Carbon Assimilation by *Claviceps purpurea* Growing as a Parasite

By A. G. DICKERSON, P. G. MANTLE AND L. J. NISBET*

Department of Biochemistry, Imperial College, London SW7

(Received 5 March 1976; revised 23 June 1976)

SUMMARY

Carbon assimilation by *Claviceps purpurea*, growing as a parasite on cereals, has been investigated by supplying the host plant with $^{14}\text{CO}_2$ in a closed system. The presence of the pathogen induced the plant to exude photosynthate which contained high levels of sucrose. During the period of $^{14}\text{CO}_2$ supply, ^{14}C was incorporated into the sucrose and so the path of carbon into the parasite could be traced. Hexoses, derived by the action of the fungal sucrase on sucrose, were assimilated by the pathogen and largely converted into polyols – mainly mannitol and, to a lesser extent, trehalose. The rate of carbohydrate metabolism decreased with maturation of the ergot, and also showed qualitative differences between the basal and apical regions of the ergot which were probably a function of nutrient supply.

INTRODUCTION

Enzymological evidence and honeydew analysis strongly suggest that growth of *Claviceps purpurea* during parasitism of rye or wheat ovaries is supported by sucrose released by the host as a phloem exudate (Dickerson, 1972). The supply of sucrose is maintained throughout the development of the parasite (Corbett, Dickerson & Mantle, 1974) and the conversion of the sucrose to other carbohydrates is a feature of the constitutive enzyme activities observed in the fungal tissues and is not a function of the host.

Our aim was to establish these conclusions independently by supplying $^{14}\text{CO}_2$ to those photosynthetic tissues of the infected host plant which contribute carbon most directly to the pathogen.

METHODS

Origin of C. purpurea strain. Strain 29/4, reselected (Nisbet, 1975) from strain 17-3 as used by Corbett *et al.* (1974), was used. The strain was highly infectious, yielding ergots between 15 and 20 mm long and 1 and 3 mm wide, and also grew well in liquid culture.

Growth of host plants. Seeds of Svalof's Fourex Rye (Swedish Seed Association, Svalof, Sweden) and Maris Dove wheat (Plant Breeding Institute, Cambridge) were grown under glasshouse conditions in pots (10 per pot) and in rows in open plots at the Chelsea Physic Garden, London. Plants from the open plots were transferred to pots 10 to 14 days before experiments.

Inoculation of host plants. This was normally carried out in early June before anthesis. For any single experiment, plants of the same age were all inoculated at the same time. Production of the inoculum and inoculating procedure were as described by Corbett *et al.* (1974).

Supply of $^{14}\text{CO}_2$ to infected plants. Figure 1 shows the apparatus used to supply $^{14}\text{CO}_2$ to plants. The gas was generated by injecting 1 ml 90% (w/v) lactic acid through a Suba-Seal

* Present address: Roche Products Ltd, Welwyn Garden City, Hertfordshire AL7 3AY.

Fig. 1. Apparatus for supplying $^{14}\text{CO}_2$ to plants parasitized by *Claviceps purpurea*. B, bubbler; G, gas generator; GM, Geiger-Müller tube; P, pump; PC, plant chamber; R, reservoir; S, stirrer; T, thermometer; TT, three-way tap. The arrows show the direction of gas flow. For further details, see Methods.

