

Carbohydrate Metabolism in Higher Plant Tissues Infected With Obligate Parasites^{1, 2}

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Introduction

Several reports (7, 8, 22) have described apparently fundamental changes in the rates and nature of respiratory pathways during infection of higher plant tissues by obligate parasites, particularly rust fungi. Although disease can cause increases of 400% in rates with a marked lowering of C_6/C_1 ratios, there is considerable uncertainty about the carbohydrate changes which accompany the respiratory alteration. Allen (1) first reported increases in sucrose and hexoses in wheat infected by powdery mildew. Gerwitz and Durbin (10) have described increases in both reducing and non-reducing carbohydrates in wheat infected by rust fungi while others (5, 15, 16) have reported marked decreases in both types of compounds. Shaw and Samborski (22) found increases at 3 and 7 days after infection of wheat with rust fungi, but at 5 and 10 days the diseased tissue contained less sucrose and hexose than healthy controls.

Inman (13) has pointed out that the amount of infection and the stage of parasite development are two factors which are important in such studies. With heavy infection of the bean rust fungus, the levels of reducing and non-reducing carbohydrates increased by 50 and 300%, respectively, just prior to sporulation. Subsequently, and within 72 hours, the levels fell to 50% of control tissue. With progressively fewer infections per unit of leaf area the changes were neither as great nor as abrupt in time. In cases of light infection the concentrations of soluble carbohydrate did not fall below the levels in control plants.

It is of interest that, despite the consistently low C_6/C_1 ratios in conjunction with high rates of respiration, only the usual hexoses and sucrose have been positively identified in detectable amounts in diseased tissue.

Failure to observe changes in concentrations in carbohydrates, especially pentoses or heptuloses, postulated to arise by reactions of an oxidative pentose pathway, is not completely unexpected because of possible kinetic difficulties (i.e. small pool sizes). It is also possible that intermediates of glucose catabolism via shunt metabolism may be sequestered as phosphate derivatives and thus not readily identified by usual chromatographic analysis.

Similar failure to detect changes in sugars have been reported for tissues in which low C_6/C_1 ratios have been induced by other means (2, 20).

In instances of disease-induced changes in C_6/C_1 ratios, there is circumstantial evidence that an oxidative pentose pathway is involved. It has been shown that low C_6/C_1 ratios occurred in the infected areas of diseased tissues, not in adjacent uninvaded tissue. The germinating spores of the parasite show low C_6/C_1 ratios and all of the enzymes of the oxidative pentose pathway are present with the possible exception of transaldolase (23).

There are two reports of alterations in carbohydrates that appear to be related to respiratory changes due to infection by obligate parasites. Jain and Pelletier (14) reported increased labelling in sedoheptulose when plants with low levels of infection metabolized $C^{14}O_2$ in the light. Wang (26) reported similar results and found that this change occurred in uninfected host cells of diseased leaves. In view of the association of respiratory changes with invaded tissue and the complicating role of light in these experiments, the increased synthesis of sedoheptulose by a pentose pathway in diseased tissue is not established.

Furthermore, it is to be noted that in both papers the evidence presented for the occurrence of sedoheptulose apparently is based chiefly on the similarities of R_f values of a radioactive spot with known compounds, usually in one dimensional chromatograms. Other workers have not mentioned the presence of sedoheptulose in rusted tissue (10, 15, 22) and separation of sedoheptulose from fructose sometimes is difficult (3). A similar criticism can be advanced for Wang's admittedly tentative identification of mannose (26), also not indicated to be present in other studies, as a major component of diseased tissue.

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In view of the possible bearing that levels of infection (13) may have in correlating respiratory and carbohydrate changes, the present study was undertaken with heavy infections of safflower and bean rust fungi. Preliminary data (13) with radioactive tracers indicated that the carbohydrate-containing fractions of rusted bean tissue contained at least one unreported additional compound with R_f value suggestive of a pentose. The experiments to be described were done in an attempt to relate products of glucose metabolism to respiratory changes involving preferential release of C_1 carbon during disease development in safflower.

