Steady State Models of Spore Cell Metabolism in
Dictyostelium discoideum*

Yoke Yin Chiew, Jacqueline M. Reimers, and Barbara E. Wright
From the Department of Microbiology, University of Montana, Missoula, Montana 59812

(Received for publication, April 26, 1985)

Young sorocarp (consisting of a mass of spore cells
resting on a stalk) were exposed to low levels of [U-14C]glucose and the spore cells were rapidly separated
from stalk cells. Metabolites were isolated from spores
and their specific radioactivities compared to these
metabolites isolated from the whole organism; i.e.
spore plus stalk cells. Based on these data, known
reaction rates, and metabolite concentrations, highly
constrained steady state models of metabolism in spore
and stalk cells were constructed. Direct evidence has
been obtained which substantiates earlier predictions
regarding cell permeability, the distribution of specific
metabolites, and the location of reactions in vivo.

The life cycle of Dictyostelium discoideum consists of two
distinct phases: growth of unicellular myxamoeba when a food
source is available and, on starvation, differentiation into a
multicellular sorocarp. The major cell types in the maturing
sorocarp are the spore cells and the stalk cells: the latter are
becoming vacuolated and dying. Investigations in numerous
laboratories over many years have accumulated a wealth of
information on the metabolism: pathways, reaction rates, and
metabolite levels in D. discoideum. Until recently, few at-
ttempts have been made to integrate all these data into a
dynamic metabolic picture of what occurs in the differentiat-
ing organism. The computer has become invaluable as a tool
in setting up steady state models to simulate metabolite fluxes
and concentrations at specific stages of differentiation (1-3).
The validity of these kinetic models to the in vivo process and
their value as an analytical tool are demonstrated by their
predictive value.

Recently, analyses of steady state models of carbohydrate
metabolism in D. discoideum (1) have resulted in a number of
predictions; viz. (a) mature stalk cells are more permeable
than presumptive spore and stalk cells to exogenous sub-
strates, (b) there is a single pool of uridine diphosphoglucose
and fructose 6-phosphate (Fru-6-P) present in the presum-
tive cell types of a sorocarp, and (c) there are separate (i.e.
cell type-specific) pools of glycogen, glucose 6-phosphate (Glu-
6-P), and glucose 1-phosphate (Glu-1-P) in the mature stalk

cells and in the presumptive spore and stalk cells (hereafter
referred to as spore cells).

The isolation and analysis of spore cells has provided direct
evidence substantiating these predictions. D. discoideum at
the young sorocarp developmental stage was exposed to low
levels of [U-14C]glucose. The spore cells were then separated
from stalk cells. The metabolites, indicated in Fig. 1, were
isolated from the spore cells (represented by the upper circular
part) and from the whole organism to determine their specific
radioactivities. Knowing their total (organismal) cellular lev-
els, many of the indicated fluxes in vivo, and the specific
radioactivities of spore-specific metabolites, the curve-match-
ing computer program TFLUX was used to determine the
specific radioactivities of stalk-specific metabolites, as well as
unknown reaction rates and the relative distribution of certain
metabolites in the two cell types. With the additional experi-
mental data available, the constraints on the model param-
ters were again examined.