cap on to 15 to 20 mg $\text{Ba}^{14}\text{CO}_3$ and heating the mixture gently (Dickerson & Edelman, 1966). The liberated CO_2 was mixed in the glass gas reservoir by an impeller, operating at 2000 rev. min^{-1} , which had two 2 cm wide 'swing-out' paddles and was sealed into the reservoir with O-rings. The 5 l reservoir used for most experiments represented about 90 % of the volume of the entire apparatus; the initial concentration of CO_2 was, therefore, about 0.08 % by volume (i.e. about 2.4 times the background concentration). This level of CO_2 was considered to be insufficient to cause aberrant metabolism of either host or parasite. Normally 500 μCi $^{14}\text{CO}_2$ was used per experiment. The gas mixture was circulated through the apparatus, excluding the plant chamber, until equilibrium, i.e. a constant reading on the rate-meter attached to the Geiger-Müller tube, was reached (normally after 10 to 15 min at a pumping rate of 1.5 l min^{-1} at a pressure of 0.30 kg cm^{-2}). Attached inflorescences ('ears'; about 8) were washed with water (except when 'stale' honeydew was being investigated) and sealed into a perspex plant chamber of rectangular section which was illuminated bilaterally using a daylight source of approximate intensity 1×10^5 lux (Philips MBRF/U; 700 W) on one side of the chamber and a mirror on the other. Gas was then circulated through the plant chamber. Honeydew from infected florets was sampled using a hypodermic needle pushed through the Suba-Seal cap on the port in the plant chamber.

Excised inflorescences with their stems in water were supplied with $^{14}\text{CO}_2$ in a modified apparatus in which the plant chamber was round in section and permitted the use of up to 25 ears. Undue misting was prevented by an additional impeller directly beneath the water vessel containing the ears. This horizontal fan impeller with a magnetic flea in the base of the spindle had sixteen 1 cm wide blades. The base of the plant chamber was sealed with a greased glass plate and placed on a magnetic stirrer to rotate the impeller.

The internal temperature of the apparatus did not rise above 30 °C in any experiment.

Honeydew collection. Honeydew appeared on the florets about 10 days after inoculation of the plant and was collected at intervals with a hypodermic syringe. Freshly exuded honeydew was obtained by washing plants free from old honeydew and collecting the subsequent exudate. Spores were removed by centrifuging the honeydew which in some cases was dialysed overnight at 4 °C against at least 2000 vols 0.05 M-sodium phosphate buffer pH 6.8. The dialysed material was either used immediately or freeze-dried.

Sampling and extraction of ergots. At intervals during $^{14}\text{CO}_2$ feeding, the plant chamber

was isolated by the three-way taps, and some ears were removed from the chamber, washed briefly in distilled water and dropped into liquid nitrogen. The chamber was then resealed and $^{14}\text{CO}_2$ supply was continued. Uninfected ovaries and developing ergots were dissected from frozen ears and extracted separately for acid-soluble material and soluble carbohydrates by the methods described by Corbett *et al.* (1974). Occasionally, tissues were not extracted directly but were blended for 1 min in a small volume of water (approximately twice the wet weight of the tissues) in a vortex mixer. The resultant slurry was freeze-dried and the residue was stored at -20°C under vacuum. For analysis of non-polar materials (i.e. carbohydrates), residues were reconstituted in water and deionized as previously described (Dickerson & Mawer, 1974).

Because of the difficulty of precisely standardizing plants and experimental conditions, absolute levels of compounds investigated and the levels of ^{14}C incorporated into the parasite varied by up to 30 % between experiments. However, variations of the ratio of incorporation of ^{14}C into the individual metabolites assayed did not exceed 10 %. The results presented are for experiments where relatively large amounts of fungal tissue were available, thus enabling the most accurate determinations of specific radioactivity to be carried out.

Incubation of ergots. Ergots (2 to 4-weeks-old; from strain 29/4 growing on wheat) and sections of ergots were briefly washed in sterile 2 mM-sodium phosphate buffer pH 6.8. Normally tissue (300 to 400 mg ml $^{-1}$) was then incubated in the same buffer containing 15 % (w/v) [$\text{U-}^{14}\text{C}$]sucrose or [$\text{U-}^{14}\text{C}$]glucose (2 to 20 μCi). Incubation was carried out in a lightly-stoppered, shaken tube maintained at 25°C . At intervals, samples (10 to 20 μl) were removed from the incubation medium for analysis. Results are given as the mean of three replicates; the overall variation did not exceed 10 %.