Materials & Methods

Safflower (*Carthamus tinctorius* L.) and bean (*Phaseolus vulgaris* L. var. Great Northern 59) were grown under controlled environmental conditions described previously (7,8). Infection of safflower hypocotyl by the rust fungus, *Puccinia carthami* L., was accomplished by dusting germinating seed with a mixture of teliospores and talc before planting. Primary leaves of bean were dusted with uredospores of *Uromyces phaseoli typica* L. before placing plants in a humidity chamber overnight.

Healthy and diseased safflower hypocotyl tissues were collected after sporulation was initiated and cut into sections of 2 to 3 mm. After washing and blotting dry, the weighed tissue samples were incubated in vessels (7) in the dark at 21 C in solutions of 0.1 M KH_2PO_4 (pH 4.6) containing radioactive glucose (usually 1 mg/ml). Details of individual experiments are given in the section on results. For each gram of safflower tissue 2 ml of liquid were employed. Because of problems with clumping of bean leaf tissue, a ratio of 1 g of bean tissue per 10 ml of solution was used in petri plates. Sections of bean tissue, 25 mm², were most convenient for this study.

► Preparation of Extracts. After suitable time periods the tissue was removed from the bathing medium by filtering through four layers of cheesecloth and washed thoroughly to remove excess radioactive glucose. It then was blended with 50 ml of 80% ethyl alcohol, filtered through a Buchner funnel and the extracts (100 ml) stored at -10 C.

Extracts were passed slowly through a 10 × 0.8 cm column of Dowex-1X (200-400 mesh) in the formate form. This was followed by slow passage over a 20 × 1 cm column of indicating mix-bed resin (Cal-Biochem Ag501-x8). Effectiveness of ion removal was checked by visual change in indicator color. The column was rinsed with approximately 30 ml of 80% ethyl alcohol. Fresh columns were used for each sample.

The extracts were reduced, with occasional additions of water, in a rotary evaporator under vacuum at temperatures no greater than 40 C until approximately 15 ml of solution remained. This material was extracted in a separatory funnel three times with approximately equal volumes of either anhydrous

ethyl ether or hexane. The non-polar solvent was washed twice with distilled water and the washings combined with the extract (approximately 50 ml). In most instances the clear, colorless extract was passed again over indicating mix-bed resins with appropriate water washes.

The neutral, lipid-free extracts were reduced as before to approximately two to three milliliters, and then made up to standard volumes (usually 5 ml). In the experiment of table II, the extracts were lyophilized and made to 1 ml.

► Paper Chromatography. Since the original weights and final volumes were known, it was possible to transfer for chromatography aliquots representing known fresh weights of tissue. To prevent overloading, it was necessary to restrict aliquots to amounts representing less than 150 mg of tissue fresh weight, applied by multispotting 2 μ l volumes to Whatman No. 1 paper. A number of solvent systems was tested for separation of a standard reference mixture of sucrose, glucose, fructose, xylose, and ribose. Of each sugar, 25 or 50 μ g were employed. Each chromatogram usually consisted of individual spots of reference compounds, extract, and extracts over-spotted with reference compounds for comparisons.

The most satisfactory resolution of the unknowns present in extracts was obtained with *n*-butyl alcohol-ethanol-water (BEW) and ethyl acetate-acetic acid-water (EAW) in volumetric proportions of 52:32:16 and 3:1:3, respectively. It is to be emphasized, however, that single or multiple passes of solvent with either small (20 cm) or large (47 cm) distances from the origin to the paper edge were not sufficient to obtain suitable resolution. It was necessary to use descending chromatograms and permit solvent to drip from the serrated lower edge for 96 to 120 hours in order to demonstrate radioactive components in diseased tissue which were distinct from those in healthy tissue. Figure 1 shows such a separation of components in diseased safflower tissue after 106 hours of development in EAW. The total length of the chromatogram from origin to edge was 45 cm. Ribose migrated approximately 38 cm. Four passes of solvent would have resulted in movement from the origin of only 17 cm.

Location of known sugars was routinely determined by phloroglucinol or aniline hydrogen phthalate sprays (3). In most instances the paper was sprayed with aniline hydrogen phthalate to locate glucose and the pentoses and then sprayed with phloroglucinol to reveal sucrose, fructose, and other ketoses.

Location of radioactive components from one dimensional chromatography was accomplished with a Tracerlab chromatogram scanner. If good resolution was obtained, the spots were cut out and counted with a flow counter. For two dimensional chromatograms (45 × 45 cm) (fig 2), the entire paper was cut into half inch squares for counting with the gas flow system.

