The University of Manitoba

UPTAKE AND TRANSLOCATION OF GLUCOSE-6-T AND GLYCINE-2-T BY *RHIZOCTONIA SOLANI* KUHN

A Thesis

Submitted to

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In Partial Fulfilment of

The Requirement for

The Degree of Master of Science

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by

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ABSTRACT

The uptake and translocation of two simple nutrients, glucose and glycine, were studied at a hyphal level in *Rhizoctonia solani* by means of an autoradiographic method involving the use of tritiated compounds, autoradiographic stripping emulsion and slide cultures.

Results obtained show that uptake is not localized in the mycelium but that it is directly related to cytoplasmic density, competetively inhibited by structurally similar compounds, saturated at high nutrient concentrations and completely inhibited by the uranyl ion. Glucose uptake was shown to be a two-step process; the first step having the properties of a facilitated diffusion and the second having the properties of an active accumulation. Comparisons have been made with better known systems in other organisms.

A study of translocation of glucose showed that movement occurs simultaneously in both directions in a hyphae. It was shown that this movement was the result of an actual translocation inside the hypha and not the result of diffusion through the medium, along the surface of the hypha or through the cytoplasm. A possible mechanism has been proposed to account for this bidirectional translocation.

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INTRODUCTION

Although there have been several studies on the uptake and translocation of ions and dyes by filamentous fungi only a few studies have been made on the uptake and translocation of common nutrients.

Uptake, however, has been studied very extensively in bacteria, yeasts and mammalian cells and a great deal is known about the nature of the mechanisms involved in these organisms, where uptake of organic molecules appears to be an active process probably consisting of two steps. The first, an energy-independent facilitated diffusion, shows the following characteristics: non-linear kinetics, stereospecificity, competetive inhibition, species or strain specificity, a frequently anomalous Q_{10}, greater rates than predicted from molecular size and lipid solubility and sensitivity to certain inhibitors; while the second involves an energy-dependent accumulation against a concentration gradient.

Very little however is known about the uptake of nutrients at a cellular level since these experiments have been conducted only in bulk. Although it is generally assumed that uptake can occur at any point on a fungal mycelium evidence of hyphal specialization suggests that the possibility of localization of uptake should also be considered.

A similar lack of information exists in the study of translocation in filamentous fungi where the movement of ions and dyes has been investigated by several workers, but very few have studied the

translocation of common nutrients.

It is generally assumed that translocation is closely related to cytoplasmic streaming but mechanisms similar to the electro osmotic activated mass flow suggested for transport in phloem cells or the counter current theory proposed for amoeboid movement must also be considered.

Thus, the present investigation was conducted to study the following questions at a hyphal level:

1. Whether uptake of glucose or glycine by Rhizoctonia solani had characteristics similar to the better known systems, and to attempt to establish whether it is a two step process;
2. Whether there is any localization of uptake sites on the mycelium;
3. Whether Rhizoctonia solani was capable of translocating glucose, and in what direction;
4. Whether a possible mechanism for the translocation could be proposed.

The very small quantities of nutrient present in the hyphae are not detectable by normal chemical means of analysis, so as a first approximation, the distribution of tritium is taken as an indication of the probable distribution of the labelled compound fed.

LITERATURE REVIEW

Owing to the great diversity of phenomena involved a comprehensive review of the literature on uptake and translocation in general has not been attempted. The review is largely devoted to papers concerned with the uptake of sugars and amino acids, and with translocation studies particularly in the fungi.

I. UPTAKE

Two recent reviews are available covering the extensive literature on amino acid and sugar uptake by bacteria and yeasts, (11,14). Very few studies however have been done on these processes in filamentous fungi.

To measure nutrient uptake several methods are in general use. One, developed by Conway and Downey (15) depends on measurement of the rate of disappearance of the nutrient from the medium, while a second and more widely used one depends on analysis of cell contents.

Methods involving the feeding of cells with radioactively labelled substances, in which the cells are filtered, collected, washed and counted directly on Millipore filters, have been described by Roberts, *et al.* (48) and Zalokar (62) while Grossbard (24) has developed a similar autoradiographic method. Photometric techniques which depend on volume changes accompanying uptake have been used extensively for protoplasts and red blood cells (39,43).

Sugar uptake by bacteria, yeasts and animal cells appear to be an active transport consisting of two separate processes; (a) an energy-independent facilitated diffusion across the membrane which accounts for

the non-linear kinetics, stereospecificity, competetive inhibition and sensitivity to certain inhibitors, such as uranyl acetate (17), followed by (b) an energy-dependent accumulation process which accounts for sensitivity to metabolic inhibitors and accumulation against a concentration gradient (6,10,11,12,37,51,52,53,61).

By feeding C^{14} compounds in the presence of common metabolic inhibitors Horecker et al. (27) have demonstrated a high initial rate of uptake, which they attribute to facilitated diffusion, which declines rapidly presumably as a result of inhibition of the accumulation step.

According to Rosenberg and Wilbrandt (51,52,53) these properties of the facilitated diffusion process can be explained only if a carrier or an enzyme or both are involved in the transport. Rothstein (49,50) has suggested that uranyl acetate, a non-penetrating specific inhibitor for sugar transport (4,49,50) acts by inhibiting sugar phosphorylation at the membrane surface. Evidence that the uranyl ion also inhibits the uptake of sugars that are not hexokinase substrates has led Cirillo (11,13) to suggest that the ion acts by inhibiting the operation of a carrier system possibly involving phosphatidic acids as carriers.

Unfortunately there is little known about the mechanism of sugar uptake by the filamentous fungi and comparisons with better known systems cannot be made.

Zalokar (62), Mathieson and Catcheside (38) and Brockman, de Bush and Wagner (8) have investigated amino acid uptake by the filamentous fungus Neurospora while Jones (30) and Jones and Watson (31) have studied

uptake by *Botrytis fabae*.

In *Botrytis* Jones (30) found that accumulation of amino acids showed the characteristics of active uptake - accumulation against a concentration gradient, saturation kinetics, inhibition by structurally similar compounds and sensitivity to inhibitors which uncouple oxidative phosphorylation. The low degree of specificity to various amino acids shown by *Neurospora* (8,38,62) and *Botrytis* (30,31) more closely parallels the situation found in animal material by Finch and Hird (21) than that described by Cohen and Monod (14) in bacteria.

Very few of these investigations of sugar or amino acid uptake have attempted to study the process as it occurs at the cellular level. For example it has always been assumed that uptake could occur at any point on a fungal mycelium (22), but even this simple assumption has never been confirmed. Since Tribe (60) and Isaac (28) have presented results which suggest that degradation of cellulose by filamentous fungi is associated with specialized side branches the possibility of localization of uptake in the mycelium should be considered also.

II. TRANSLOCATION

Until recently the problem of translocation of food materials by filamentous fungi has received very little attention. It has generally been assumed that translocation is very closely associated with cytoplasmic streaming especially in the moulds, (1,18). Buller investigated the streaming in both septate and non-septate fungi and concluded that food translocation was directly connected to streaming (9).

There is some evidence of bidirectional streaming of the protoplasm in fungal hyphae. In *Rhizopus nigricans* Buller (9) occasionally observed a thin peripheral layer moving in the opposite direction to the bulk of the cytoplasm, while Isaac (29) has recently reported evidence in *Rhizoctonia solani* of a movement of dry matter in one direction and implied a counter flow of fluids in the opposite direction. Schutte (54) however has been able to demonstrate only very limited bi-directional translocation in *Rhizopus oryzae*.

Lawes, Gilbert and Warrington (36) and Shantz and Piemeisel (55) were able to detect translocation of H_2O, N_2 and K in the subterranean mycelium of fairy rings. Schutte (54) has also conducted detailed studies on translocation of dyes in agaric fructifications and found that translocation occurs in the direction of cytoplasmic streaming, although a cause and effect relationship could not be demonstrated.

The translocation in mould fungi such as *Aspergillus niger* and *Mucor mucedo* can be measured respirometrically (5) since the respiration rates of these fungi drop rapidly when they are placed in H_2O. However, this method is not suitable for fungi which store food reserves.

Schutte (54) has developed three simple methods of studying the translocating ability of moulds. In these methods the fungi are grown under conditions such that they must grow across an area of agar deficient in an essential nutrient. Any mould that can grow across such an area is considered to be a 'translocating' fungus capable of translocating the nutrient from an area of normal nutrient agar across

an area of agar that lacks the nutrient. Moulds that were incapable of growing over this deficient area were termed 'non-translocating'.

Recently radioactive tracers have been used extensively to study trasnlocation of ions by Rhizoctonia solani (42), mycorrhizae (40) and several other fungi (25).

Although much valuable data has been obtained from these experiments very little is known about translocation at the cellular level, the processes involved and the factors that govern it.

MATERIALS AND METHODS

I. FUNGUS

Rhizoctonia solani Kuhn was used for the entire investigation. This fungus was obtained from the stock culture (No.R-3) collection of the Botany Department of the University of Manitoba.

II. CULTURE MEDIA

The stock cultures were stored on potato-carrot agar (35), but during the course of the investigation the fungus was maintained by successive transfers at 20 - 25°C on a sucrose peptone medium.

sucrose	10 g.
peptone	5 g.
KH_2PO_4	2 g.
$MgSO_4 \cdot 7H_2O$	.5 g.
H_2O	1000 ml.

III. SLIDE CULTURES

a) Preparation of Slides

The fungus was grown on a nutrient film on a microscope slide using a modification of methods developed by Kohler (34), Tomkins (59), Paris (45), Pauli (46) and Gow (23). Standard 1" x 3" microscope slides were sterilized in nutrient agar media in a pyrex storage dish (Canlab 29594). They were removed while the agar was still molten, allowed to drain, and placed in a petri plate on the surface of a solidified layer of 1.5% agar as recommended by Gow (23) and Isaac (28) to maintain a high humidity.

The petri plates were then placed over H_2O in a dessicator jar. A small quantity of copper sulfate was added to the H_2O to prevent the growth of contaminants, and filter paper strips were placed along the sides and dipping into the solution to help maintain the high humidity. Using this procedure the thin film on the slide could be kept for a number of weeks without any visible drying occurring.

The slides were inoculated toward one end with a disc of inoculum cut with a #2 cork borer from a 5 - 7 day old culture of _R. solani_ growing on sucrose-peptone.

b) Media

For most uptake and translocation experiments the slides were sterilized in a medium containing;

agar	15 g.
H_2O	1000 ml.

To test the effect of structurally similar compounds on glucose or glycine the following medium was used.

test substrate	10 g.
agar	15 g.
H_2O	1000 ml.

IV. FEEDING

Because of the high resolution that can be obtained with tritiated compounds, glucose-6-T (sp.act. 7.3 mc/mg.) and glycine-2-T

(sp.act. 2.4 mc/mg.) (Radiobiochemical Centre, Amherst, England) were used for the uptake and translocation experiments.

a) Uptake

The first experiments were carried out by dipping the slide cultures in a 1µc/ml. solution of the radioactive chemical. Considerable difficulty was encountered with the agar film washing off the slide so the feeding procedure was modified in two ways:

1. A radioactive solution (1µc/ml.) was applied by dipping a strip of Whatman #1 filter paper in the solution and laying it on the surface of the slide culture.
2. The compounds were applied in a 1.5% agar block with the radioactive compound at a concentration of 2µc/ml. of agar. With this method longer feeding times were required but a much clearer background could be obtained.

b) Translocation

For translocation experiments the compounds were applied in an agar disc cut with a #2 cork borer rather than as a solution to eliminate the possibility of the compounds spreading along the mycelium as a moisture film.

The positions at which the cultures were fed were marked by a circle enscribed on the slide with a diamond tipped objective mounted slide marker.

V. AUTORADIOGRAPHY

After feeding the radioactive compounds in the hyphae were detected by washing the slides with the film of nutrient and hyphae in distilled H_2O for 2 - 3 minutes to remove the free radioactivity from the surface of the hyphae and the agar and then applying Kodak A.R.-10 autoradiographic stripping emulsion (Kodak Co., London).

The procedures described in the Kodak instruction sheets were followed for applying and processing the stripping emulsion.

An exposure period of 1 - 2 days was found to be adequate to detect the activity present in the hyphae after a feeding period of 16 minutes (using the filter paper method) or 1/2 - 1 hour (using the agar block method) while the background remained relatively clear.

For developing the following standard procedure was used.

Kodak D-19b	4 minutes
H_2O	rinse
'Quick-fix'	twice time required for clearing
H_2O	rinse

The slides were then mounted in glycerin-jelly (7) with no cytological staining.

VI. DETERMINATIONS OF HYPHAL MASS

Determinations of hyphal mass were made with a Dyson interference microscope (Vickers Instruments, Model No. M.2595).

The measurements were made using white light with the microscope adjusted for uniform illumination with one colour fringe spread to fill the entire field (maximum fringe width)(26).

Several authors (3) have pointed out the difficulties of making total mass measurements of cells due to their irregular form. Fungal hyphae however are particularly suitable for these measurements because of their simple geometrical shape.

Total mass can be readily calculated from a determination of the mass/unit area as measured at the thickest point by means of the formula:

$$M/l = \frac{\emptyset . D \pi}{X . 4}$$

where M = mass (pica grams)
l = length (μ)
$\emptyset$ = optical path difference (μ)
D = diameter (μ)
X = 0.17 (specific constant)

All diameter measurements were made with an image splitting eye-piece (Vickers-Dyson)(19).

The positions at which the measurements were made were recorded in order that the same portion of the mycelium could be located again for the subsequent measurement of radioactivity.