Extraction of enzymes from whole ergots. Ergots grown on wheat in the glasshouse were harvested 4 weeks after inoculation and suspended overnight at 25°C in 2 mM-sodium phosphate buffer pH 6.8 (40 g fresh wt/100 ml buffer). They were then extracted for 5 h at 25°C by grinding with glass in a pestle and mortar. The resultant slurry was cooled in ice for 1 h and centrifuged at 4°C and 17000 g for 30 min. The supernatant was freeze-dried and yielded 5.1 g residue which was stored under vacuum at -20°C . Before incubation, the freeze-dried residue was reconstituted in 50 mM-sodium phosphate buffer pH 6.8, and dialysed overnight at 4°C against the same buffer. The soluble protein concentration of the dialysed solution, measured at 280 nm after clearing by centrifuging at 20000 g, indicated that the freeze-dried residue contained 11 % (w/w) protein.

Enzyme incubation. [$\text{U-}^{14}\text{C}$]glucose (0.1 M) and [$\text{U-}^{14}\text{C}$]sucrose (0.044 M) were incubated at 25°C with the above enzyme extract; the final concentration of soluble protein in the incubation mixture was 0.26 mg ml $^{-1}$. For polyol synthesis, the incubation mixture also contained 0.02 M-NADPH, 0.01 M-ATP and 0.02 M-MgCl $_2$. 6H $_2$ O. Samples were removed for analysis at intervals over a period of 5 h. Results are given as the mean of three replicates; the overall variation did not exceed 5 %.

Carbohydrate estimation. Carbohydrates were identified and estimated as described previously (Corbett, Dickerson & Mantle, 1974, 1975).

Chromatography. The methods used were essentially those described by Corbett *et al.* (1974, 1975). Estimations by gas-liquid chromatography were carried out on a Pye 104 chromatograph with a 3 % SE 52 column programmed at 130°C for 4 min, then rising by 6°C min^{-1} to 250°C and a final run of 15 min isothermally. Silyl derivatives of carbohydrates were prepared as before and identification was carried out by reference to markers using both retention time and temperature of emergence from the column.

Assay of radioactivity. This was carried out as described by Corbett *et al.* (1975) using a

Fig. 2. Levels of ^{14}C in sugars in honeydew exuded by rye plants, parasitized by *Claviceps purpurea*, during a period of $^{14}\text{CO}_2$ supply to the plant. Sugars in the honeydew were separated by chromatography and assayed for radioactivity. The radioactivity in individual sugars in each sample is expressed as a percentage of the total radioactivity for all the sugars determined for that sample. $\circ$, Sucrose; Δ , fructose; $\blacktriangle$, glucose; $\blacksquare$, oligosaccharides.

Beckman LS 200B counter. In the apparatus used to supply $^{14}\text{CO}_2$ to plants, radioactivity was monitored with a model E Mini-instruments monitor (type 5.10); the Geiger-Müller tube (Mullard MX 123) was modified so that only the face of the tube was enclosed in the apparatus. The efficiency of the tube when the apparatus contained 0.5 mCi of thoroughly mixed gas was 2 % and, as the efficiency did not exceed 3 % even at low isotope levels, radioactivities are recorded without correction.

RESULTS

In a typical experiment over 90 % of the CO_2 supplied to infected ears had been assimilated after 5 h. Monitoring showed that nearly all the ^{14}C supplied was retained in the ear. Rates of assimilation were the same irrespective of whether ears were still attached to the plant or isolated standing in water; metabolism (see below) with respect to both host and parasite was also similar. Evans *et al.* (1972) found that if CO_2 was supplied to the flag leaf of the plant alone, instead of the ears, only a small proportion of the ^{14}C appeared in the ear; in our experiments, the amount of ^{14}C translocated to the ears was less than 10 % of that supplied to the flag leaf.