Results

Preliminary separation, with two passes in several solvents of extracts from healthy safflower tissue which had metabolized glucose- $U-C^{14}$ (0.5 mg/ml, 10^6 cpm/ml) for 4 hours indicated that healthy tissue contained fructose, glucose, sucrose, and several poorly resolved components near the origin which produced a yellow color with phloroglucinol but did not react with aniline hydrogen phthalate. Radioactivity was present only at the sucrose, glucose, and fructose areas. Chromatograms of similar amounts of extracts from diseased tissue showed the same components to be present but the smaller spot sizes indicated lower concentrations per unit of tissue. The distribution of radioactivity on chromatograms from diseased tissue was different from that from healthy tissue. The sucrose area was more highly radioactive, while the glucose and fructose areas were not satisfactorily delineated with respect to radioactivity. In addition, the area corresponding to xylose migration was highly radioactive. The results were similar to those of Wang (26). However, no color was developed at this region by phloroglucinol or aniline hydrogen phthalate sprays.

A radioactive tracing of the more effective method of descending drip chromatograph (fig 1) resulted in separating radioactivity of sucrose from that of a slower moving component (A) possessing most of the activity previously thought to be entirely in su-

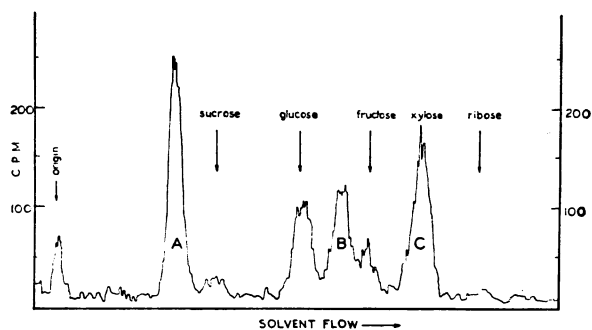


Fig. 1. Radioactive components present in infected safflower hypocotyls after exposure to uniformly labelled glucose. Arrows indicate positions of known sugars overspotted on extract. A, B, and C designate peaks of activity not observed in comparable healthy chromatograms.

crose. Similarly a second component (B) was observed between glucose and fructose, corresponding to the expected position for sedoheptulose (3). A third component (C) migrated nearly identically with xylose. A comparable healthy tissue extract showed appreciable radioactivity only in glucose and fructose with a small amount of radioactivity in sucrose. The three peaks A, B, and C did not react with ketose or aldose spray reagents. Four additional spots re-

acting with phloroglucinol and lying between the origin and unknown A (presumably ketoses such as raffinose, or similar homologs) did not contain detectable radioactivity and will not be discussed.

Figure 2 shows a two-dimensional chromatogram of extract representing 100 mg of rust-affected safflower tissue collected at a stage of development 3 days earlier than the tissue extracts of figure 1. In this instance arabinose, N-acetyl glucosamine and xylulose also were used as reference compounds but did not correspond to any of the unknowns.

In phenol, separation of C from xylose and xylulose⁴ was accomplished. Ribulose, located on the basis of R_f values supplied by Dr. W. A. Wood, did not correspond to B. The R_f of A was similar to maltose, but a comparison of the R_f values of maltose, trehalose, sucrose and the unknown with more prolonged (136 hr) development of one dimensional chromatograms in BEW, indicated maltose migrated more slowly than either unknown A or trehalose.

It was necessary to determine if any or all of the unknown compounds present in diseased, but not healthy tissue, arose by reactions involving removal of the C_1 carbon of glucose. Six gram samples of healthy and diseased tissue were incubated for 6 hours in 12 ml of 0.1 M KH_2PO_4 containing 1 mg/ml of glucose labelled either in the 1, 2, or 6 positions (specific activities 1.6, 0.72, and 1.3×10^6 cpm/mg). During the 3rd and 4th hours, the radioactivity of respired CO_2 was determined as described previously (7). The percentage recoveries are given in table I. The C_6/C_1 ratio for diseased tissue was much lower than that of healthy tissue and the C_2/C_6 ratio is in accord with the operation of a pentose pathway (4).