VII. GRAIN COUNTS

For qualitative experiments the radioactivity was recorded as present (+) or absent (-), but for quantitative experiments grain counts were made by counting the number of silver grains visible in the emulsion above a length of hypha and subtracting from this value the number of

silver grains in a comparable area in the background.

EXPERIMENTAL RESULTS

I. UPTAKE

a) Localization of Uptake Sites

Experiments were carried out to determine whether sites for the uptake of glucose or glycine were localized in any portion of the mycelium of *Rhizoctonia solani*.

Young hyphae (0 - 3 days) on plain agar slide cultures were fed uniformly using the filter paper method and a 16 minute feeding period.

Development after a one day exposure period showed a relatively uniform distribution of silver grains in the emulsion above the hyphae on all parts of the slide with no apparent localization (Fig. 1).

To eliminate the effect of any translocation that might occur from the original sites of uptake during the 16 minute feeding period a shorter feeding period (30 seconds) and a longer exposure (7 days) was used.

Again no localization of uptake could be detected.

b) Relationship Between Mass and Uptake

Older mycelia (7 - 14 days) showed non-uniform uptake as shown in Fig.2.

To determine whether the non-uniform uptake was related to the uneven distribution of cytoplasm which occurs in older hyphae measurements were made of the mass per unit length of 10 day old hyphae before feeding. After feeding, exposing and processing the amount of uptake was

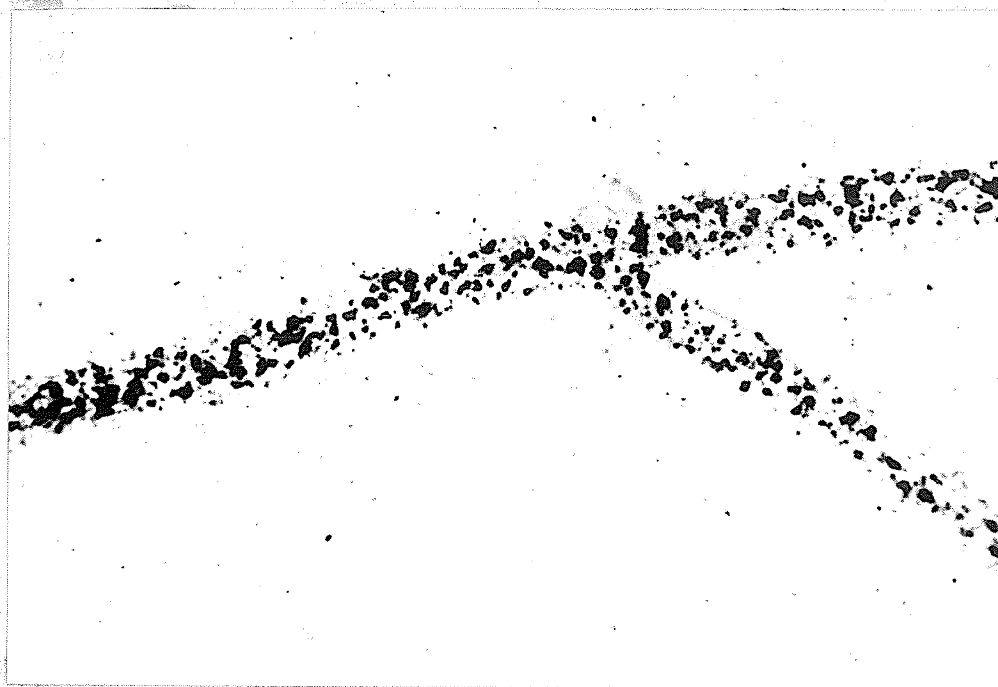


FIGURE 1. Uniform distribution of silver grains in emulsion above young hyphae (0-3 days) after a 16 minute feeding period. (X 1000)

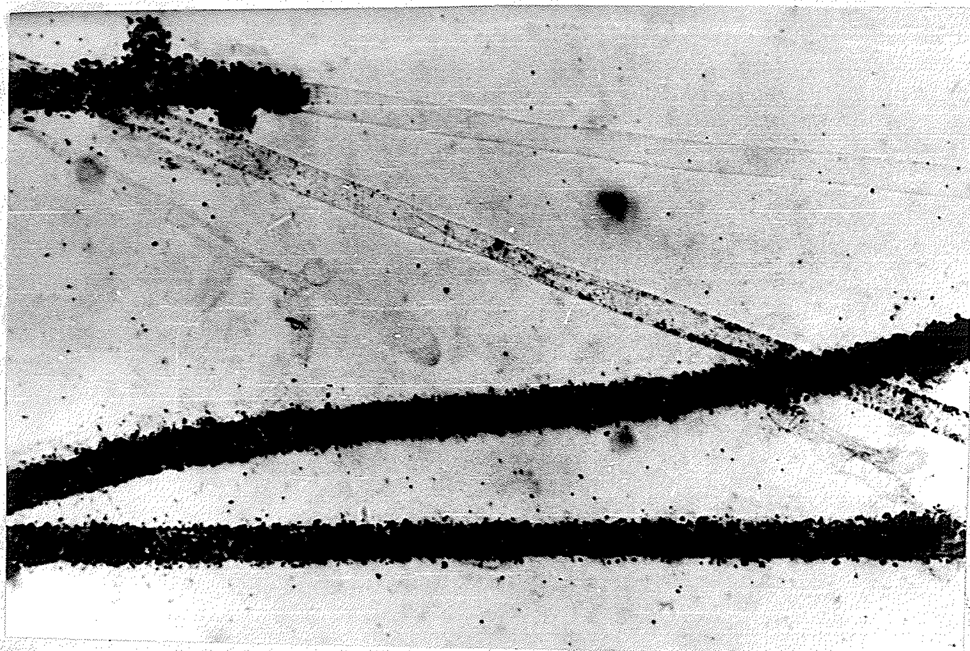


FIGURE 2. A portion of a 10 day old culture showing non-uniform deposit of silver grains. (X 500)

estimated by counting the silver grains in the emulsion at the same points where the density had been measured.

For the measurements of mass, areas were selected that could be readily identified and located again for the subsequent grain counts (Figs. 3-6). These areas generally showed a wide range in hyphal mass. In each field chosen, every hypha was measured. The results are recorded in Table A (see Appendix).

The relationships between mass and grain count, diameter and grain count and diameter and mass are shown by scatter diagrams, (Figs. 7-9).

Since the distributions of mass and grain count were not normal and since there was an obviously significant relationship between mass and diameter the associations between mass and grain count and diameter and grain count were determined by partial regressions using the form:(2)

$$Y = a + b_1x_1 + b_2x_2$$

where Y = grain count

x_1= mass (pg/unit length of hypha)

x_2= diameter (μ)

b_1= regression coefficient for mass = 3.84 ± 0.53

b_2= regression coefficient for diameter = 1.04 ± 2.11

From the standard deviations of the regression coefficients it is clear that there is no detectable relationship between diameter and grain counts but there is a significant association ($P < 0.001$) between

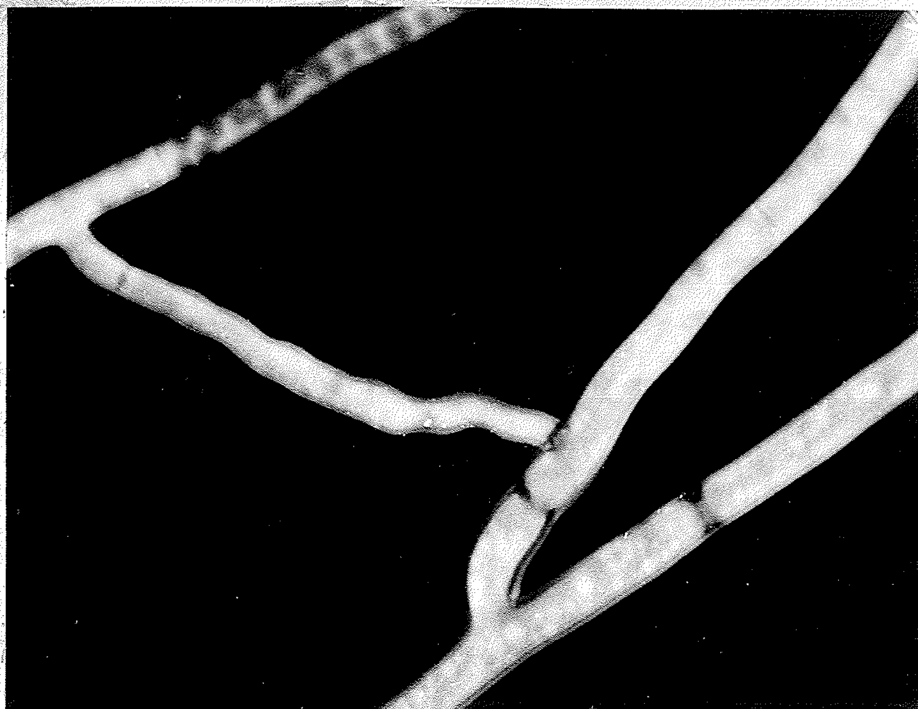


FIGURE 3. Photograph taken through interference microscope using monochromatic light (background black position). (X 1000)

FIGURE 4. Same area after feeding and processing showing silver grains in emulsion above hyphae. (X 1000)

FIGURE 5. Similar to Figure 3 but showing a different area of mycelium. (X 1000)

FIGURE 6. Similar to Figure 4 but showing the same area as Figure 5. (X 1000)

FIGURE 7. Scatter diagram showing relationship between mass (X) and grain count (Y) in mature hyphae (10 days).

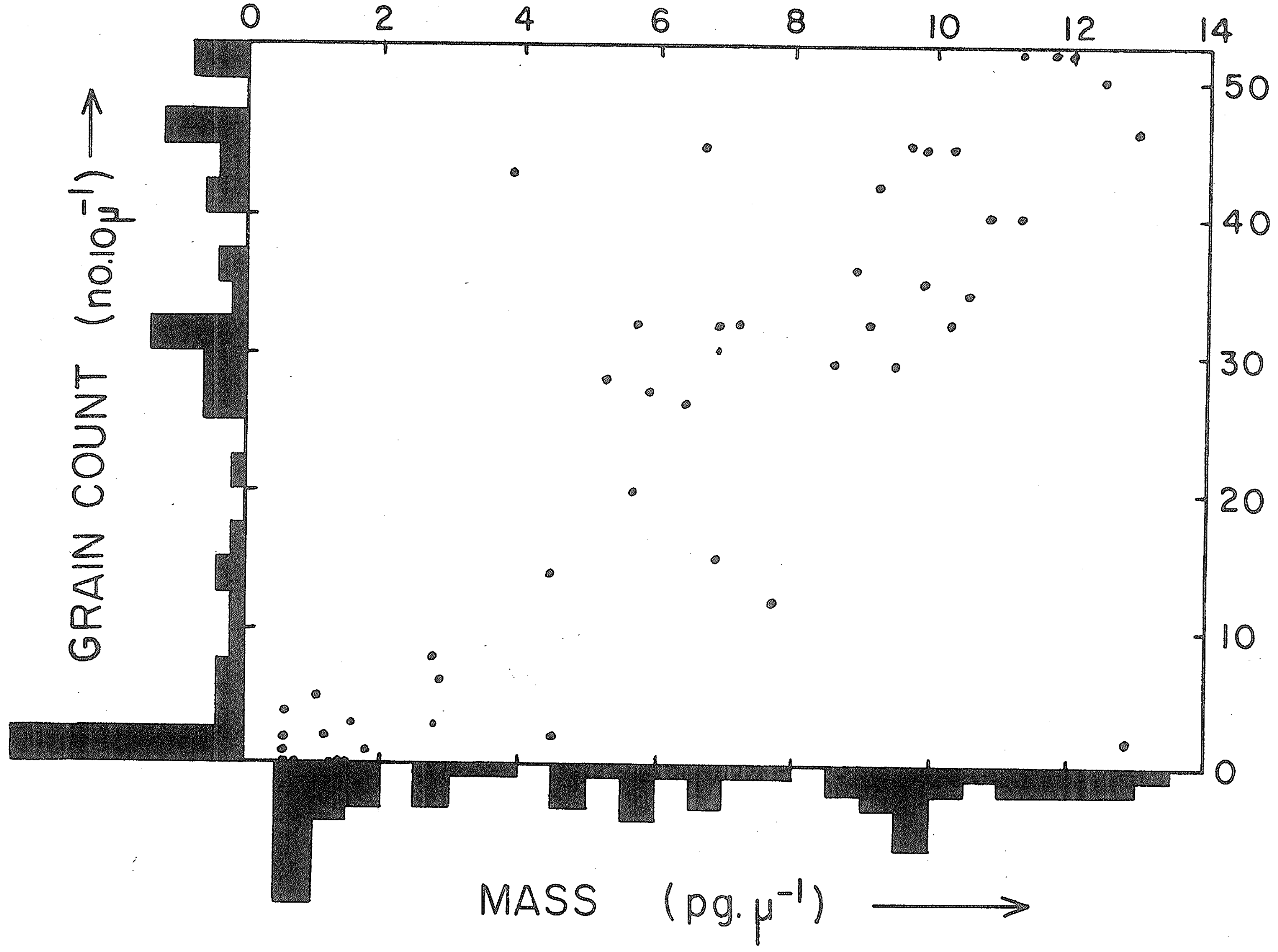

GRAIN COUNT (no.10μ⁻¹) →
MASS (pg.μ⁻¹) →
0
2
4
6
8
10
12
14
0
10
20
30
40
50

FIGURE 8. Relationship between diameter (X) and grain count (Y).