Directly after a period (normally 5 h) of $^{14}\text{CO}_2$ feeding, the level of ^{14}C in the developing ergot was found to be between 4 and 17 times higher (on a tissue freeze-dried weight basis) than that in the uninfected, developing seeds in the same ear. The difference tended to be lower as the ergot approached maturity. Considerable variation also occurred depending on the relative positions of the parasite and developing seed within the ear. Dissemination of ^{14}C into metabolites was greater in the fungal tissues which showed high levels in many compounds after 5 h. In contrast, in most of the uninfected seeds 90 % or more of the ^{14}C was in sucrose.

Samples of honeydew that exuded from the ears during CO_2 assimilation were removed at intervals and the sugars in the exudate were assayed for radioactivity. Sucrose was the first

Table 1. *Specific activity of carbohydrates in honeydew removed from the inflorescence of rye during a period of $^{14}\text{CO}_2$ supply*

Carbohydrate	Fresh* honeydew		Stale† honeydew	
	Concn (mg ml ⁻¹)	Specific radioactivity at 1 h ($\mu\text{Ci mg}^{-1}$)	Concn (mg ml ⁻¹)	Specific radioactivity at 1 h ($\mu\text{Ci mg}^{-1}$)
Fructose	50	0.05	486	0.008
Glucose	26	0.14	278	0.022
Sucrose	380	0.25	3	29.3
Oligosaccharides	—	—	1.5	1.3

* Freshly exuded honeydew; equivalent to honeydew at its first appearance on the floret following infection, i.e. 10 to 11 days.

† Honeydew equivalent to that found 6 days after its first appearance, i.e. 16 to 17 days after infection.

compound to become labelled (Fig. 2). Glucose, fructose and oligosaccharides subsequently became labelled in a manner consistent with their formation by the action of the fungal sucrase. The structures of the oligosaccharides were the same as those reported previously for *C. purpurea* (Dickerson, 1972).

Freshly exuded honeydew, especially material that had only been in brief contact with the infected ovary and was uncontaminated with fungal spores, contained up to 40 % (w/v) of free sugar, mostly (90 to 100 %) sucrose (see below). The specific radioactivities of the various sugars in honeydew collected when $^{14}\text{CO}_2$ was being assimilated were more similar than those in staler honeydew. The staler honeydew occurred on ears that were not washed before supplying $^{14}\text{CO}_2$ and thus were contaminated with sphacelial conidia and had been in contact with mycelia. Consequently, sucrase activity of the fungal tissues led to higher proportions of monosaccharide and a relatively lower sucrose content (Table 1).

At intervals during and after supplying the plants with $^{14}\text{CO}_2$, three or four infected ears were removed from the apparatus, and the ergots were dissected out, divided transversely into three equal portions and extracted. Soluble sugars and polyols in the deionized extracts were assayed and their ^{14}C levels were determined (Table 2). Even if all the CO_2 assimilated during a single experiment was converted to carbohydrate, the total carbohydrate level in all the ears together would be no more than about 3 mg higher. Therefore the differences in the weights of the carbohydrates observed at any one sampling time were due to the normal distribution within the ergot.

On the 18th day after inoculation, mannitol, fructose and trehalose showed the highest levels in the ergots. Mannitol was fairly uniformly distributed, fructose accumulated towards the apex and trehalose towards the base of the ergot. Glucose showed apical accumulation but arabinitol and sucrose were uniformly distributed. At the 25th day after inoculation, ergots showed fairly high levels of mannitol and trehalose, both of which accumulated towards the base. In these ergots, other sugars and arabinitol were uniformly low.

The increases in the specific radioactivities of the sugars were always greatest towards the base of the ergot. The younger ergots showed the greatest increases. In most cases, the specific activities of glucose, fructose and mannitol were similar at 1 h but by 5 h that of mannitol was substantially greater than the others. The high specific activity of sucrose in a few ergots was possibly due to some sucrose being carried over on the outside of the ergot despite washing before extraction.