Aliquots representing 125 mg fresh weight were over-spotted with reference compounds and chromatographed in BEW for 90 hours. After the usual location of areas by color reactions or radioactivity, each area was cut into three or four segments and the time required for total of 10^4 counts determined for each segment. The data in table II are expressed

⁴ Samples kindly supplied by Drs. B. L. Horecker and W. A. Wood.

Table I
Percentage Recoveries* of Radioactivity
in Carbon Dioxide**

| | Percentages | | | |
|---|-------------|-------|-------|-----------|
| | C_1 | C_2 | C_6 | C_6/C_1 |
| H | 1.10 | 1.09 | 0.80 | 0.72 |
| D | 5.53 | 2.36 | 1.34 | 0.23 |

* CO_2 collected in 0.2 ml 10% KOH during the middle 2-hour period of a 6-hour exposure to glucose. Recoveries expressed as cpm per total cpm of supplied glucose $\times 100$.

** From healthy (H) and diseased (D) safflower hypocotyls supplied glucose labelled at the 1, 2, or 6 positions.

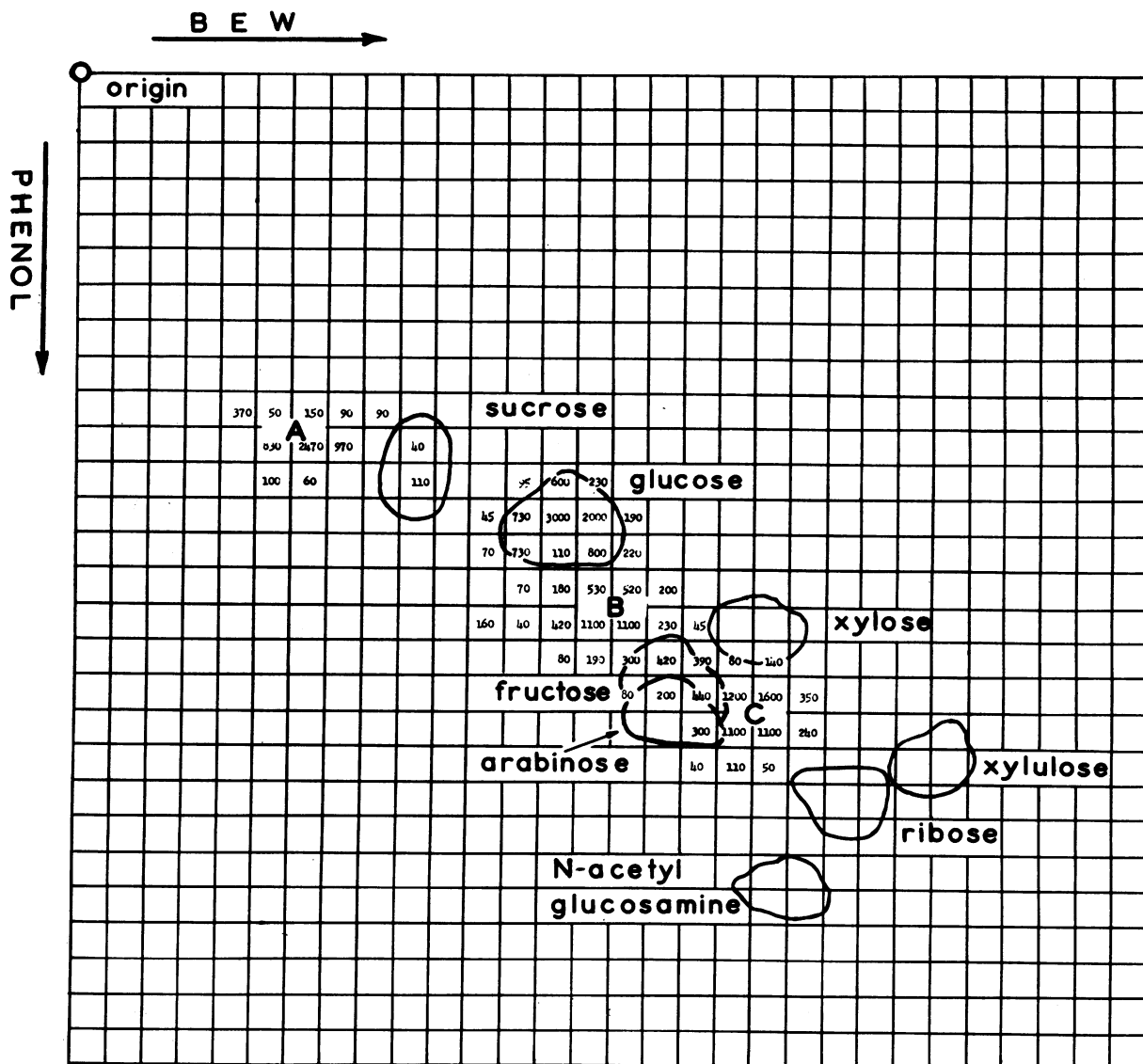


Fig. 2. Two dimensional separation of radioactive components from infected safflower hypocotyls. Note the unknown C of figure 1 does not correspond to known five carbon sugars.

as cpm for the entire area of each spot after correction for background and differences in initial specific activity of glucose.

There are several factors which handicap attempts to make meaningful comparisons of the absolute amounts of radioactivity from individual carbons into specific compounds present in healthy and diseased tissue: the different rates of sugar uptake and subsequent metabolism, the greater losses of certain carbon atoms via respiration (table I), randomization effects, siphoning of specific carbons into other metabolic cycles and recycling of carbon during a 6-hour exposure of tissue to glucose. A more immediately useful comparison is the distribution of label expressed as a percentage of the total on the chromatogram.