MATERIALS AND METHODS

Growth and Incubation Conditions

D. discoideum strain NC-4 (ATCC 24697) was grown with Esche-
richia coli on nutrient agar at 23 °C as previously described (4).
Amoeba were harvested, washed free of bacteria, and spread on 1.5%
agar containing 10 mM phosphate buffer, pH 6.5, and 1 mM EDTA.
When the cells had differentiated to the young sorocarp stage, ap-
proximately 2.5 g dry weight were removed from the agar and incu-
batated for 40 min in 100 ml of 5 mM phosphate buffer, pH 6.5, at
23 °C, in the presence of about 10 μmol of [U-14C]glucose (ICN; 1.7 
× 105 cpm/μmol). The exact specific radioactivity of the [U-14C]
glucose was determined by counting a sample in 5 ml of Aqualos 2
(New England Nuclear), using a Beckman Liquid Scintillation Coun-
ter (Model LS7500) and by quantitation of glucose using a coupled
hexokinase/Glu-6-P dehydrogenase assay (Sigma). The cells were
then pelleted by centrifugation (1000 × g for 3 min). A third of the
cell pellet was washed twice with 10 mM MES buffer, pH 5.5, and
stored at -60 °C. The rest of the cells were resuspended in one cell-
volume of 10 mM MES buffer and vortexed at maximum speed for 1
min (using a Vortex Genie, Science Industries Inc.). Four cell-volumes
of buffer were then added, rapidly mixed, and the cell suspension
filtered through Whatman No. 114 paper by vacuum suction. The
cells in the filtrate were pelleted by centrifugation and washed once
in MES buffer. This preparation of spore cells was stored at -60 °C.
The stalk cells and some trapped spore cells retained on the filter
paper were discarded. We were unable to devise a rapid method for
obtaining a clean stalk preparation.

Permeability Experiment

The penetration of trypan blue dye (M, ~ 960,500) into the cells
was used as an indication of cell permeability. Samples (0.5 ml) of
cell preparations were suspended in 0.1 ml of 0.4% trypan blue
solution. After 5 min the cells were examined with a light microscope
dye penetration.
Intracellular metabolites were extracted from the frozen cell pellet with 12% trichloroacetic acid and kept in an ice bath. The pellet was extracted twice with the acid. The combined acid supernatants were extracted twice with ether and the glycogen was precipitated with ethanol as described previously (1). The final supernatant was prepared for anion-exchange chromatography and sugar and sugar phosphates separated on a borate column according to a modification of the procedure of Thompson (5) as described by Wright et al. (1). UDP-Glc was eluted from the column with a linear gradient of sodium chloride solution (0.4-0.7 M).

Sugar Phosphates

Radioactive fractions eluted at the known locations of the various metabolic intermediates were separately desalted using a AG 50W-X8 column. The eluents were lyophilized and the remaining borate removed by repeated methanol evaporation. The final preparation of each intermediate was dissolved in 100 μl of water. Glu-6-P, Fru-1-P, and Glu-1-P were purified by descending paper chromatography on Whatman No. 1 paper for two, consecutive 40-h runs in a solvent containing 1-butanol:pyridine:water:formic acid (v/v, 6:4:3:1.05). The paper was dried between runs. The particular sugar phosphate was eluted from the paper, lyophilized, and then treated with acid phosphatase. The liberated sugar was isolated by descending paper chromatography for 48 h in the same above described solvent system. The sugar was eluted, lyophilized, and finally purified by high performance liquid chromatography (HPLC) using a Perkin-Elmer Series 4 HPLC instrument. The sugar was eluted from a Bio-Sil Amino-5S column, eluting with 90% acetonitrile. After each purification step, the particular sugar phosphate or sugar was quantitated by enzymic assays and a small aliquot counted. Enzymes used were obtained from Sigma. Glu-6-P was assayed using Glu-6-P dehydrogenase; Fru-6-P using Glu-6-P dehydrogenase and Glu-6-P isomerase; Glu-1-P using Glu-6-P dehydrogenase and phosphoglucomutase; glucose using hexokinase and Glu-6-P dehydrogenase; and fructose with hexokinase, Glu-6-P isomerase, and Glu-6-P dehydrogenase. Internal standards were used in all assays, and corrections were made for any inhibition due to the presence of residual solvents or salts. Samples were counted in 5 ml of Aquasol 2 in precounted glass vials.

Trehalose

Trehalose was isolated from desalted trehalose containing fractions by HPLC using a Bio-Sil Amino-5S column, eluting with 90% acetonitrile. The trehalose was then converted to glucose with trehalase prepared according to the method of Killick (6). The liberated glucose was isolated by HPLC on the same column described above and quantitated as described.

Glycogen

Glycogen precipitated from the trichloroacetic acid extract with ethanol (see above) was dissolved in 2 ml of 1% lithium chloride. The specific radioactivity of the glycogen was determined by the following methods.