When isolated ergots bisected transversely were incubated in media containing 15 % (w/v) sucrose (this level was used as it is frequently found in the parasitic condition in honeydew),

Table 2. *Incorporation of ^{14}C into various carbohydrates of ergots following supply of $^{14}\text{CO}_2$ to the plant they were parasitizing*

Ergots were divided transversely into three equal portions; soluble sugars and polyols in extracts of these portions were assayed and the ^{14}C contents were determined (see Methods).

	Top segment			Middle segment			Bottom segment		
	$10^3 \times$ ^{14}C content (μCi^*)	Concn (mg *)	$10^3 \times$ Specific radioactivity ($\mu\text{Ci mg}^{-1}$)	$10^3 \times$ ^{14}C content (μCi^*)	Concn (mg *)	$10^3 \times$ Specific radioactivity ($\mu\text{Ci mg}^{-1}$)	$10^3 \times$ ^{14}C content (μCi^*)	Concn (mg *)	$10^3 \times$ Specific radioactivity ($\mu\text{Ci mg}^{-1}$)
Ergots analysed 18 days after inoculation of plants									
1 h sample									
Arabinitol	0.6	0.8	7.1	0.2	0.6	3.0	4.1	0.9	45.0
Mannitol	3.2	15.3	2.1	22.3	7.9	28.0	203.4	14.0	145.0
Fructose	0.5	16.6	3.1	4.7	1.9	25.0	46.9	2.1	223.0
Glucose	0.7	2.7	2.4	2.4	1.0	24.0	24.4	1.2	203.0
Sucrose	0.8	0.5	16.7	0.8	0.3	23.1	4.9	0.3	153.0
Trehalose	1.4	3.2	4.3	7.2	10.0	7.2	70.4	11.9	59.3
5 h sample									
Arabinitol	0.1	0.9	1.3	1.0	0.8	12.0	12.8	0.6	213.0
Mannitol	44.9	10.7	42.0	298.3	7.7	387.0	2148.6	13.0	1653.0
Fructose	3.3	13.8	2.4	20.3	2.0	102.0	170.2	2.0	851.0
Glucose	3.7	3.2	12.0	6.4	0.9	71.0	64.4	1.1	586.0
Sucrose	0.7	0.4	16.6	2.8	0.3	98.1	16.5	0.4	381.0
Trehalose	17.8	3.2	55.2	79.4	12.8	61.9	741.7	15.9	466.0
Ergots analysed 25 days after inoculation of plants									
1 h sample									
Arabinitol	0.1	0.3	3.3	0.1	0.8	0.9	0.1	0.3	3.0
Mannitol	0.9	2.9	3.1	1.6	5.9	2.7	28.9	7.9	37.0
Fructose	0.2	1.2	1.5	0.4	1.2	3.4	3.4	0.7	49.0
Glucose	0.1	0.6	1.4	0.2	0.3	5.0	1.4	0.4	35.0
Sucrose	0.4	0.3	11.5	0.5	0.04	140.0	0.6	0.1	80.0
Trehalose	6.6	5.4	12.3	1.6	9.7	1.7	9.8	11.2	8.7
5 h sample									
Arabinitol	0.03	0.2	1.6	0.04	0.7	0.6	1.2	0.2	60.0
Mannitol	0.8	1.8	4.6	20.5	4.5	46.0	458.8	7.3	629.0
Fructose	0.5	1.6	2.9	0.9	1.0	9.0	23.1	1.3	178.0
Glucose	0.6	0.4	15.0	0.3	0.3	8.0	6.7	0.4	168.0
Sucrose	0.4	0.2	24.7	0.6	0.1	85.7	2.9	0.1	317.0
Trehalose	0.4	3.1	1.4	3.2	9.5	3.4	117.7	11.3	104.5

* Per 100 mg freeze-dried weight of tissue.