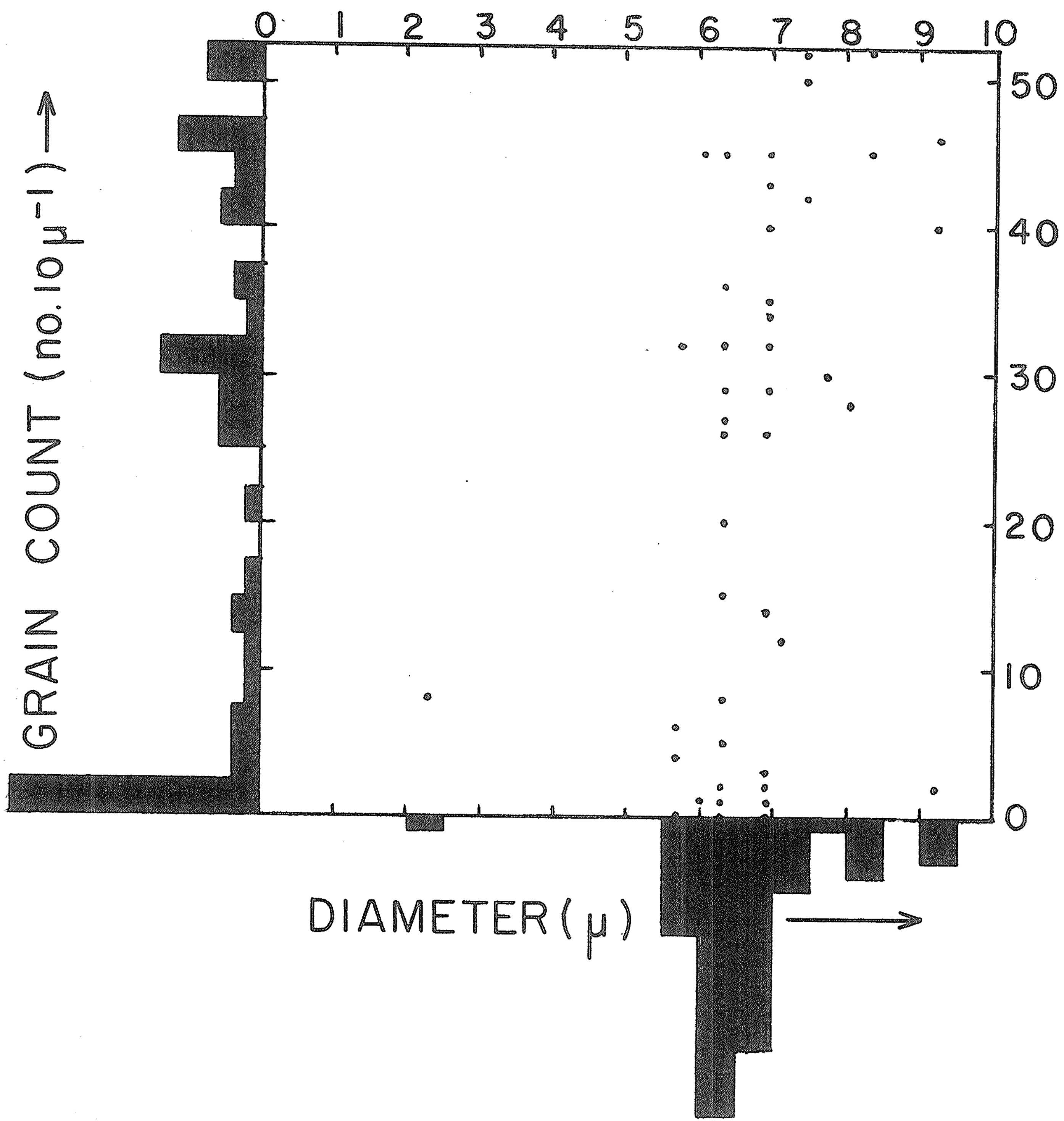
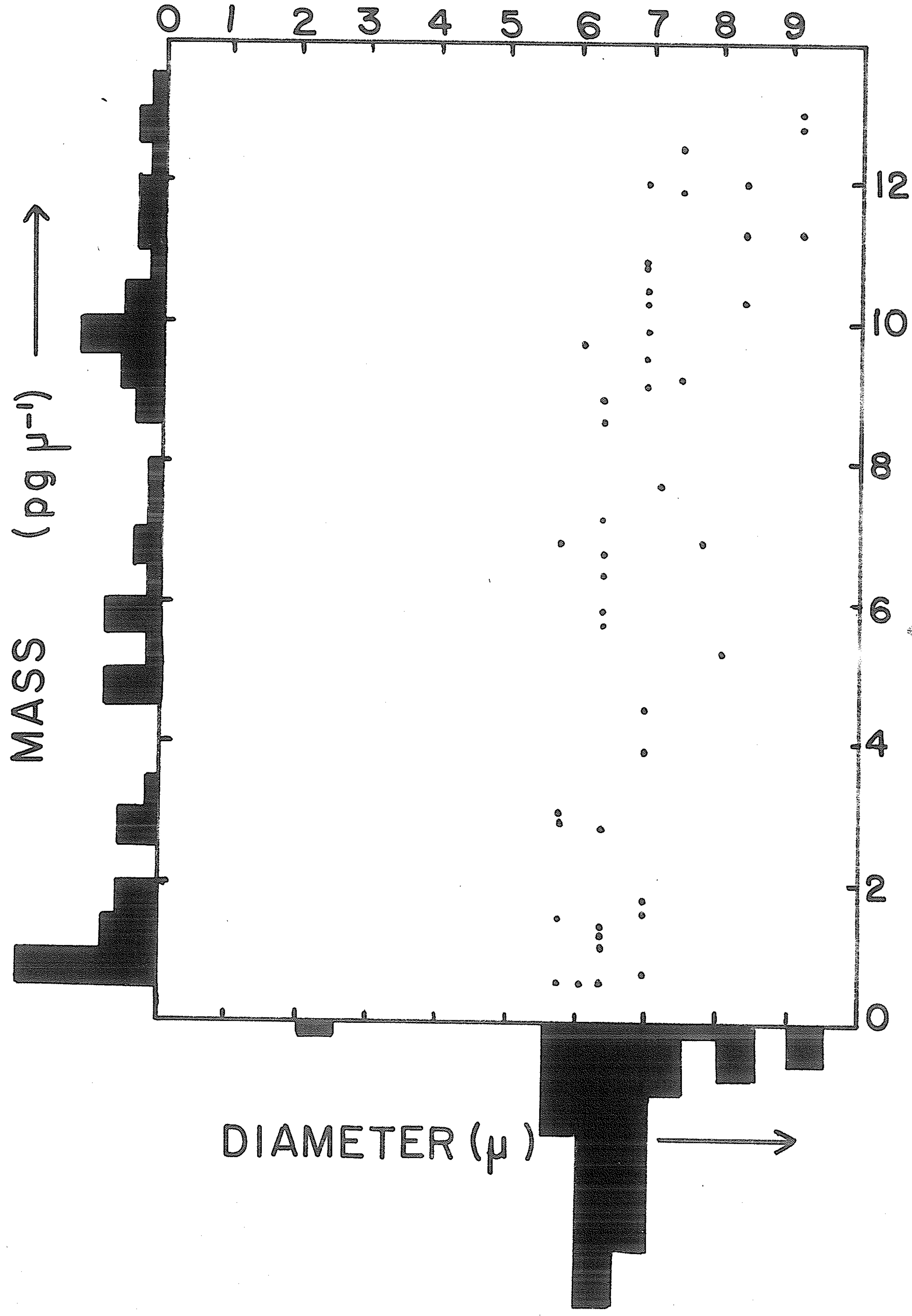

0 1 2 3 4 5 6 7 8 9 10
0 10 20 30 40 50
GRAIN COUNT (no. 10 μ-1)
DIAMETER (μ)

FIGURE 9. Relationship between diameter (X) and mass (Y).



MASS (pg μ⁻¹)
DIAMETER (μ)
0 1 2 3 4 5 6 7 8 9
0 2 4 6 8 10 12

mass and grain count.

c) Competetive Inhibition

The effect of structurally similar compounds on the uptake of glucose-6-T and glycine-2-T is shown in Table I.

d) Pre-incubation

The effect of short periods of pre-incubation with solutions of unlabelled glucose or glycine on the uptake of tritiated glucose and glycine was tested by applying a 1% solution of the test compound in a filter paper strip for periods of varying lengths before the radioactive compound was fed (Table II).

Darkening and mottling of the hyphae and darkening of the septa was occasionally observed as shown in Fig. 10. By mounting with glycerin-jelly, allowing a few minutes for the mountant to set and then applying pressure sideways on the coverslip, the emulsion could be displaced from the hyphae below and the darkening could be shown to be in the hyphae themselves and not in the emulsion (Fig.11). The cause of this darkening has not been determined, but it has been confirmed that it does not take place in the absence of the autoradiographic emulsion or the photographic processing.

e) Inhibitors

Several metabolic inhibitors were tested by adding them to the aqueous solution or agar block containing the radioactive compound and feeding simultaneously. The results are shown in Table III.

TABLE I

The Effect of Structurally Similar Compounds on the Uptake of Glucose-6-T and Glycine-2-T

Medium (Conc.1%)	Radioactive Compound (Conc. 1 μc/ml)	Uptake
Glucose	Glucose-6-T	-
Mannose	"	-
Galactose	"	-
Fructose	"	-
Ribose	"	+
Glycine	"	+
Sucrose	"	+
Control	"	+
Glycine	Glycine-2-T	-
Alanine	"	-
Serine	"	-
Sodium glutamate	"	-
Glucose	"	+
Control	"	+

TABLE II

The Effect of Pre-feeding with Unlabelled Compounds (1%) on the Uptake of Glucose-6-T and Glycine-2-T

Substrate	Pre-treatment		Uptake
Glucose-6-T (1 μc/ml)	Glucose 1% (unlabelled)	4 min.	+
"	"	8 min.	+
"	"	16 min.	-
"	"	1 hr.	-
Glycine-2-T (1 μc/ml)	Glycine 1% (unlabelled)	4 min.	+
"	"	8 min.	+
"	"	16 min.	-
"	"	1 hr.	-

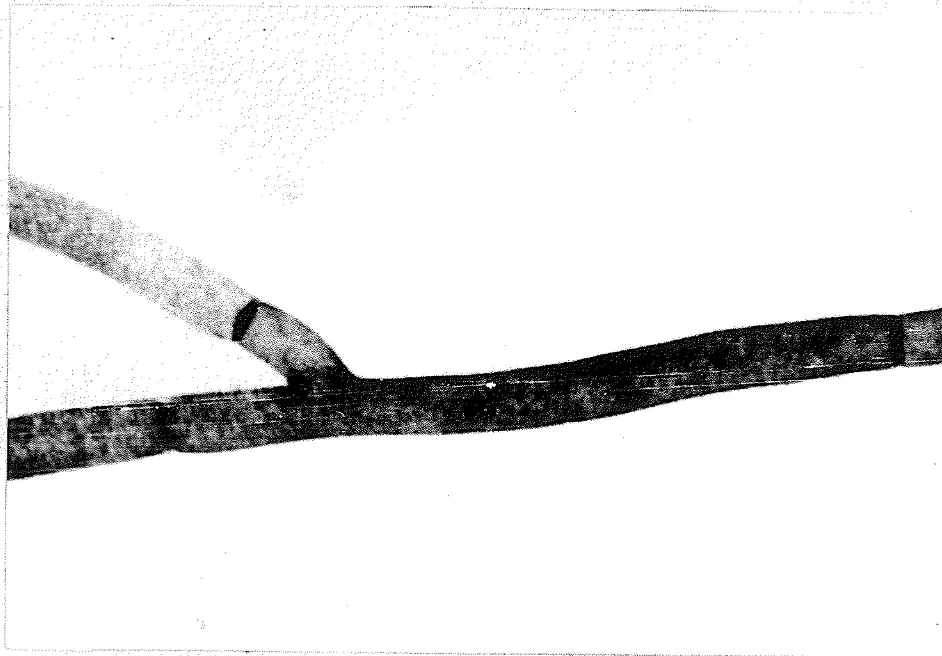


FIGURE 10. Portion of mycelium after feeding and processing showing darkening and mottling that occasionally occur. (X1000)

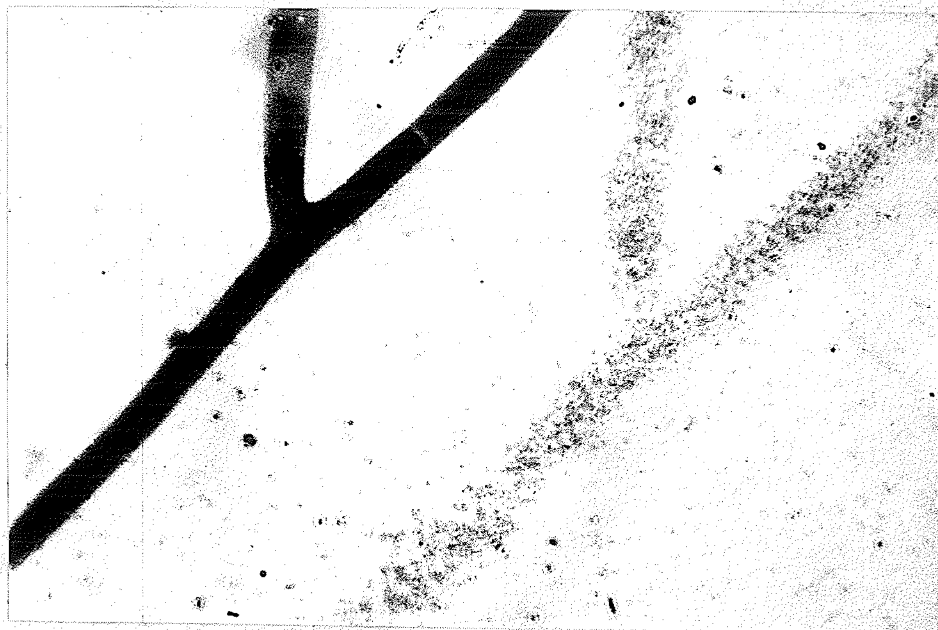


FIGURE 11. A portion of a slide on which the emulsion containing the silver grains has been displaced sideways showing the darkened hyphae and the track of silver grains to the right (focussed on hyphae). (X 500)

TABLE III

The Effect of Inhibitors on the Uptake of
Glucose-6-T and Glycine-2-T

Substrate		Concentration	Uptake
Glucose-6-T (2 μc/ml)	+ 8-hydroxyquinoline	$3x10^{-3}$ M	+
"	+ diethyldithiocarbamate	"	+
"	+ sodium azide	"	+
"	+ dinitrophenol	"	+
"	+ sodium fluoride	"	+
"	+ uranyl acetate pH 3.5	$3x10^{-4}$ M	-
Glycine-2-T (2 μc/ml)	+ 8-hydroxyquinoline	$3x10^{-3}$ M	+
"	+ diethyldithiocarbamate	"	+
"	+ sodium azide	"	+

f) The Effect of DNP

Inhibition of accumulation of glucose by DNP (3×10^{-4} M.) was measured by using glucose-U-C^{14} (.02 µc/ml liquid culture), feeding, filtering and collecting on a filter paper and determining the radioactivity with a thin mica end window Geiger-Muller counter connected to a Philips scaler. Inhibition after 1 hour was 76%, but after 12 hours was 93%.