It is to be noted, however, that the percentages can be misinterpreted unless it is remembered that appreciable loss of radioactivity from one component will affect the percentage distribution among others. In both healthy and diseased tissue, only one of the four ketose compounds contained radioactivity. Since both the absolute levels and percentage are higher in healthy tissue, its synthesis in diseased tissue probably represents a continuation, at lower rates, of normal host metabolism.

In healthy tissue, exogenous glucose was accumulated to an appreciable extent and synthesis of sucrose occurred readily. There was detectable but low activity at the region of xylose and unknown C from all labelled carbons, while the region at unknown

Table II
Separation of Radioactive Neutral Components*

| Chromatogram area | Healthy | | | Diseased | | |
|-----------------------|-------------------|----------------|----------------|----------------|----------------|----------------|
| | C ₁ ** | C ₂ | C ₆ | C ₁ | C ₂ | C ₆ |
| Ketose | 130(1.2) | 90(1.0) | 80(1.0) | 35(0.2) | 45(0.2) | 45(0.3) |
| Unknown A | ... | ... | ... | 3,380(21.7) | 4,000(18.5) | 2,500(17.6) |
| Sucrose | 3,400(32.4) | 3,200(36.7) | 2,300(29.4) | 1,380(8.9) | 1,400(6.5) | 1,070(7.5) |
| Glucose | 6,300(59.6) | 5,100(56.8) | 5,000(64.0) | 7,730(49.4) | 9,700(44.9) | 6,900(48.6) |
| Unknown B | ... | ... | 50(0.6) | 1,030(6.6) | 1,550(7.2) | 680(4.8) |
| Fructose | 670(6.3) | 540(6.0) | 360(4.6) | 1,150(7.4) | 1,430(6.6) | 970(6.8) |
| Unknown C (xylose) | 60(0.6) | 40(0.4) | 20(0.3) | 750(4.8) | 3,400(15.7) | 2,000(14.1) |
| Ribose | ... | ... | ... | 70(0.4) | 90(0.4) | 40(0.3) |
| | 10,560(100) | 8,970(100) | 7,810(100) | 15,570(100) | 21,600(100) | 14,200(100) |

* Produced from glucose -1, -2 or -6C substrates in healthy and diseased safflower hypocotyls. Aliquots representing 125 mg of fresh tissue chromatogrammed in BEW for 96 hours. Data corrected for differences in initial specific activity of glucose and for background. Unbracketed figures are cpm. Parenthesis indicates percentage of total counts on each chromatogram.

** Sucrose, glucose, fructose, xylose, and ribose areas determined by over-spotting with reference compounds.

B was radioactive in extracts from C₆-labelled glucose but with a low percentage.