Table 3. *Synthesis of oligosaccharides by segments of ergots*

Ergots (strain 29/4; 20-days-old) from wheat were washed in buffer (2 mM-sodium phosphate pH 6.8), bisected transversely and the apical and basal halves were incubated separately in lightly-stoppered, shaken tubes at 25 °C. The incubating medium (1 ml per 300 to 400 mg tissue) contained 15 % (w/v) [^{14}C]sucrose (2 $\mu\text{Ci ml}^{-1}$) in the same buffer. Total oligosaccharide content is expressed as a percentage of the total carbohydrates in the incubation medium as determined by ^{14}C content.

Sampling time (h)	Total oligosaccharide content (% w/v)	
	Apical half	Basal half
0	0.6	0.5
$\frac{1}{2}$	2.9	1.6
1	5.2	2.8
2	13.2	5.1
5	25.7	6.6

Table 4. *Synthesis of oligosaccharides by cell-free extracts of ergots in the presence or absence of added monosaccharides*

The enzyme extract, prepared as described in Methods, was incubated in shaken tubes at 25 °C with [^{14}C]sucrose (10 $\mu\text{Ci ml}^{-1}$; final concentration 0.044 M) in the presence or absence of a mixture of glucose and fructose both equimolar to the sucrose. Results are expressed as in Table 3.

Sampling time (min)	Incubated with ...	Total oligosaccharide content (% w/v)	
		Sucrose alone	Sucrose and monosaccharides
0		0.3	0.2
5		0.9	0.8
10		1.3	1.1
20		2.2	1.7
40		4.0	2.9
80		6.5	4.7
120		8.4	6.0

the rate of sucrose breakdown by both the apical and basal segments was the same, $0.22 \mu\text{mol h}^{-1}$ (mg fresh wt tissue) $^{-1}$. However, the oligosaccharides formed a larger proportion of the products in the top half of the ergot (Table 3). After 5 h both halves of the ergot had assimilated 5 to 7 % of the hexose liberated, mostly as glucose (this value also includes hexose fermented during the same period; see Nisbet, 1975). Similarly, segments of ergots incubated with 15 % (w/v) glucose instead of sucrose assimilated up to 10 %. The assimilated hexose gave rise to mannitol in the tissues, as previously noted during stromatal incubation (Corbett *et al.*, 1975).

When a partially-purified extract of whole ergots was incubated with 1.5 % (w/v) sucrose, the levels of products were essentially the same as those obtained by incubating the basal segments of whole ergots. In the presence of a mixture containing glucose and fructose, both equimolar to the sucrose, the rate of sucrose breakdown was slightly depressed [0.74 to $0.85 \mu\text{mol min}^{-1}$ (mg protein) $^{-1}$] and even lower levels of oligosaccharide were obtained (Table 4).

Suppression of oligosaccharide formation was also observed when honeydew, which contains some extracellular sucrase (Dickerson, 1972), was incubated with [^{14}C]sucrose (final concentration 10 %, w/v). Removal of free hexose by dialysis of honeydew before addition of sucrose resulted in nearly nine times as much oligosaccharide being formed

Table 5. *Action of enzymes in honeydew from rye on sucrose*

Honeydew from rye infected with *C. purpurea* (strain 29/4), collected 17 days after infection, was incubated with 10 % (w/v) [U-¹⁴C]sucrose (10 μ Ci ml⁻¹) at 25 °C. Honeydew that had been dialysed (see Methods) was incubated under similar conditions. Results are expressed as μ mol labelled product (ml incubation medium)⁻¹ (mg protein)⁻¹; protein concentrations of honeydew, as measured at 280 nm, were 3.4 mg ml⁻¹ before dialysis and 2.7 mg ml⁻¹ after dialysis.