The apparent uptake of glucose-6-T in the presence of DNP (3×10^{-4} M.) which was shown by the autoradiographic method was examined further by relating the amount of uptake (grain count) to the measurements of mass and diameter of the hyphae (Table B, see Appendix).

No highly vacuolated hyphae were measured to assure that the diameter measurement could be considered to also be a measure of the surface area of the cytoplasm.

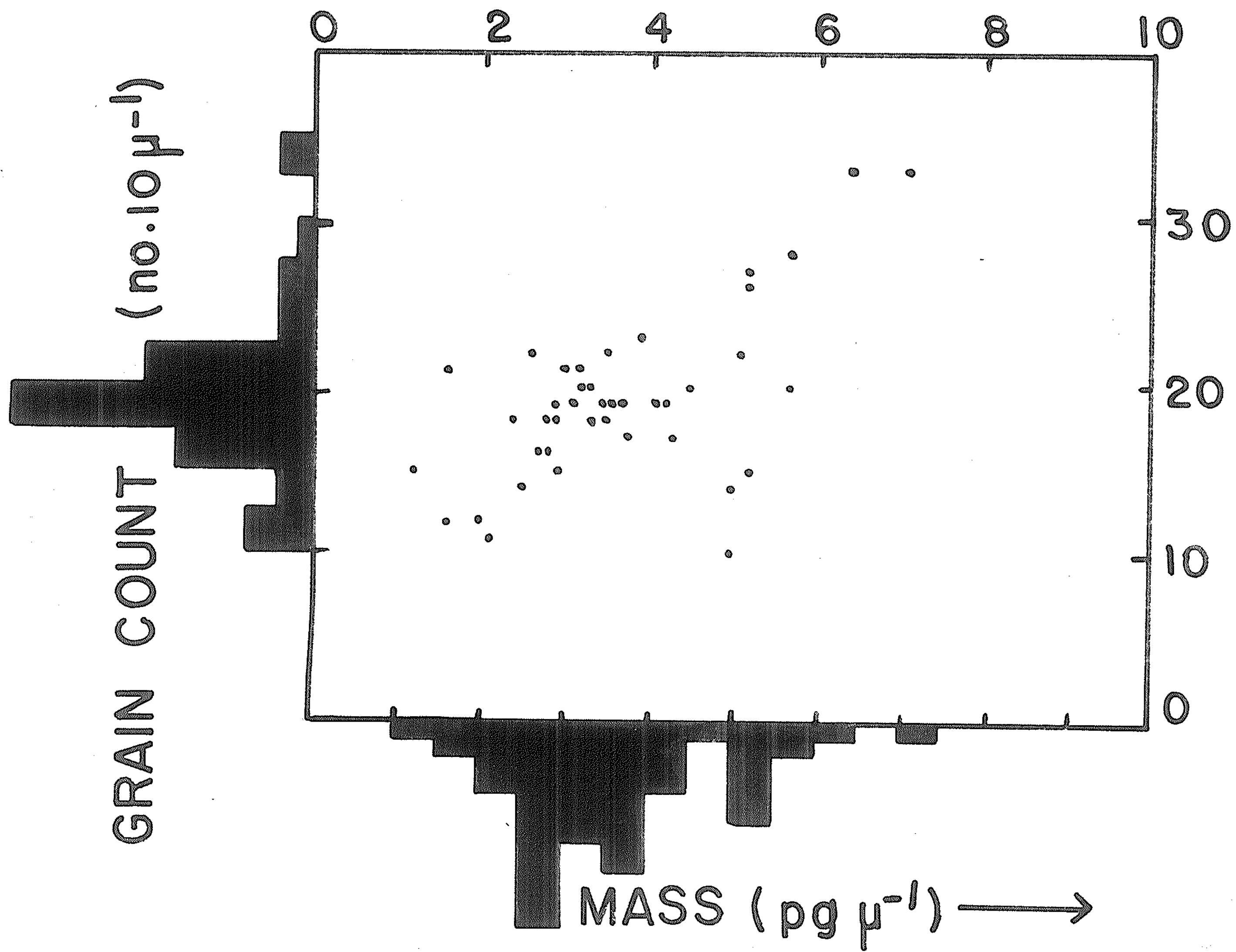
The results are shown in scatter diagrams (Figs. 12-14).

Since the grain count (1), hyphal mass (2) and hyphal diameter (3) were normally distributed, correlation coefficients were calculated(2) to test the association between the factors.

Grain Count/ Mass	$r_{12} = 0.60$ ($P < 0.001$)
Grain Count/ Diameter	$r_{13} = 0.81$ ($P < 0.001$)
Mass/Diameter	$r_{23} = 0.61$ ($P < 0.001$)

Since the mass diameter correlation was significant these factors were tested separately, eliminating the effect of the other by partial

FIGURE 12. Scatter diagram showing relationship between mass (X) and grain count (Y) in the presence of DNP 3×10^{-4} M.



0
2
4
6
8
10
30
20
10
0
GRAIN COUNT (no.10 μ⁻¹)
MASS (pg μ⁻¹) ⟶

FIGURE 13. Relationship between diameter (X) and grain count (Y) in the presence of DNP $3x10^{-4}$ M.

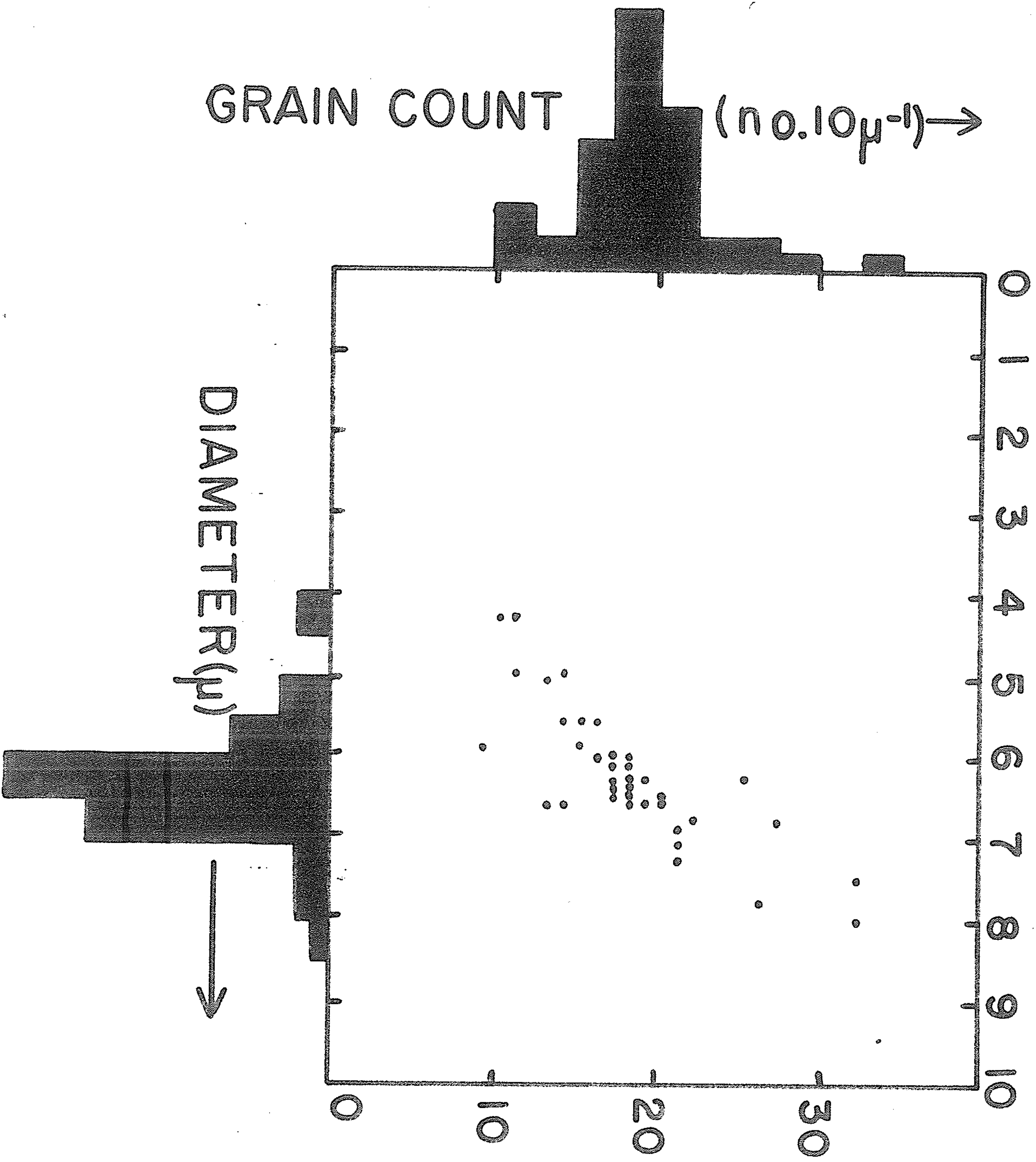
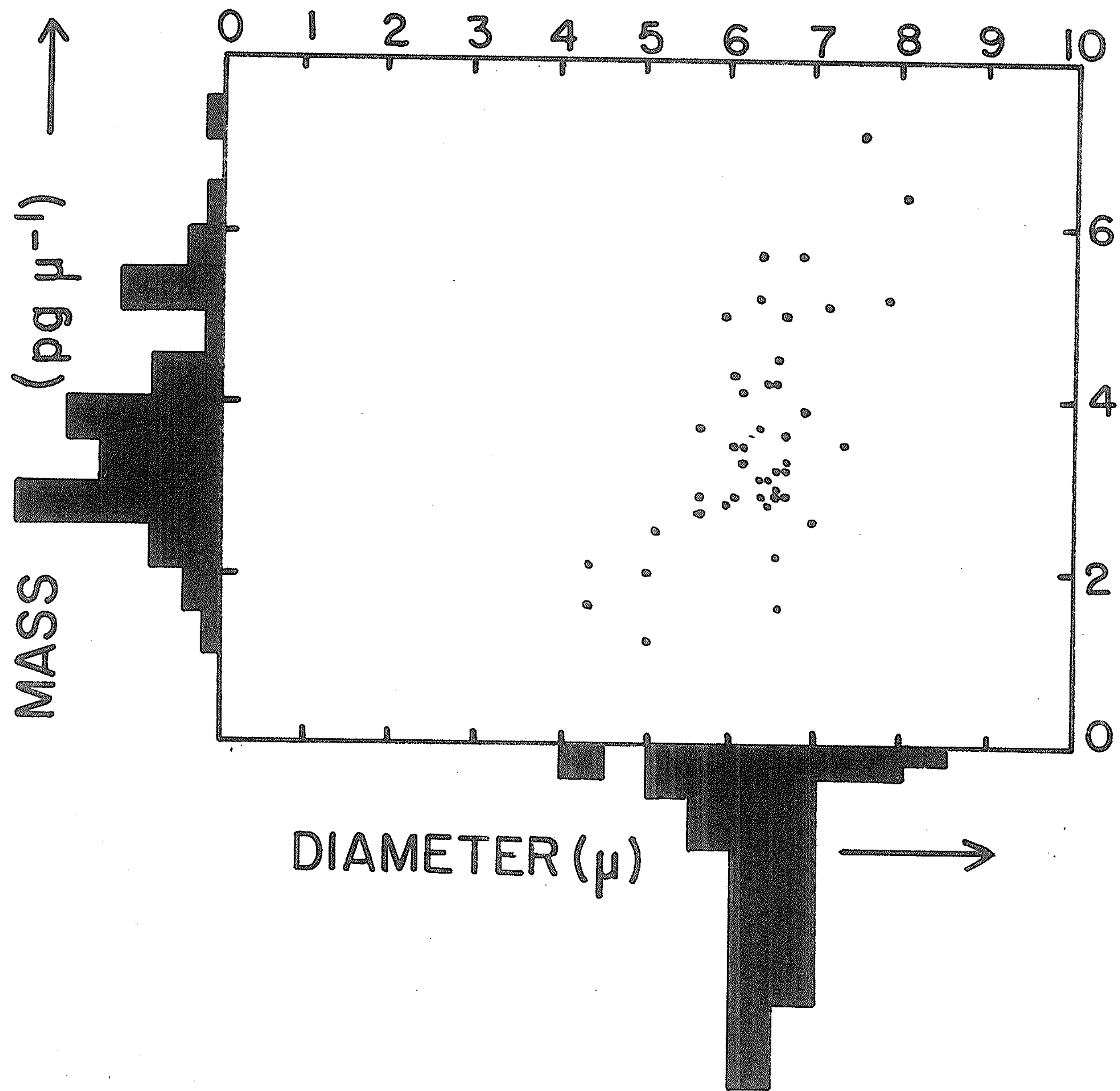

GRAIN COUNT (no.10μ⁻¹)
DIAMETER(μ)
0
1
2
3
4
5
6
7
8
9
10
0
10
20
30

FIGURE 14. Relationship between diameter (X) and mass (Y) in the presence of DNP $3x10^{-4}$ M.