In diseased tissue, A was highly tagged but approximately in equal percentages from C₁, C₂, and C₆, suggesting that this material may be formed by direct incorporation of glucose into a disaccharide. The lower activity at the sucrose region, although higher than in other experiments (fig 2), suggests lower rates of synthesis in diseased plants than in healthy tissue. It is to be noted that incorporation into unknown B, which has an R_f similar to sedoheptulose is higher from C₁ and C₂ than from C₆.

The most significant difference in C₁, C₂, and C₆ incorporation for diseased tissue is in unknown C which had lower total activity and percentage from glucose-1-C¹⁴. This suggests that this compound arises from the metabolic system connected with the preferential release of C₁ as carbon dioxide. The R_f also is suggestive of a pentose material. At the present time it is not possible to explain the appearance of approximately five per cent of the total activity from C₁ in this region of the chromatogram. Several possibilities exist—there may be other components which co-chromatograph with C, randomization of label (as for example in an aldolase reaction), or reactions analogous to the glucuronic acid pathway (11).

Similar materials were found in bean leaves infected by rust fungi. Table III compares the radioactivity incorporated from glucose-U-C¹⁴ into components from 100 mg of tissue separated by two-dimensional chromatography. At the flecking (5 days after inoculation) and sporulation (8 days after inoculation) stages only sucrose, glucose, and fructose reacted with the spray reagents employed. It is to be noted that in this infection (136 pustules per cm²) some activity was found in the unknowns shortly before sporulation, but the activity was increased 3

days later at sporulation when C₆/C₁ ratios are low (7). The cpm given for unknown A at sporulation is approximately one-half the true value since this amount of radioactive area was inadvertently destroyed before counting. The levels of endogenous glucose were small and this may have been due to rapid conversion of exogenous glucose to sucrose in healthy tissues.

► Some Properties of the Unknowns. One difficulty encountered repeatedly was the absence of definite reactions with spray reagents commonly employed in chromatography of carbohydrates. Negative or uncertain results were obtained with aniline hydrogen phthalate, phloroglucinol, ammonium molybdate, ammoniacal silver nitrate, and orcinol sprays

Table III
Assay of Radioactivity (cpm) From
2-Dimensional Chromatograms*

| Compound | Flecking State | | Sporulation | |
|----------|----------------|-------|-------------|---------|
| | H | D | H | D |
| A | ... | 560 | ... | 1,300** |
| Sucrose | 1,600 | 1,350 | 1,000 | 2,450 |
| Glucose | 90 | 150 | ... | 80 |
| B | ... | 325 | ... | 1,300 |
| Fructose | ... | ... | ... | ... |
| C | ... | 300 | ... | 670 |
| Xylose | ... | ... | ... | ... |
| Ribose | ... | ... | ... | ... |
| Xyulose | ... | ... | ... | ... |

* Of extracts representing 100 mg of healthy (H) and diseased (D) bean leaves. Plants sampled after a 15 hour dark period 5 and 8 days after inoculation. 1 g of tissue placed in 10 ml 0.1 M KH₂PO₄ containing 1 mg/ml glucose-U-C¹⁴ (1.4 × 10⁶ cpm/mg) for 5 hours.

** Minimal value. Approximately one-half of radioactive area lost prior to counting.

(3). Permanganate periodate (3) produced a strong color with unknown C in some extracts, but in some instances a positive reaction with B and C was difficult to obtain because of the background color of the chromatogram paper.

Since it was possible that the amounts were too low for reaction, concentration of the unknowns was attempted by eluting the radioactive areas from chromatograms prepared by applying a thin band of extract and developing for approximately 100 hours in BEW. The extract was the same as for figure 1. Spraying spots representing a tenfold increase in concentration gave good reactions with permanganate periodate for all compounds and, in addition, there were faint colors produced with phloroglucinol and aniline hydrogen phthalate. However, the latter were probably the result of contamination of B with fructose and glucose and of A with sucrose (see fig 1).

Since A had a migration rate characteristic of an oligosaccharide, several tests with different preparations from band separations were carried out to determine component sugars. Hydrolysis in 1.5 N HCl for 2.5 hours at 100 C was followed by one dimensional chromatography. Approximately 93 % of the activity was obtained at the glucose area and 7 % remained at the origin (table IV).