Sampling time (min)	Products of incubation with undialysed honeydew			Products of incubation with dialysed honeydew		
	Fructose	Glucose	Oligosaccharide	Fructose	Glucose	Oligosaccharide
10	9.0	14.2	1.3	7.1	10.2	3.0
20	13.4	20.5	1.6	11.8	16.3	5.1
40	21.6	30.9	2.4	19.0	27.3	8.7
80	36.8	46.5	3.0	24.4	41.6	13.1
300	57.1	65.8	3.2	60.3	85.0	27.8

Table 6. *Synthesis of mannitol by cell-free extracts of ergots*

The enzyme extract, prepared as described in Methods, was incubated in shaken tubes at 25 °C with 0.1 M-[U-¹⁴C]glucose (20 μ Ci ml⁻¹), 0.02 M-NADPH, 0.02 M-MgCl₂.6H₂O and 0.01 M-ATP. Results are expressed as μ mol hexose or hexose equivalent (ml incubation medium)⁻¹.

Sampling time (min)	Glucose	Sugar phosphate	Mannitol
0	100	0	0
10	95.7	3.6	0.6
20	94.3	4.8	0.9
40	92.7	6.0	1.3
80	92.0	6.2	1.8
120	91.8	6.0	2.2
300	91.5	5.8	2.8

(Table 5). With honeydew collected on the first day after its appearance on the inflorescences (Table 1), oligosaccharide formation was also suppressed [the corresponding 5 h values being 8.3 and 13.2 μ mol ml⁻¹ (mg protein)⁻¹ for the undialysed and dialysed honeydew respectively]. Wheat honeydew gave similar results.

Incubation of the enzyme extract with 0.1 M-glucose in the presence of NADPH, ATP and Mg²⁺ ions yielded mannitol and sugar phosphate. With a limiting concentration of ATP (0.01 M) a nearly theoretical 10 % conversion of glucose to sugar phosphate plus mannitol was approached (Table 6). When either NADPH or ATP was omitted from the incubation mixture no polyol synthesis occurred.

DISCUSSION

Infected ears of rye or wheat, when supplied with CO₂ in the enclosed system, assimilated the gas down to a compensation level. Measurements of radioactivity showed that most of the carbon fixed appeared in fungal tissue rather than in uninfected developing seeds in the same ear. Directional flow of metabolites from plants specifically to fungal pathogens has been well known since the early work of Yarwood & Jacobsen (1955) and is established for rusts, smuts and powdery mildews (Smith, Muscatine & Lewis, 1969); it also accounts for the fact that seeds from ears of cereals infected by ergot fungi have a lower mean dry weight than those from uninfected ears (Corbett *et al.*, 1974).

As expected, young developing sclerotia (18 days after inoculation of the plant) were more active metabolically than 25-day-old ergots. The basal part of the ergot appeared to be more active than the apex; but studies on isolated ergots and enzyme extracts of ergots suggest that, with respect to sucrase, this was largely a result of the host's nutrient supply being more available to the basal region. The absence of a differentiated translocation system and the plectenchymatic nature of the fungal tissue (Mantle, 1972) might impede the flow of material passing up the ergot, thus causing an accumulation around the base and a higher rate of metabolism in this area.

Carbon fixed by the plant from CO₂ reaches the ergot mainly as sucrose. Sucrase (Dickerson, 1972; Mower & Hancock, 1975) provides a supply of free hexose which the parasite can assimilate. Differences in the levels of oligosaccharide formed on incubating washed ergots suggest that the mode of action of the enzyme is modified by its position within the ergot. Evidence from the cell-free and honeydew incubations showed that the presence of an increased level of monosaccharide suppressed oligosaccharide formation; as most of the sucrase is cell-bound (Dickerson, 1972), its activity might be affected by the distribution of metabolites. It would appear that such metabolites need not always be carbohydrates, since only trehalose shows consistent polarization of levels within the ergot. In common with other fungi, particularly fungal pathogens of plants (Smith *et al.*, 1969; Lewis, 1974), fixed carbon – in this case in hexose – is converted within the fungal tissues to a number of products, chiefly mannitol and, to a lesser extent, trehalose. This is also similar to the 'Nostoc pattern' in lichens, symbionts which show similarities to ergot-type parasitism, reported by Richardson, Smith & Lewis (1967). The pathway to mannitol involves phosphorylated intermediates. It is likely that the pathway would include the mediation of glucose 6-phosphate and fructose 6-phosphate as in that proposed for *C. purpurea* by Cooke & Mitchell (1970).