MASS (pg μ⁻¹)
DIAMETER (μ)
0 1 2 3 4 5 6 7 8 9 10
0 2 4 6

correlation (2), to give:

Grain Count/Mass $r_{12.3} = 0.228$ $(P > 0.1)$

Grain Count/Diameter $r_{13.2} = 0.705$ $(P < 0.001)$

indicating that in the presence of DNP (3×10^{-4} M.) the relationship between grain count and diameter was significant but the association between grain count and mass was lost.

For this experiment mature mycelium (10 days) was used since young mycelium has a very high correlation between mass and diameter which would tend to obscure the results obtained with DNP.

Measurements of a young mycelium (3 days) are shown in Table C (see Appendix) and the relationship between mass and diameter is shown by a scatter diagram (Fig.15).

II. TRANSLOCATION

a) Distance of Translocation

The furthest point from the area of feeding (glucose-6-T) at which the radioactivity could be detected in individual hyphae is shown in Fig.16. Three different feeding times were used: 15 min., 30 min., 60 min.

b) Measurement of Radioactivity Along Translocation Path

Grain counts were made on consecutive 40μ lengths along trunk hyphae from the edge of the feeding area to the furthest points at which activity could be detected, forward toward the apices and backward toward the inoculum (Figs. 17 and 18).

FIGURE 15. Scatter diagram showing relationship between diameter (X) and mass (Y) in young hyphae (3 days).

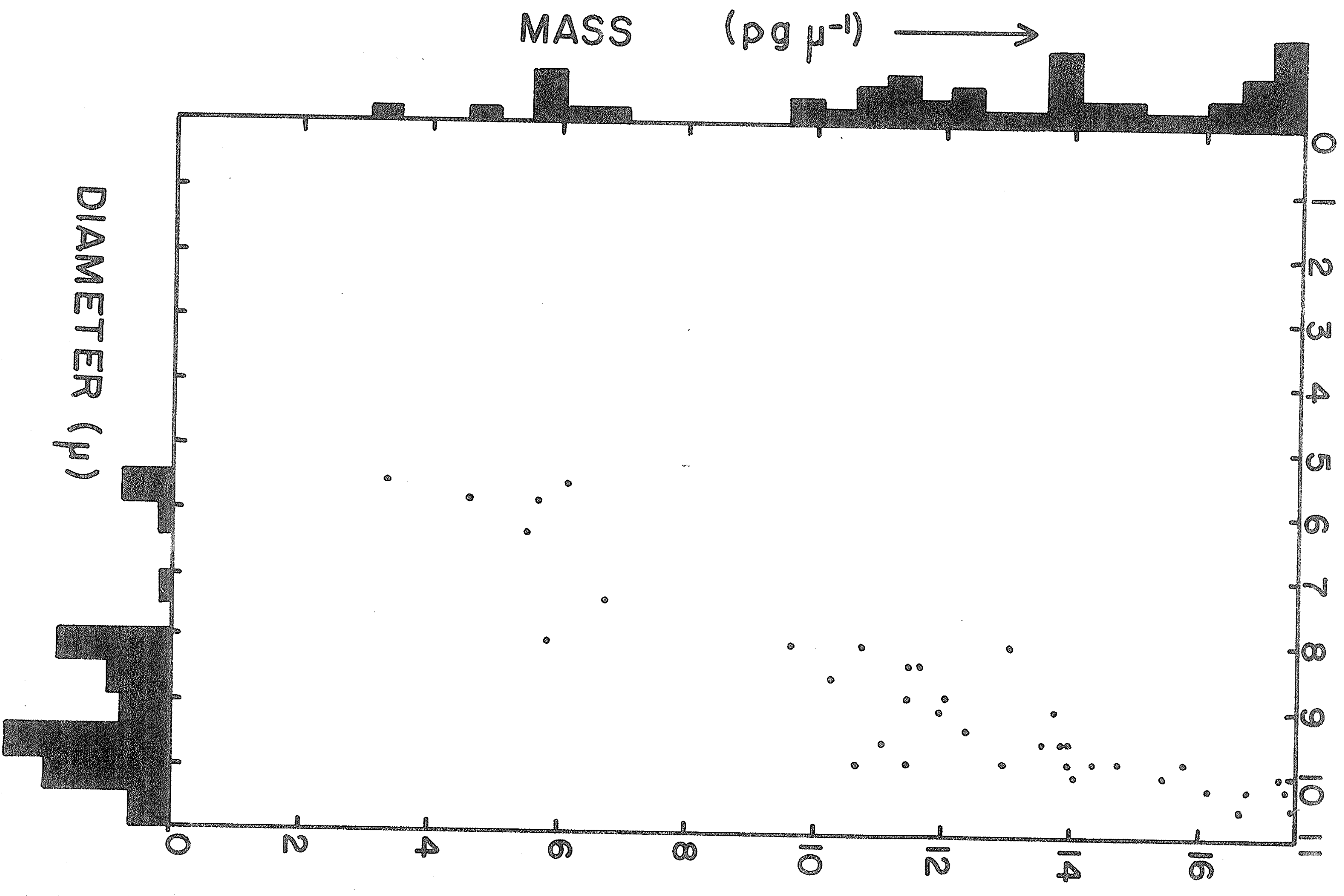
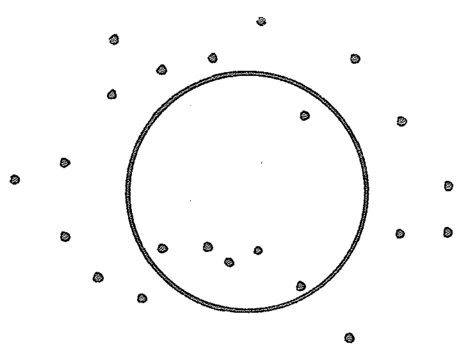

MASS (pg μ-1)
DIAMETER (μ)
0 2 4 6 8 10 12 14 16
0 1 2 3 4 5 6 7 8 9 10 11

FIGURE 16. Distance of translocation in hyphae.

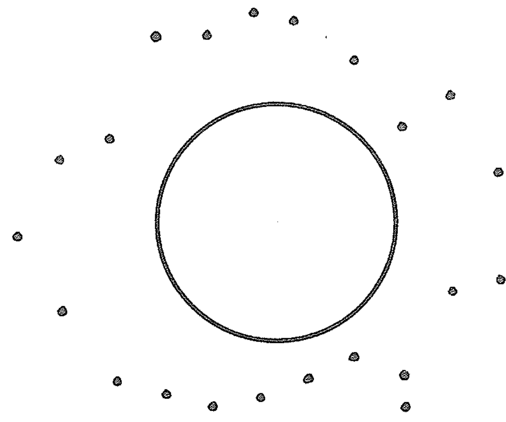
● - furthest point from feeding area at which radioactivity could be detected in the hyphae.

Circle indicates feeding area (agar disc cut with #2 cork borer).

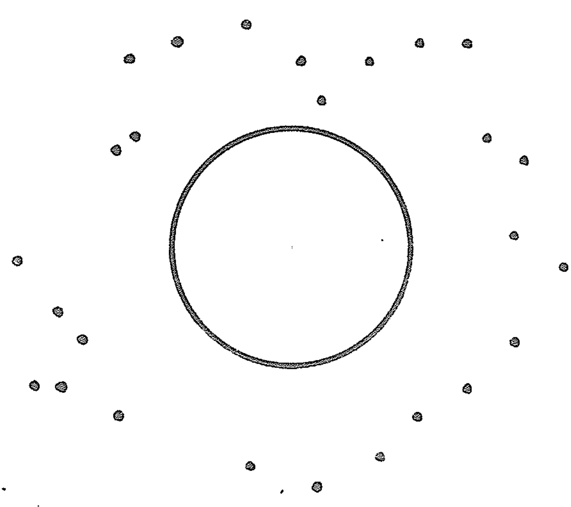
← APICES INOCULUM →



15 min.



30 min.



60 min.

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← 5 0 0 5 →

DISTANCE FROM FEEDING AREA (mm.)

FIGURE 17. The relationship between the grain count $40^{-1}\mu$ and the distance from the feeding area along two selected hyphae.

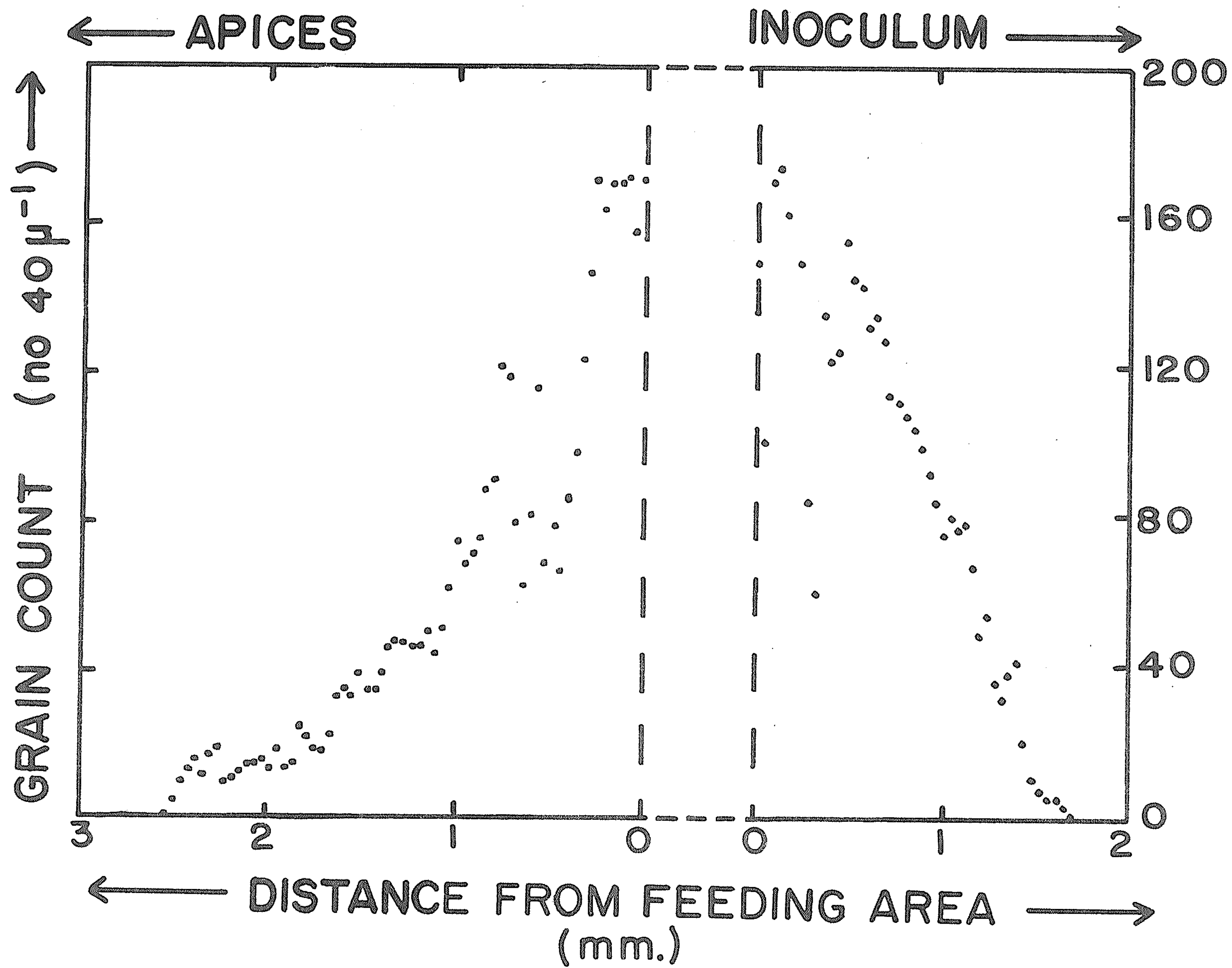
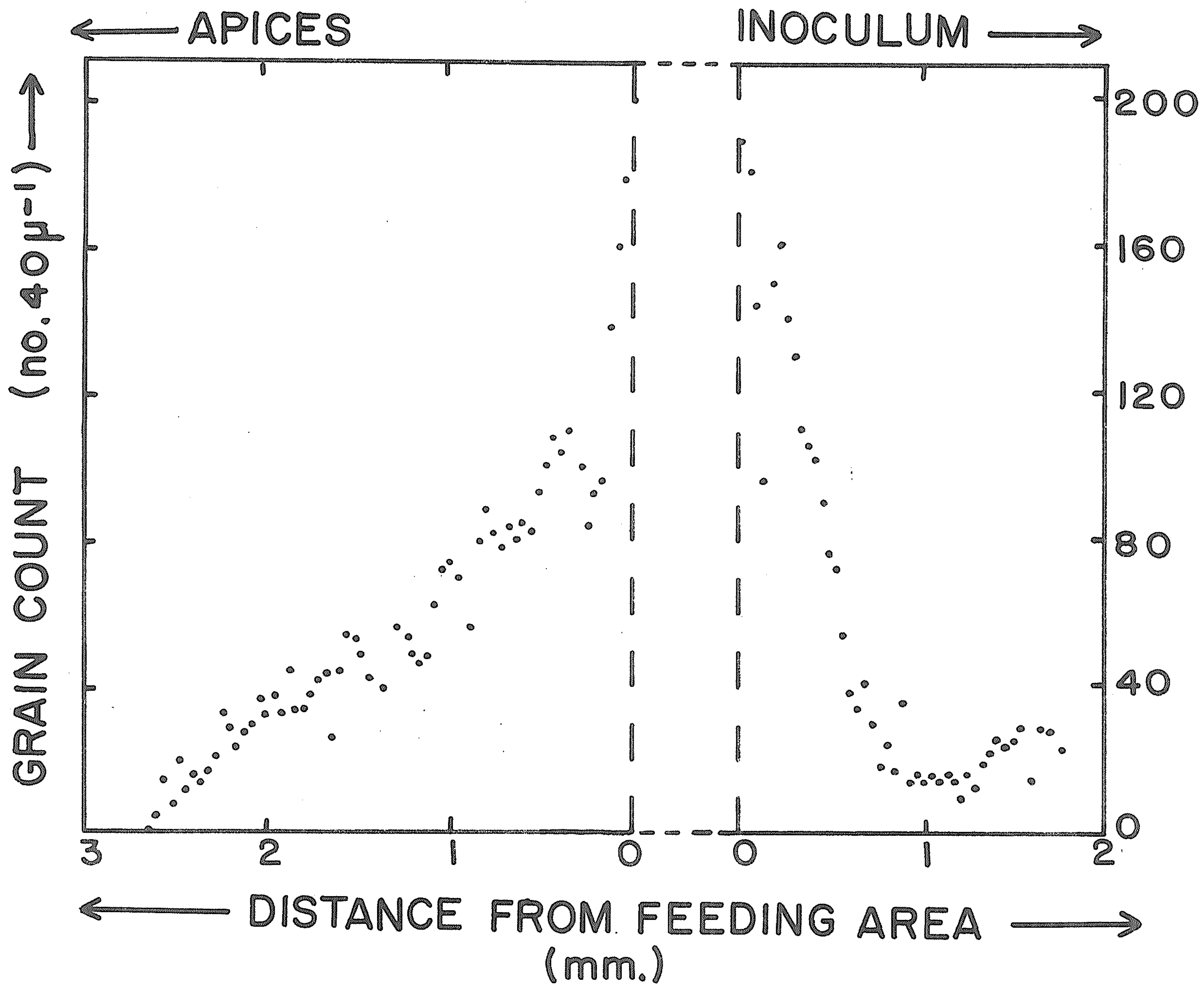

