounds while others like K, Fe, Mn and Cu may be characteristic of growing tissues.

H. Growth regulators: Goodey (1948) was the first to suggest that plant growth substances induce the gall or giant cell formation. Later it was shown that extracts of rye plant infected with *D. dipsaci* significantly increased the length of *Helianthus* seedlings suggesting that a plant growth substance which induced the increase of stock length also caused galling in plant (Nolte & Kohler 1952). Bird (1962) also reported that extracts of galled tomato roots promoted the growth of wheat coleoptile. Dropkin et al. (1969) showed that addition of indolacetic acid (IAA) did not increase galling in tomato roots infected with *Meloidogyne* spp., whereas Myuge and Viglierchio (1975) reported that IAA promoted root mass and galling by 3 and 5 fold, respectively, in tomato plants parasitized by *M. incognita* but not when infected with *M. hapla*. They further observed that glutathione significantly increased gall numbers in *M. hapla*-infected seedlings while malic hydrazide drastically inhibited shoot growth and gall formation in plants infected with either of the species of *Meloidogyne*.

Although, Myuge (1956a) could not show auxins in the extracts of galled tomato roots but an alcohol extract of such roots caused galling in beans (Myuge 1956b). It was found by latter workers that root-knot galls contained more auxin than normal roots (Bird 1962, Yu & Viglierchio 1964, Setty & Wheeler 1968). These workers suspected the presence of organic acids in the medium and chromatographic studies revealed the presence of IAA. IAA-like substance has also been shown in extracts of *M. javanica*-infected *Abelinoschus esculentus* (Balasubramanian & Rangaswami 1962). Viglierchio and Yu (1968) reported that nematode species influenced the type of auxin present in the infected plants. Thus, Brussels sprout infected with *M. incognita* showed the presence of indolbutyric acid, reduction of indolacetonitrile (IAN), and that proportion of IAA and indolacetic acid ethyl ester (IAE) remained unchanged as compared to healthy tissues. But on infection with *M. javanica* IAE was found absent, IAA increased and IAN remained unchanged. Auxins are known to increase the plasticity and permeability of cell walls which results in cell enlargement, however, Viglierchio and Yu (1965) are of the view that the infection with *D. dipsaci* probably inactivates the auxins which consequently results in stunting of stem apex. On the other hand, other workers suggested that a higher level of endogenous auxins (Cutler & Krusberg 1968) or of auxins and cytokinins (Kochaba & Samish 1972) or of exogenously applied cytokinins (Dropkin et al. 1969, Kochaba & Samish 1971) are associated with the formation of giant cells and galls. Since cytokinins regulate cell enlargement, karyokinesis and DNA synthesis (Webster 1975), and the fact that susceptible peach roots possessed more endogenous level of

cytokinins than resistant roots (Kochaba & Samish 1972), its involvement in regulating giant cell formation becomes apparent.

It has been proposed that root growth is controlled by a balance between auxins, kinins and gibberellins (Street 1961). It was found that *Meloidogyne* larvae contained too little auxin to account for the excess found in galled roots, and it is possible that this extra auxin arises from bound form or its precursor in plant cells (Setty & Wheeler 1968). Although there is no convincing evidence for the origin of increased auxins in plants due to nematode infection, it has been speculated that nematode secretions release protein-bound auxin (Webster 1967, Johnson & Viglierchio 1969). Giebel (1970), on the other hand, suggested that nematodes interfere with the auxin synthesis.

Seasonal changes in growth promoters and inhibitors in nematode-infected plants have been studied by Hanks and his coworkers (Feldman et al. 1966, Hanks & Feldman 1968b, 1969, 1972). Hanks and Feldman (1969) found decreased level of gibberellin in shoots of citrus tree infected with *R. similis* than uninfected tree. Similarly, decreased production of neutral and acidic gibberellins and also of cytokinin was caused in tomato plants by *M. incognita* infection (Brueske & Bergeson 1972). Some workers found greater amount of gibberellin in terminals collected from citrus trees infected with *R. similis* than those from healthy plants and proposed this increase to be seasonal. Increase in hespiridine, a growth hormone, was observed during October to December in *R. similis*-infected citrus trees (Hanks & Feldman 1968b). No mechanism is known for such an increase, especially at a distance quite far from the infection site. It has been reported that gibberellins were bound to proteins (Mc Comb 1961) and it has been suggested that hydrolytic enzymes, secreted by nematodes, release gibberellin and other growth substances which, in turn, stimulate the plant tissue response (Webster 1969).

Digby et al. (1964) and Webster (1969) reported that gibberellin and IAA increase the quantity of sugars and amino acids, probably by stimulating their synthesis. It was also proposed that gibberellin promotes the conversion of tryptophan to IAA (Sastry & Muir 1965), functions as growth promoting substance (Brian & Hemming 1957, Sirois & Parups 1965) or increase the activity of hydrolytic enzymes (Paleg 1961). Mountain (1960) suggested that larval proteolytic enzymes hydrolyze IAA-protein complex and release IAA and tryptophan. Tryptophan reacts with phenolics to form auxins and also is converted to IAA via indoxyl-3-acetaldehyde (Webster 1969). Orion and Minz (1969) reported that ethylene, another plant growth regulator, caused increase in gall size due to proliferation of root parenchyma. Webster (1975) proposed that probably auxin triggers the synthesis of ethylene.