Of known oligosaccharides, trehalose appeared to be most similar to A and a series of comparisons of the unknown, trehalose, and maltose was made. The R_f values in BEW and EAW were identical to trehalose as was the reaction to spray reagents. Both trehalose and the unknown produced strong color only with permanganate periodate. The time required for reaction with both compounds was much slower than for other disaccharides tested. According to Pigman and Goepf (18), trehalose is resistant to hydrolysis when compared with other oligosaccharides. A comparison of the ease of hydrolysis of A, trehalose, and maltose is shown in table IV.

In this experiment, preparations of knowns and A were mixed with equal volumes of 2 N HCl. Im-

mediately after mixing (0 hr) and at 0.5, 2.0, and 4 hours, samples of maltose and trehalose were analyzed for reducing sugars. An aliquot of A was placed on chromatogram paper and dried with warm air. After 0.5, 2, and 4 hours, additional aliquots were removed. The chromatogram was developed in BEW and checked by the strip scanner for radioactivity. The active areas were removed and counted with a flow counter.

This sample originally contained sucrose as a contaminant since even at zero hours amounts of glucose and fructose equivalent to 3 % sucrose were present. During hydrolysis the amount of glucose increased while fructose decreased. This is to be expected since it has been shown that fructose loss occurs during acid hydrolysis (25). Corrections for sucrose hydrolysis based on the amount of fructose present show (table IV) that the maximum hydrolysis of A was only 3.0 % in 4 hours. Actually it probably was less since fructose destruction would minimize the correction factor. By comparison, maltose was hydrolyzed to the extent of 36 % while no detectable breakdown of trehalose was found.

It was thought that perhaps unknowns B and C might correspond to sugar alcohols since they are frequently found both in higher plants and fungi. A comparison of the migration rates of the unknowns with mannitol, dulcitol, sorbitol, arabitol, ribitol, xylitol, and erythritol was made in BEW. The spots were located with permanganate-carbonate and brom cresol purple sprays for sugar alcohols. B migrated with the hexitols while C had migration rates similar to the pentitols arabitol or ribitol, but not xylitol.

Discussion

The data presented clearly indicate that following metabolism of added glucose, diseased tissues differ significantly from healthy tissues with respect to compounds present in neutral, lipid-extracted fractions. Although the evidence obtained is provisional, and definite proof must await isolation of sufficient quantities for chemical characterization, trehalose appears to be one major component synthesized. Reports of the occurrence of trehalose in higher plants are limited and conflicting, but its presence in fungi is well documented (11, 18). The occurrence of trehalose in diseased tissue is further evidence for a significant contribution of the parasite to metabolism of the host-parasite complex (8). Failure to detect trehalose previously may reside in the light infections employed, the relative lack of reactivity toward carbohydrate reagents used as sprays and the difficulty in obtaining good separations from accompanying sucrose and maltose. Krog et al. (15) using procedures involving 60 to 72 hours of irrigation mention the presence in wheat of materials migrating as di- or trisaccharides which did not react with specific sugar reagents and which were not analyzed. Whether these occurred in rusted or healthy tissues is not stated.

Table IV
Comparison of Rates of Hydrolysis of A,
Trehalose, & Maltose

| | Distribution of radioactivity | | | | |
|---------------|-------------------------------|--------|------|------|--------------------|
| | 1 N HCl at 66 C | | | | 1.5 N HCl at 100 C |
| | 0 | 0.5 hr | 2 hr | 4 hr | 2.5 hr |
| A | 96.7 | 95.5 | 95.0 | 92.2 | 7 |
| Glucose | 1.7 | 3.3 | 4.0 | 6.8 | 93 |
| Fructose | 1.5 | 1.2 | 0.9 | 0.9 | Not detected |
| | % Hydrolysis | | | | |
| A (Corrected) | 0.1 | 1.1 | 1.6 | 3.0 | |
| Trehalose* | ... | 0 | 0 | 0 | |
| Maltose | ... | 7.6 | 24.8 | 36.5 | |

* Trehalose and maltose hydrolysis determined by Nelson's modification of Somoygi's procedure (17)

The same technical factors, especially lack of reactivity toward reagents, make identification of B and C difficult. However, in our experience, the same difficulties occur with sugar alcohols and the positive permanganate-periodate reaction suggests a Polyol structure. Furthermore, compound C apparently is formed after C_1 oxidation and migrated as did arabitol and ribitol, suggesting a pentitol. Prentice and Cuendet (19) have established, by derivative formation, that wheat rust spores contain at least 3% by weight of arabitol. Either arabitol or ribitol could be formed by reduction of ribulose-5-phosphate arising by pentose pathway reactions. Although it was not mentioned (26), Wang's chromatograms for infected wheat show a radioactive band at a position similar to that we have found for compound C in infected bean and safflower.