APICES
INOCULUM
GRAIN COUNT (no 40μ⁻¹)
200
160
120
80
40
0
3
2
1
0
0
1
2
DISTANCE FROM FEEDING AREA
(mm.)

FIGURE 18. Similar to Figure 16 but taken from a different slide.


APICES
INOCULUM
GRAIN COUNT (no. 40 μ⁻¹)
200
160
120
80
40
0
3
2
1
0
0
1
2
DISTANCE FROM FEEDING AREA
(mm.)

Only hyphae that were clearly in focus (i.e., on the surface of the agar and therefore in good contact with the emulsion) along their entire length were used for the grain counts.

c) The Effect of a Near-by Source of Glucose on the Translocation of Glucose-6-T

The effect of a near-by source of a relatively high concentration (1%) of unlabelled glucose on the translocation of glucose-6-T was demonstrated by placing three 2 mm - wide strips of agar across the slide culture:

1.	glucose (unlabelled 1%) + 1.5% agar	15 mm from inoculum
2.	glucose-6-T (2 µc/ml) + 1.5% agar	18 mm from inoculum
3.	glucose (unlabelled 1%) + 1.5% agar	21 mm from inoculum

A feeding period of 2 hours was used.

The results are shown in Fig.19.

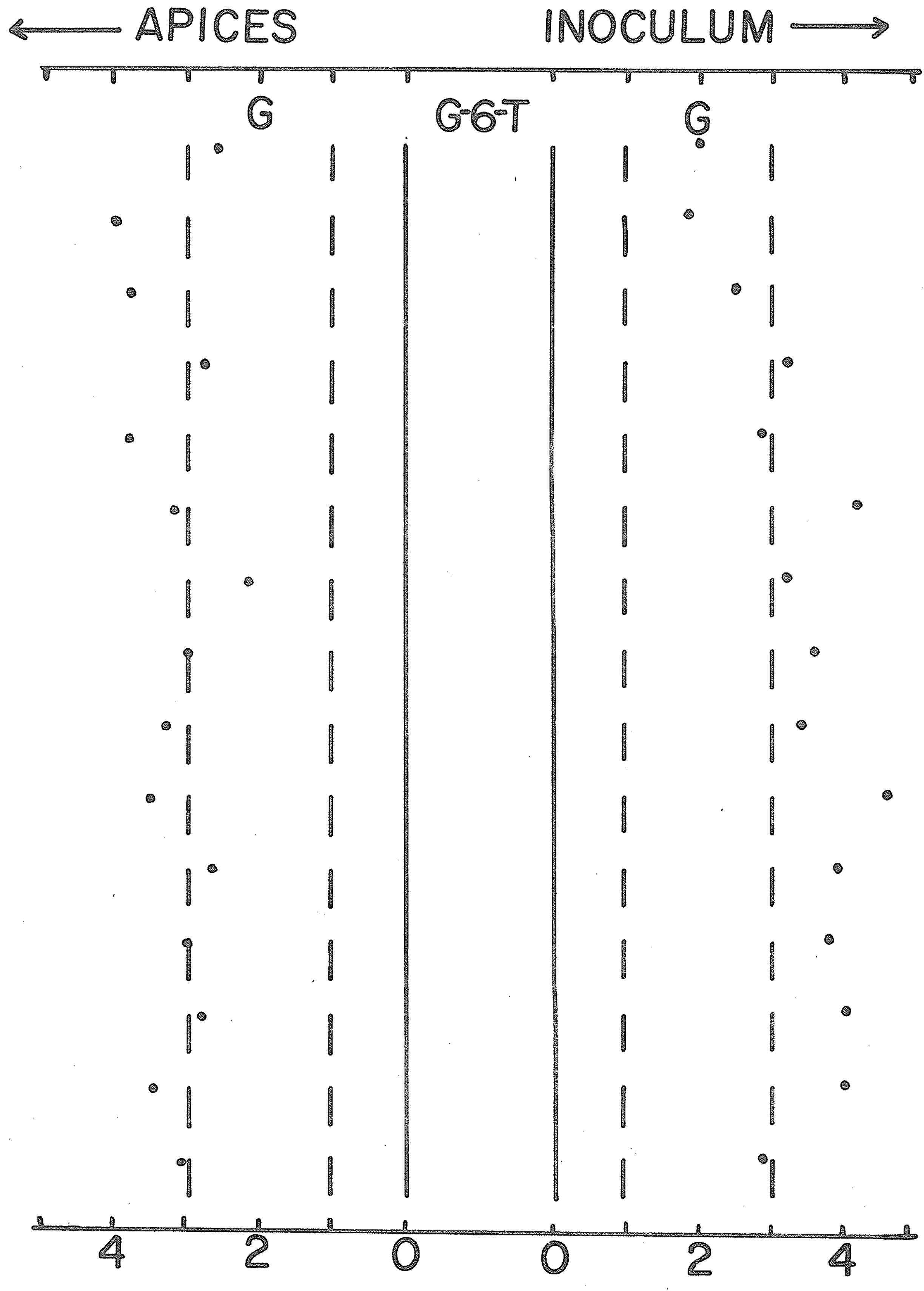
d) Diffusion

The effect of the hyphae on a slide culture on the diffusion through the layer of agar on the slide was tested by placing a 5 mm. disc of 1.5% agar containing fluorescein on a slide culture, leaving for three hours and then observing under u/v light. The hyphae in the agar did not interfere with the diffusion in any direction. The circular distribution obtained is shown in Fig. 20.

To determine the effect a small septal pore might have on the rate of diffusion through the cytoplasm of a fungal hypha the diffusion of fluorescein down three strips of agar was studied. The strips were

FIGURE 19. The effect of a near-by source of relatively high concentration (1%) unlabelled glucose on the translocation of glucose-6-T.

● = furthest point along hypha at which radioactivity could be detected.


APICES
INOCULUM
G
G-6-T
G
4
2
0
0
2
4
DISTANCE FROM FEEDING AREA (mm.)

all the same depth but differed in width: 1 inch, 1/8 inch and 1 inch with frequent constrictions to 1/8 inch. A block of agar equal to the width of the strip was placed at the end of each. The strips were then placed in a petri dish and stored over H_2O in a dessicator to prevent drying. After one week the strips were observed under u/v light. The fluorescein had travelled approximately the same distance in the 1 inch and 1/8 inch strips but had been markedly slowed in the strip with the constrictions (Fig.21).

e) Translocation of Glucose-6-T Through and Beyond an Area Where Uranyl Ion Has Been Applied.

To eliminate the possibility that the glucose-6-T might be diffusing through the agar and then taken up by the hyphae along the way three, 2 mm. strips of agar were placed across the slide culture:

1. uranyl acetate ($3x10^{-4}$ M. pH 3.5) + 1.5% agar   15 mm. from inoculum
2. glucose-6-T (2 μc/ml.) + 1.5% agar   18 mm. from inoculum
3. uranyl acetate ($3x10^{-4}$ M. pH 3.5) + 1.5% agar   21 mm. from inoculum

A feeding period of 2 hours was used. Radioactivity could be detected in the hyphae in and beyond the area where uptake would be inhibited by uranyl ion.

The results are shown in Figure 22.

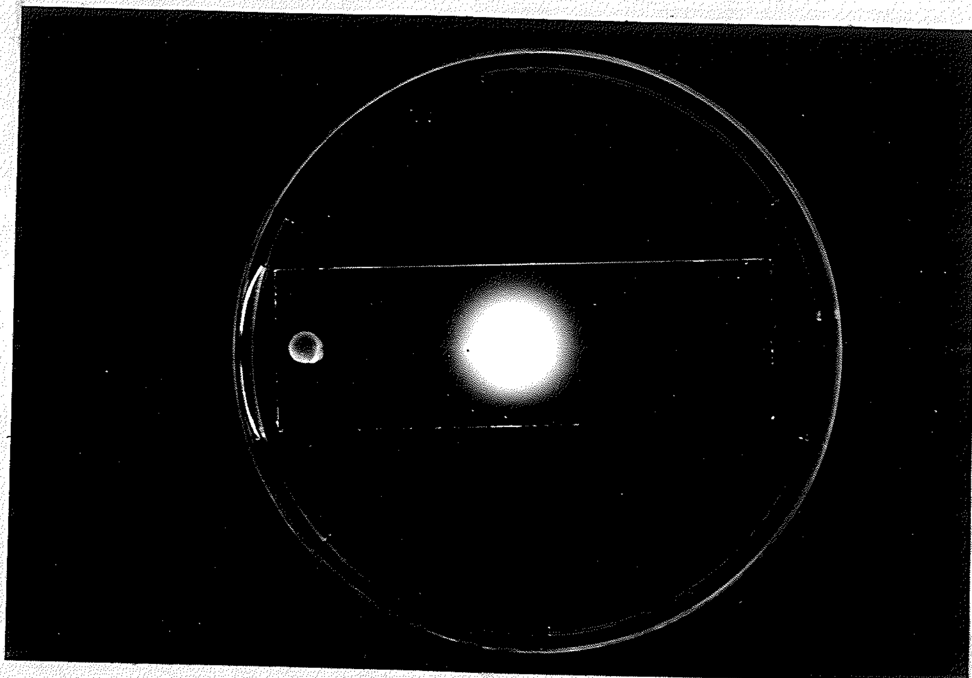


FIGURE 20. Circular distribution of fluorescein diffusing through the agar on a slide culture from a 5 mm. disc of fluorescein-agar placed on the surface.