Thus, the overall situation is that the nematode infection disbalances the levels of plant growth regulating substances which result in either stunting of plants or formation of giant cell and galls. This disbalance may also result in the production of stimuli which cause increase in metabolic activities of plants such as synthesis of more enzymes and inorganic and organic substances.

Conclusion

The overall response of plant to nematode infection has been diagrammatically summarised in figure 2. Whereas hydrolases play significant role in host-parasite relationship (Roy 1980), role of oxidative enzymes of intermediary metabolism and energy metabolism is not yet understood. Lyases seem to play some role in nematode-plant association. It appears that the biochemical response of the plant to nematode infection is mediated

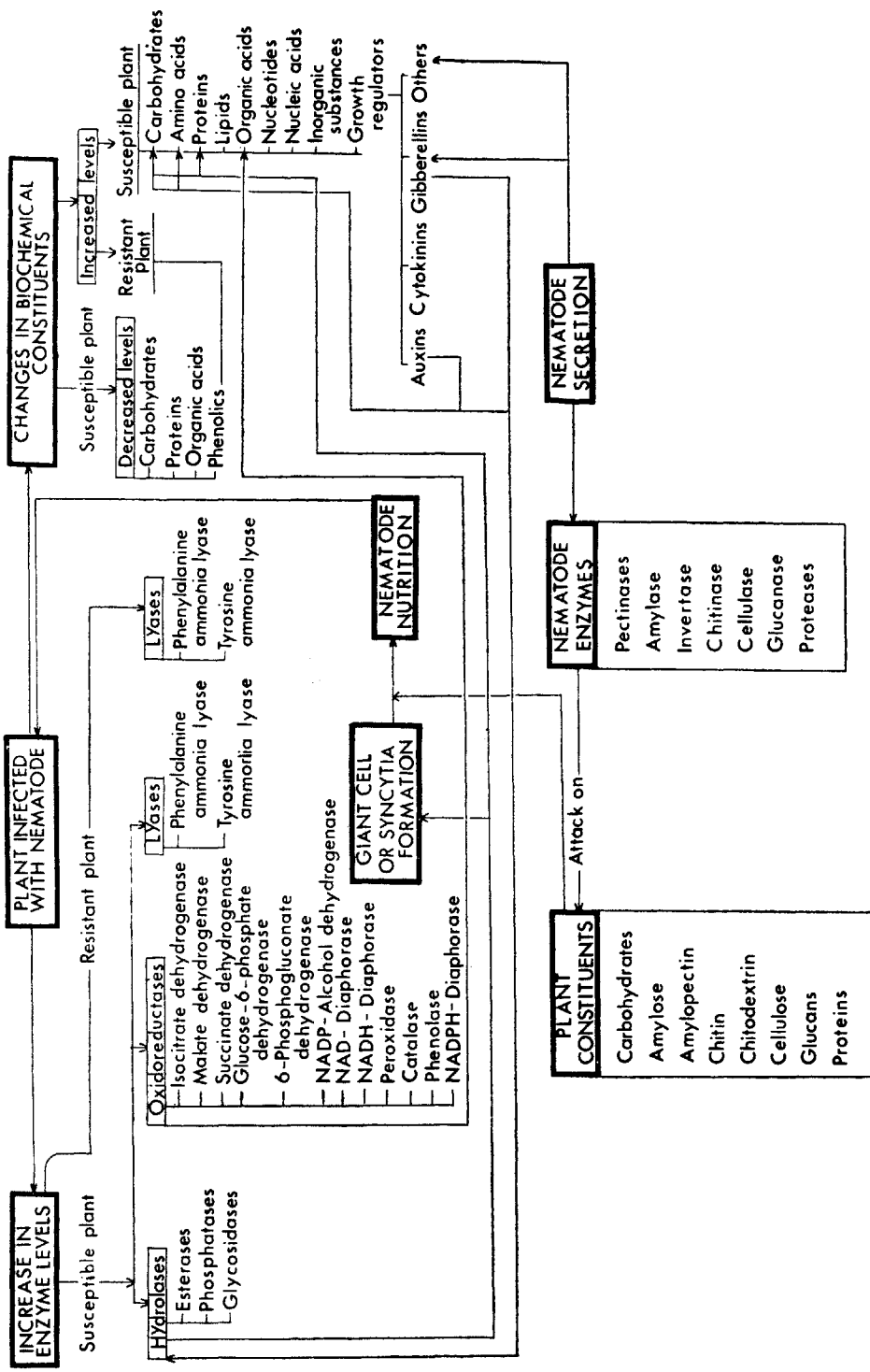

Figure 2 Diagramatic representation of nematode-plant relationshlps indicating the interrelationship between various plant substances, affected by infection, and the nematode

through the change in plant growth regulators. Since changes in levels of some enzymes and organic constituents of plant are interrelated, origin of any single organic component or its contribution in the host-parasite system cannot exactly be pinpointed. The system is much complex to interpret conceivably especially in situations where contradictory observations are reported by different workers using same host-parasite system or same host but different species of the same parasite and vice versa. More studies are needed especially in enzymes of lyase group and system involving ectoparasitic nematodes.

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