The possible nature of B is more obscure, especially in view of the published reports claiming mannose or sedoheptulose (14, 26) in infected leaves. It may be of significance that these two reports stress results obtained by radioautography, but other papers do not mention the detection of either sugar in rusted tissue. In the present study, compound B did not give the reactions of sedoheptulose nor mannose. Further, in our solvents mannose migrates nearly identically with fructose.

It is possible that B may correspond to a hexitol but D-manno-D talo-heptitol (sedoheptitol, volemitol) has also been found in fungi (18). If B arose by a transketolase reaction with pentoses as substrate and acceptor, the greater amount of radioactivity (table II) from C_2 labelled glucose when compared to C_6 is as expected. The relatively high amount of tagging from both C_2 and C_1 is not expected unless hexose (i.e. fructose-6-phosphate) acts either as an acceptor or donor to form an octulose (9). Such compounds have been observed in plant tissues (6). As indicated previously, randomization effects impose limitations on the interpretation of the relatively slight differences observed.

Recent studies have demonstrated the existence of dehydrogenases which convert pentuloses, hexuloses, and heptuloses to corresponding alcohols (27). A greater specificity has been claimed for a TPN dehydrogenase while the DPN system converts a variety of substrates to alcohols. Since oxidative pentose metabolism is TPN linked, specificity of TPN dehydrogenases might limit the number of possible alcohols occurring in diseased tissues. Previous workers have found that the sugar alcohols have a pronounced effect on the development of rust. Samborski and Forsyth (21) and Silverman (24) have inhibited rust development by applications of sorbitol, ribitol, arabitol, dulcitol, and mannitol without toxic effects on the host. Silverman found that the effect could be readily overcome by removal of the alcohol and subsequent application of a suitable sugar to the tissues. One possible explanation would involve sugar alcohol formation as a metabolic sink to maintain low TPNH/TPN ratios during high rates of

respiration via the pentose pathway (4). A supply of exogenous sugar alcohol would act reversibly to inhibit oxidative pentose metabolism and inhibit rust development.

Although the major emphasis in the literature has been on wheat rust diseases, while the present study used bean and safflower as host, the existence of similar materials in all species indicates common pathways (7). The possibility that trehalose or sugar alcohols may be present in rusted tissue would necessitate re-examination of previous analytical data on carbohydrate levels, especially in comparisons of resistant and susceptible plants. Mild analytical procedures for non-reducing sugars, designed to detect sucrose, probably would fail to detect trehalose. Similarly, analytical data based on elution of spots revealed by sprays also might be misleading. Positive quantitative identification of these compounds is essential and is in progress.

Summary

Rust-affected safflower and bean tissues, characterized by high rates of respiration and low C_6/C_1 ratios, have been shown to contain at least three components resembling carbohydrates which are not detectable in healthy tissues. Chromatograms of neutral, lipid-free extracts from diseased tissue which had been fed uniformly labelled glucose showed high levels of activity at regions corresponding to a disaccharide (A), hexose (B), and pentose (C). Through the use of glucose labelled in the 1, 2, or 6 positions, it was shown that C probably arises after the removal of the first carbon of glucose and, therefore, is directly related to the low C_6/C_1 ratios observed during infection. Compound A, however, appeared to be synthesized from the intact glucose molecule and yielded glucose on acid hydrolysis. The metabolic origin of B is uncertain.

Although none of the materials could be detected by the usual carbohydrate spray reagents, all gave positive permanganate periodate reactions indicating polyol structures. On the basis of R_f values and ease of hydrolysis, A appeared to be trehalose, a disaccharide found in fungi. It is suggested that since C co-chromatographs with arabitol, previously found in rust spores, unknown C may be a sugar alcohol produced by metabolic reduction of intermediates of the pentose pathway. The other unknown, B, also has properties and R_f values similar to known polyols. The significance of the results is discussed with respect to previous interpretation of carbohydrate levels in rusted tissues.

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