Particularly during the later stages of the experiments in which ¹⁴CO₂ was supplied to infected plants, the specific radioactivity of mannitol usually exceeded that of the hexoses. Thus, assuming hexose is the precursor for most of the mannitol, the mannitol would need to be derived from only part of the hexose in the tissues; this hexose would have to be separated from the rest and have a higher specific activity probably arising from a greater rate of turnover. Furthermore, enrichment of the specific activity of mannitol over that of the hexose from which it is derived could be achieved by the reactions of the pentose phosphate pathway (Mitchell & Shaw, 1968; Holligan & Jennings, 1972), especially if there is unequal isotopic labelling of the carbon atoms of the hexose.

We thank the Science Research Council for the award of a Research Studentship to L. J. N.

REFERENCES

- COOKE, R. C. & MITCHELL, D. T. (1970). Carbohydrate physiology of sclerotia of *Claviceps purpurea* during dormancy and germination. *Transactions of the British Mycological Society* **54**, 93–99.
- CORBETT, K., DICKERSON, A. G. & MANTLE, P. G. (1974). Metabolic studies on *Claviceps purpurea* during parasitic development on rye. *Journal of General Microbiology* **84**, 39–58.
- CORBETT, K., DICKERSON, A. G. & MANTLE, P. G. (1975). Metabolism of the germinating sclerotium of *Claviceps purpurea*. *Journal of General Microbiology* **90**, 55–68.
- DICKERSON, A. G. (1972). A β -D-fructofuranosidase from *Claviceps purpurea*. *Biochemical Journal* **129**, 263–272.
- DICKERSON, A. G. & EDELMAN, J. (1966). The metabolism of fructose polymers in plants. *Journal of Experimental Botany* **17**, 612–619.
- DICKERSON, A. G. & MAWER, J. (1974). Purification of inulobiose obtained by acid hydrolysis of inulin. *Carbohydrate Research* **39**, 162–163.

- EVANS, L. T., BINGHAM, J., JACKSON, P. & SUTHERLAND, J. (1972). Effect of awns and drought on the supply of photosynthate and its distribution within wheat ears. *Annals of Applied Biology* **70**, 67-76.
- HOLLIGAN, P. M. & JENNINGS, D. H. (1972). Unexpected labelling patterns from radioactive sugars fed to plants containing mannitol. *Phytochemistry* **11**, 3447-3451.
- LEWIS, D. H. (1974). Micro-organisms and plants: the evolution of parasitism and mutualism. *Symposia of the Society for General Microbiology* **24**, 367-392.
- MANTLE, P. G. (1972). An unusual parasitic association between *Claviceps purpurea* and rye. *Transactions of the British Mycological Society* **59**, 327-330.
- MITCHELL, D. & SHAW, M. (1968). Metabolism of glucose-¹⁴C, pyruvate-¹⁴C and mannitol-¹⁴C by *Melampsora lini*. II. Conversion to soluble products. *Canadian Journal of Botany* **46**, 453-460.
- MOWER, R. L. & HANCOCK, J. G. (1975). Mechanism of honeydew formation by *Claviceps* species. *Canadian Journal of Botany* **53**, 2826-2834.
- NISBET, L. J. (1975). *Differentiation of Claviceps purpurea during parasitic and axenic culture*. Ph.D. thesis, University of London.
- RICHARDSON, D. H. S., SMITH, D. C. & LEWIS, D. H. (1967). Carbohydrate movement between the symbionts of lichens. *Nature, London* **214**, 879-882.
- SMITH, D., MUSCATINE, L. & LEWIS, D. (1969). Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. *Biological Reviews* **44**, 17-90.
- YARWOOD, C. E. & JACOBSEN, L. (1955). Accumulation of chemicals in diseased areas of leaves. *Phytopathology* **45**, 43-48.