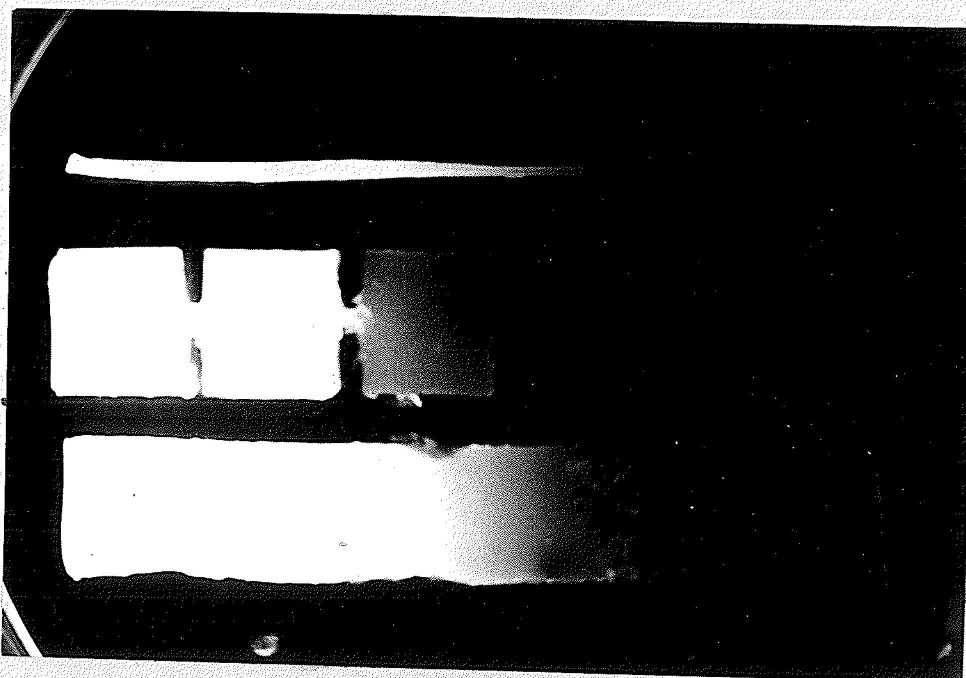
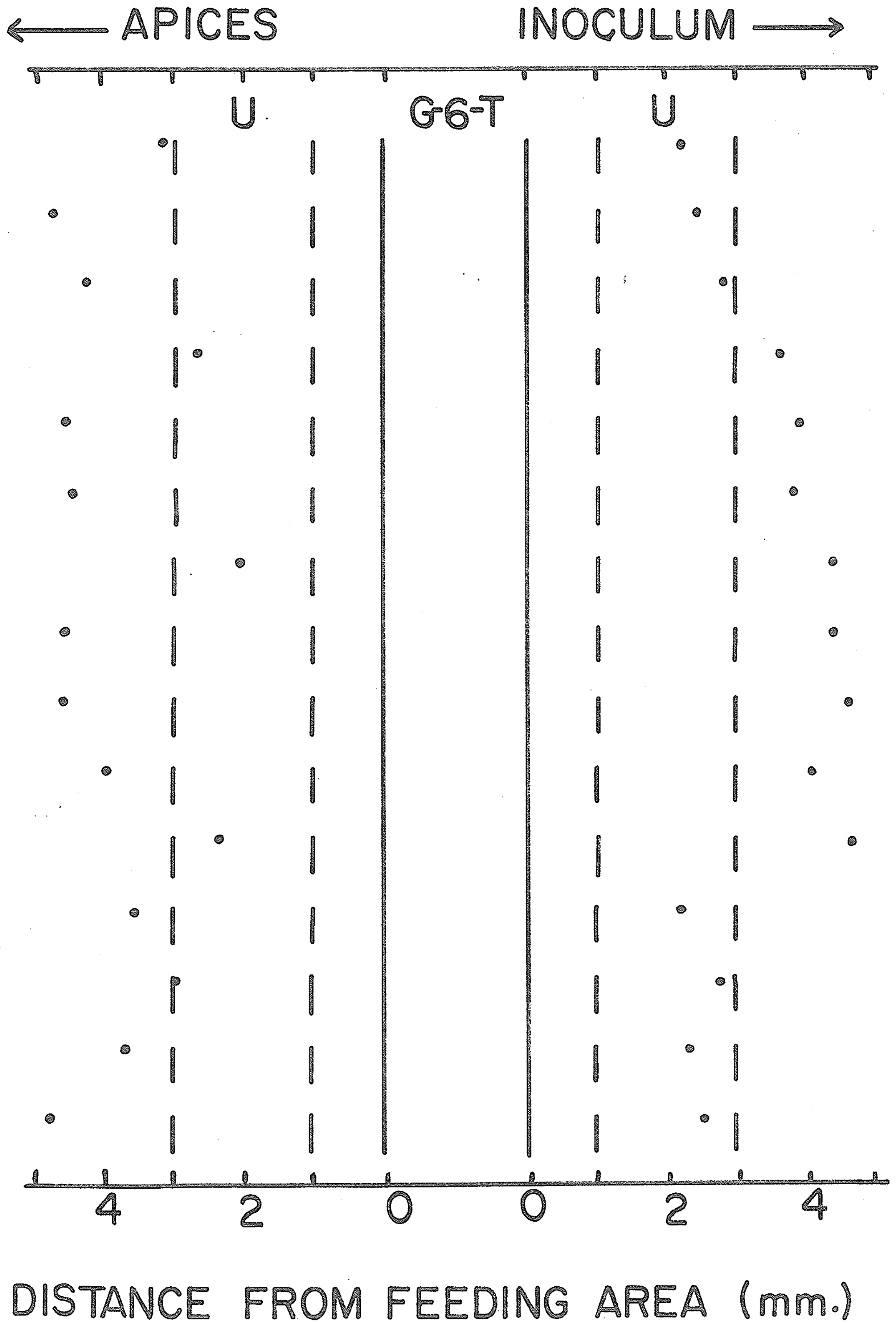


FIGURE 21. The effect of frequent constrictions on the rate of diffusion of fluorescein along strips of agar.

FIGURE 22. Translocation of glucose-6-T through and beyond an area in which uptake has been inhibited by uranyl acetate.

● = furthest point along hypha at which radioactivity could be detected.


APICES
INOCULUM
U
G-6-T
U
4
2
0
0
2
4
DISTANCE FROM FEEDING AREA (mm.)

DISCUSSION

The autoradiographic method has proven suitable for the investigation of uptake and translocation as it occurs at a hyphal level and it has been possible to study several properties of these phenomena which have previously been investigated only in bulk experiments.

I. UPTAKE

After short feeding periods, glucose-6-T and glycine-2-T can be detected in the hyphae of *Rhizoctonia solani*. Uniform feeding experiments indicate no localization of the compounds in young hyphae (Fig.1). Since translocation can be shown to occur at no more than 30-40 µ/hr. in this fungus (33) the lack of localization after a 1/2 minute feeding period indicates that the uptake sites must be less than 15-20µ apart.

That this is an actual uptake and not just adsorption on the cell wall is shown by the fact that the cells from which the cytoplasmic contents have been withdrawn show no activity (Fig.2). As further support of this the amount of uptake of glucose-6-T can be demonstrated to be directly related to the density (mass measurement) of the cytoplasm which suggests that the uptake is probably an active cytoplasmic transport rather than a passive diffusion (Fig.7).

The competetive inhibition shown between structurally similar compounds (Table I) is also characteristic of an active process (16,17).

There appears to be a common pathway for the uptake of the hexoses, glucose, galactose, fructose and mannose but the pentose, ribose, and

the disaccharide, sucrose,must have separate uptake mechanisms. This is similar to the results obtained with mammalian tissue culture cells by Rickenberg and Maio (47) who found that all the six carbon sugars tested inhibited galactose uptake but that there was no inhibition in the presence of ribose or any of the disaccharides that they tested.

The four amino acids tested showed competetive inhibition and therefore probably share a common uptake pathway. This low degree of specificity for amino acids differs from the systems described in bacteria (14) but resembles those of Botrytis (30,31) and Neurospora (8,38,62) and also animal tissues (21).

The possibility, that inhibition of uptake in the presence of a nutrient at a concentration of 1% might have resulted from the production of impermeable walls, can be eliminated by the fact that very short periods of pre-feeding with unlabelled glucose or glycine will inhibit the uptake of glucose-6-T or glycine-2-T probably as a result of the saturation of uptake sites (Table II).

The effects of metabolic inhibitors as shown in Table II suggest that uptake of glucose is a two step process similar to the one suggested for microorganisms and animal cells (11,61) and consisting of an energy-independent facilitated diffusion followed by an energy-dependent accumulation. The autoradiographic technique provides a convenient means of testing this hypothesis.

The uranyl ion, a non-penetrating, specific inhibitor for sugar uptake is believed to function by blocking the initial step of the

uptake process (4,49,50). If this step were blocked no uptake could be detected by the autoradiographic emulsion and this is in fact the result obtained when uranyl acetate and glucose-6-T are applied simultaneously.

Since the second step in the uptake process is energy-dependent it is sensitive to metabolic inhibitors which uncouple oxidative phosphorylation. The accumulation of glucose-U-C^{14} can be shown to be markedly inhibited by DNP (3×10^{-4} M.).

The autoradiographic emulsion cannot differentiate between the radioactive glucose which has been taken up by facilitated diffusion and that which has been actively accumulated in the bulk of the cytoplasm. If only the second step were blocked the facilitated diffusion would be unaffected and glucose-6-T would be detected in the hyphae.

However if the accumulation process were blocked the amount of glucose entering the facilitated diffusion should be proportional to the number of uptake sites and therefore the surface area and the grain count would be expected to be related to the hyphal diameter rather than to the density of the cytoplasm. If the active accumulation were not inhibited the amount of uptake would be expected to be related to the concentration of the cytoplasm and therefore the measurement of mass.

This is found to be the case (Figs. 12 and 13). In the absence of any inhibitors the uptake of glucose-6-T is proportional to the density, but in the presence of DNP uptake is no longer associated with the mass but is now directly related to the diameter of the hyphae.

Therefore the results obtained are all consistent with the view that uptake is a two step process consisting of an energy-independent facilitated diffusion across the membrane which accounts for the competetive inhibition and sensitivity to certain inhibitors such as uranyl acetate, followed by an energy-dependent accumulation process which accounts for sensitivity to uncoupling of oxidative phosphorylation and for accumulation against a concentration gradient.

II. TRANSLOCATION

Although Schutte (54) was able to demonstrate only very limited bi-directional translocation in the fungi that he studied Monson and Sudia (42) have shown that ions are readily translocated in either direction in *Rhizoctonia solani*. However a simultaneous movement in opposite directions has been demonstrated only in phloem cells (44).

Using the autoradiographic technique it has been possible to show that translocation of glucose in the mycelium of *R. solani* occurs simultaneously in all directions for an approximately equal distance from the initial point of feeding (Fig.16). The elliptical distribution of the silver grains around the point of feeding may be explained by the orientation of the hyphae. To move laterally in the mycelium the radioactive compounds must travel a greater distance through the hyphal branches than to move an equal distance toward the apices or inoculum along the trunk hyphae.

Grain counts indicate that the distribution of glucose-6-T along the hyphae from the point of feeding is not unlike the concentration

distribution that might be expected to result from diffusion along the hyphae but the scatter is too great to permit a mathematical analysis of the results (Fig.17).

The possibility that the glucose might be diffusing through the agar on the slide or a moisture film on the mycelium and then taken up by the hyphae along the way may be eliminated by two facts:

1. Diffusion of fluorescein through the agar film on the slide culture is not affected by the presence of the hyphae and is equal in all directions from a circular feeding area (Fig.20). Therefore a circular distribution of the radioactivity in the hyphae would be expected rather than the elliptical distribution obtained.
2. Activity can be detected in hyphae in and beyond an area where uranyl ion has been applied. The uranyl ion has been shown to completely inhibit any uptake of glucose (Fig.22). Therefore any radioactivity detected in the hyphae in these regions must have been taken up in an area that is not inhibited by uranyl ion and translocated through the hyphae.

However it seems unlikely that the glucose is simply diffusing through the cytoplasm after the initial uptake. The structure of the fungal hyphae with frequent septa with only a very small septal pore would be expected to slow the diffusion to such an extent as to make this mechanism impossible as is shown by the marked decrease in the distance travelled by fluorescein diffusing along an agar block with

frequent constrictions (Fig.21).

The simplest explanation would therefore appear to be that an active translocating mechanism is operating.

For translocation in phloem cells Spanner (57,58) and Fensom (20) have suggested an electro osmotic activated mass flow with the seive plate acting as a pump. Such a system however could not account for the bi-directional translocation that is found in R. solani.

It has often been suggested that translocation in the fungi is very closely associated with cytoplasmic streaming (9,25,54) but it seems unlikely that the low concentration of glucose-6-T used (.00028 mg/ml.) would induce cytoplasmic streaming in both directions from the point of feeding and near-by sources of a much higher concentration (1%) of unlabelled glucose do not affect translocation (Fig.19).

Isaac (29) has reported evidence of a movement of dry matter in one direction and implied a counterflow of fluids in the opposite direction in the hyphae of Rhizoctonia solani. Such a mechanism, similar to the counter current theory proposed for amoeboid movement by Kavanau (32) might account for the simultaneous translocation of glucose in both directions from the point of feeding.

SUMMARY

1. An autoradiographic method using tritiated compounds, autoradiographic emulsion and slide cultures has been developed to study the uptake and translocation of glucose and glycine by *Rhizoctonia solani* at a hyphal level.

2. There was found to be no localization of uptake sites.

3. Uptake was shown to have properties of an active transport; as demonstrated by the sensitivity to certain inhibitors, competetive inhibition by structurally similar compounds and saturation at high concentrations.

4. The relationships between uptake and cytoplasmic density and between uptake and diameter in the presence or absence of DNP have indicated that glucose uptake is a two-step process.

5. Translocation of glucose was found to occur simultaneously in both directions from the point of uptake and to be unaffected by feeding 1% unlabelled glucose or uranyl acetate at another point on the mycelium.

6. Some of the properties of normal diffusion have been studied.

7. A possible mechanism for translocation has been proposed.

BIBLIOGRAPHY

1. Arthur, J.C. The movement of protoplasm in coenocytic hyphae. Ann. Bot. 11. 491-507. 1897.

2. Bailey, N.T.J. Statistical Methods in Biology. John Wiley and Sons Inc., New York. 1959.

3. Barer, R. Phase, Interference and Polarizing Microscopy. Analytical Cytology. McGraw-Hill Co. 1959.

4. Barron, E.S.G., Muntz, J.A. and Gasvoda, B. Regulatory mechanisms of cellular respiration. I. The role of cell membranes: uranium inhibition of cellular respiration. J. Gen. Physiol. 32. 163-178. 1948.

5. Bennet-Clarke, T.A. and La Touche, C.J. The utilization of organic acids by Aspergillus niger. New Phytologist. 34. 211-231. 1935.

6. Bowyer, F. The kinetics of the penetration of non-electrolytes into the mammalian erythrocyte. Intern. Rev. Cytol. 6. 469-511. 1957.

7. Boyd, G.A. Autoradiography in Biology and Medicine. Academic Press, New York. 1955.

8. Brockman, H.E., de Busk, A.G. and Wagner, R.P. Mutant associated antagonisms in Neurospora crassa. Arch. Biochem. Biophys. 84. 455-461. 1959.

9. Buller, A.H.R. Researches on Fungi. Vol. 5. 1933. Longmans, Green and Co., London.

10. Burger, M., Hejmova, L. and Kleinzeller, A. Transport of some mono- and di-saccharides into yeast cells. Biochem. J. 71. 233-242. 1959.

11. Cirillo, V.P. Sugar transport in microorganisms. Ann. Rev. of Micro. 15. 197-218. 1961.

12. Cirillo, V.P. Transport of non-fermentable sugars across the yeast cell membrane. Membrane Transport and Metabolism. 343-351. 1960. Kleinzeller and Kotyk eds. Academic Press, London and New York.

13. Cirillo, V.P. Mechanisms of arabinose transport in Tetrahymena pyriformis. Journal of Bacteriology. 84. 754-758. 1962.

14. Cohen, G.N. and Monod, J. Bacteriological permeases. Bacteriol. Revs. 21. 169-194. 1957.

15. Conway, E.J. and Downey, M. An outer metabolic region of the yeast cell. Biochem. J. 47. 347-355. 1950.

16. Crane, R.K. Intestinal absorption of sugars. Physiol. Revs. 40. 789-825. 1960.

17. Danielli, J.F. Morphological and molecular aspects of active transport. S.E.B. Symposium VIII (Active Transport and Secretion) 502-516. 1954.

18. de Vries, H. Uber die bedeutung der circulation und der rotation des protoplasma fur stofftransport in der pflanze. Bot. Zeit. 43. 1-6, 16-26. 1885.

19. Dyson, J. Precise measurements by image-splitting. J. Opt. Soc. Amer. 50. 754. 1960.

20. Fensom, D.S. An electokinetic theory of transport. Canadian J. Bot. 35. 573-582. 1957.

21. Finch, L.R. and Hird, F.J.R. The uptake of amino acids by isolated segments of rat intestine. Biochem. Biophys. Acta. 43. 268-287. 1960.

22. Garrett, S.D. Soil Fungi and Soil Fertility. p.31. Pergamon Press. 1963.

23. Gow, C. M.Sc. thesis, University of Manitoba. 1958.

24. Grossbard, E. Autoradiography of fungi through a layer of soil and in agar culture. Nature. 182. 854-856. 1958.

25. Grossbard, E. and Stranks, D.R. Translocation of cobalt-60 and caesium-137 by fungi in agar and soil cultures. Nature. 184. 310-314. 1959.

26. Hale, A.J. The Interference Microscope in Biological Research. E. and S. Livingstone Ltd., Edinburgh and London. 1958.

27. Horecker, B.L., Osborn, M.J., McLellan, W.L., Avigad, G. and Asensio, C. The role of bacterial permeases in metabolism. Membrane Transport and Metabolism. 378-387. 1960. Kleinzeller and Kotyk eds. Academic Press, London and New York.

28. Isaac, P.K. Physiological specialization in the mycelium of Rhizoctonia solani Kuhn. Can. J. Microbiol. in press.

29. Isaac, P.K. Cytoplasmic streaming in filamentous fungi. Can. J. Bot. in press.

30. Jones, O.T.G. The accumulation of amino acids by fungi with particular reference to the plant parasitic fungus Botrytis fabae. J. Exptl. Bot. 14. 399-411. 1963.

31. Jones, O.T.G. and Watson, W.A. The uptake of amino acids by the fungus Botrytis fabae. Nature. 194. 947-948. 1962.

32. Kavanau, J.L. Protoplasmic streaming as a process of jet propulsion. Developmental Biology 7. 22-37. 1963.

33. King, M.K. and Isaac, P.K. Translocation of glucose-6-T in the mycelium of Rhizoctonia solani Kuhn. in preparation.

34. Kohler, E. Zur kenntnis der vegetativen anastromosen der Pilze II. (Mitterlung). Planta Bd. 10. 495-522. 1930.

35. Langeron, M. Precis de Mycologie. Paris, Masson et Cie. 1945.

36. Lawes, J.B., Gilbert, J.H. and Warrington, R. Contributions to the chemistry of fairy rings. Lond. Chem. Jour. 43. 208-233. 1883.

37. Le Fevre, P.G. The evidence for active transport of monosaccharides across the red cell membrane. S.E.B. Symposium VIII (Active Transport and Secretion) 118-135. 1954.

38. Mathieson, M.J. and Catcheside, D.G. Inhibition of histidine uptake in Neurospora crassa. J. Gen. Microbiol. 13. 72-83. 1955.

39. Mitchell, P. and Moyle, J. Osmotic functions and structure in bacteria. Soc. Gen. Microbiol. Symposium VI (Bacterial Anatomy). 150-180. 1956.

40. Melin, E. and Nilsson, H. Transfer of radioactive phosphorus to pine seedlings by means of mycorrhizal hyphae. Physiol. Plantarum. 3. 88-92. 1950.

41. Melin, E. and Nilsson, H. Translocation of cations to seedlings of Pinus virginiana through the mycorrhizal mycelium. Bot. Gaz. 119. 243-246. 1958.

42. Monson, A.M. and Sudia, T.W. Translocation in Rhizoctonia solani. Bot. Gaz. 124. 440-443. 1963.

43. Orskov, S.L. Investigations on the permeability of yeast cells. Acta Path. et Microbiol. Scand. 22. 523-559. 1946.

44. Palmquist, E.M. The simultaneous movement of carbohydrates and fluorescein in opposite directions in the phloem. Amer. J. Bot. 25. 97-105. 1938.

45. Paris, G.K. A simple nuclear stain and staining technique for Helminthosporia. Phytopath. 34. 700-702. 1944.

46. Pauli, F. Heidenhaim's Haemotoxylin in cytological studies of Penicillium notatum. St. Tech. 31. 91-95. 1956.

47. Rickenberg, H.V. and Maio, J.J. The accumulation of galactose by mammalian tissue culture cells. Membrane Transport and Metabolism. 409-422. 1960. Kleinzeller and Kotyk eds. Academic Press, London and New York.

48. Roberts, R.B., Abelson, P.H., Cowie, D.B., Bolton, E.T. and Britten, R.J. Studies of biosynthesis in Echerichia coli. Carnegie Inst. Wash. Publ. No. 607. 1-521. 1957.

49. Rothstein, A. Enzyme systems of the cell surface involved in the uptake of sugars by yeast. S.E.B. Symposium VIII (Active Transport and Secretion) 165-201. 1954.

50. Rothstein, A. and Meirer, R.C. The relationship of the cell surface to metabolism VI. The chemical nature of uranium complexing groups on the cell surface. J. Cellular Comp. Physiology. 38. 245-270. 1951.

51. Rosenberg, T. The concept and definition of active transport. S.E.B. Symposium VIII (Active Transport and Secretion) 27-41. 1954.

52. Rosenberg, T. and Wilbrandt, W. The kinetics of membrane transports involving chemical reactions. Exptl. Cell Research. 9.(1). 49-62. 1955.

53. Rosenberg, T. and Wilbrandt, W. Uphill transport induced by counterflow. J. Gen. Physiol. 41. 289-296. 1958.

54. Schutte, K.H. Translocation in fungi. New Phytologist. 55. 164-182. 1956.

55. Schantz, H.L. and Piemeisel, R.L. Fungus fairy rings in eastern Colorado and their effect on vegetation. J. Agri. Res. 11. 191-245. 1917.

56. Somers, E. Uptake of copper by fungal cells. Ann. Appl. Biol. 51. 425-437. 1963.

57. Spanner, D.C. The translocation of sugar in the seive tubes. J. Exptl. Bot. 9. 332-342. 1958.

58. Spanner, D.C. A note on the velocity and energy requirements of translocation. Annals of Botany. 26. 511-516. 1962.

59. Tomkins, R.G. Growth of moulds. Proc. Royal. Soc. Series B. 105. 375-401. 1930.

60. Tribe, H.T. Ecology of microorganisms in soils as observed during their development upon buried cellulose film. Soc. for General Microbiol. Symposium VII (Microbial Ecology) 287-298. 1957.

61. Widdas, W.F. Facilitated transfer of hexose across the human erythrocyte membrane. J. Physiol. 125. 163-180. 1954.

62. Zalokar, M. The kinetics of amino acid uptake and protein synthesis in Neurospora. Biochem. Biophys. Acta. 46. 423-32. 1961.

A P P E N D I X

TABLE A

Determinations of Diameter, Mass and Grain Count
- Feeding Glucose-6-T to Mature Hyphae
(10 days)

Diameter (μ)	Mass (pica grams/μ)	Grain Count (no./μ)
6.9	4.5	2
7.4	11.9	52
7.4	12.5	50
7.1	7.7	12
6.9	10.5	34
2.3	1.1	2
6.3	8.9	36
8.3	10.3	45
6.3	5.7	20
7.7	6.9	30
6.9	11.9	30
6.9	9.5	29
5.7	6.9	32
5.3	8.6	29
6.3	7.2	32
6.9	10.3	32
6.9	12.0	26
6.0	9.7	45
6.9	9.9	35
6.3	5.9	27
6.3	5.9	15
6.0	.6	1
6.0	9.7	45
6.9	10.8	40
6.9	9.9	45

TABLE A CONTINUED

Diameter (µ)	Mass (pica grams/µ)	Grain Count (no./µ)
6.3	6.4	26
6.3	.6	2
6.3	.6	0
6.3	5.7	32
5.7	.6	0
5.7	.6	4
5.7	1.5	0
6.9	1.6	3
6.3	.6	1
5.7	2.9	6
6.3	6.7	45
6.9	1.8	1
8.3	12.0	52
6.9	9.1	32
6.9	3.9	43
6.9	.7	0
6.3	1.4	0
6.3	.6	0
8.3	11.3	52
9.1	11.3	40
9.1	12.8	2
6.9	4.5	14
6.9	4.5	14
6.3	1.1	5
6.3	2.8	8
9.1	13.0	46
6.3	1.3	0
7.4	9.2	42

TABLE A CONTINUED

Diameter (μ)	Mass (pica grams/μ)	Grain Count (no./μ)
7.4	9.2	42
5.7	2.9	0
5.7	3.0	0
5.7	.6	0
8.0	5.3	28

TABLE B.

Determinations of Diameter, Mass and Grain Count
- Feeding Glucose-6-T in the Presence of
DNP 3x10-4M to Mature Hyphae (10 Days)

Diameter (µ)	Mass (pica grams/µ)	Grain Count (no./µ)
6.3	3.2	20
6.3	4.5	20
6.3	4.2	19
5.6	3.7	17
6.8	5.7	28
6.6	2.9	15
6.6	5.0	14
6.3	5.7	20
6.5	1.6	21
6.5	3.0	21
4.3	1.6	12
6.9	2.6	22
6.6	3.3	20
6.6	3.6	19
6.5	2.9	19
6.1	3.3	18
6.5	2.9	19
5.1	2.5	14
4.3	2.1	11
7.5	7.1	33
6.3	2.9	18
6.3	5.2	15
6.3	5.2	26
6.5	2.2	18
6.5	2.2	18
7.1	5.1	22

TABLE B CONTINUED

Diameter (μ)	Mass (pica grams/μ)	Grain Count (no./μ)
6.4	2.8	18
7.3	3.5	22
5.6	2.7	16
6.4	3.1	19
5.9	2.8	16
5.6	2.9	15
6.4	4.2	19
7.8	5.2	27
6.3	3.1	19
8.0	6.4	33
6.0	3.5	18
6.0	3.5	18
6.0	4.3	17
5.0	1.2	15
6.6	3.2	21
6.0	2.9	19
6.0	2.9	18
5.9	5.0	10
5.0	2.0	12
6.1	4.1	19
6.8	3.9	23
6.8	3.9	23
6.3	3.7	19
6.1	3.5	19

TABLE C

Determination of Diameter and Mass in Young Hyphae
(3 days)

Diameter (µ)	Mass (pica grams/µ)
6.3	5.5
10.2	17.3
10.2	16.7
8.8	11.4
7.3	6.7
15.8	4.6
8.5	10.2
9.8	17.2
9.8	17.2
8.8	10.6
8.0	5.8
8.0	5.8
8.0	9.6
8.0	9.6
10.2	16.7
10.2	16.7
5.8	5.7
8.3	11.4
9.8	14.7
10.2	16.1
9.8	14.7
10.0	15.4
10.5	16.6
9.5	13.5
5.5	3.3
9.8	13.9

TABLE C CONTINUED

Diameter (μ)	Mass (pica grams/μ)
9.5	13.9
9.5	11.0
10.2	16.1
8.0	10.7
10.5	17.4
10.5	17.4
8.3	11.6
8.0	10.7
8.0	13.0
10.0	14.0
9.0	13.7
9.0	13.7
9.8	15.7
5.5	6.1
8.8	12.0
10.0	17.4
10.0	17.4
8.8	12.0
9.5	13.8
9.3	12.3
9.8	12.9
9.0	11.9
9.8	14.3