**Acid-extracted Glycogen**—Duplicate samples, 0.2 ml each, were removed to a screw-topped tube. To each sample was added 0.5 ml of 1% lithium chloride and 1.0 ml of 95% ethanol. The solution was mixed thoroughly. The tube was capped tightly and then boiled for 5 min, after which it was kept at -16 °C for 20 min. The solution was centrifuged for 10 min at 1000 × g at 4 °C. The supernatant was discarded and the pellet redisolved in 0.5 ml of lithium chloride and 1.0 ml of ethanol. The procedure was repeated twice. The final pellet was dissolved in 2.0 ml of water and the amount of glycogen present was estimated using the anthrone method (7) with glucose as standard. A sample was removed for counting in 5 ml of Aquasol 2.

**Alkali-extracted Glycogen**—Duplicate samples, 0.2 ml each, of the glycogen solution in 1% lithium chloride were removed to screw-topped tubes. Each sample was thoroughly mixed with 0.5 ml of 95% ethanol, the tube capped, and the contents boiled for 5 min. The tube was then kept at -16 °C for 20 min after which glycogen was pelleted by centrifugation (1000 × g, 10 min). The pellet was dissolved in 0.3 ml of water and 0.3 ml of 60% potassium hydroxide was added. The solution was boiled for 30 min, cooled, and then centrifuged. The supernatant was removed to a screw-topped tube, 0.5 ml of 95% ethanol was added, mixed well, and the tube capped tightly. The tube was then kept at -16 °C for 20 min. Glycogen was recovered as the pellet after centrifugation and washed twice in lithium chloride and ethanol as described for acid-extracted glycogen. The glycogen was finally dissolved in 2.0 ml of water and its specific radioactivity determined as described.

**UDP Glucose**

The UDP-glucose eluted from the column was adsorbed on acid-washed charcoal, eluted, and isolated by two-dimensional thin layer chromatography on prewashed Bakersflex polyethyleneimine cellulose plates according to the procedure of Pannbacker (8). The specific radioactivity of UDP-Glc was determined by measuring the A400 of the eluted UDP-Glc spot and counting the entire sample. The specific radioactivity of UDP-Glc was also ascertained by hydrolyzing a purified sample with 0.1 N HCl at 100 °C for 20 min. The acid was removed by rotary evaporation and the liberated glucose isolated by paper chromatography or HPLC. The specific radioactivity of the glucose moiety was determined as described above.

**Computer Simulation Using TFLUX**

Values of most of the model input conditions, e.g. reaction rates and metabolite concentrations, were derived from experimental data presented here or from Wright et al. (1). Those conditions not determined experimentally (e.g. unknown fluxes) were adjusted until a match with the experimental labeling patterns (Table I) was obtained using the computer program TFLUX. The TFLUX program is described in detail elsewhere (9). Briefly, if we assume that the
The specific radioactivity of cellular metabolites following a 40-min incubation in the presence of [U-\(^{14}\)C]glucose

| Experiment | Incubation medium (EXGLU) | Cell type | Specific radioactivity |
|------------|---------------------------|-----------|-----------------------|
|            | Molarity                  | Specific radioactivity | Glc-6-P | Fru-6-P | Glu-1-P | UDP-Glu | TRE     |
| A          | 0.115                     | 1610.0    | 8.3      | 2.3     | 12.0     | 12.3    | 23.1    |
| B          | 0.098                     | 1750.0    | 19.6     | 3.8     | 26.0     | 12.6    | 24.4    |
| C          | 0.100                     | 1690.0    | 3.9      | 1.3     | 4.0      | 3.2     | 3.5     |
| D          | 0.200                     | 900.0     | 5.0      | 3.9     | 2.0      | 4.3     | 15.7    |

*The abbreviations used are: EXGLU, exogenous radioactive glucose; TRE, trehalose.

The system is in a metabolic steady state, then a set of linear differential equations can be used to describe the specific radioactivities of the metabolites over time. To use TFLUX, the user specifies a number of pools, giving the name, size (sum of labeled plus unlabeled), and initial specific radioactivities of each pool (in this case, 0 for all pools except exogenous glucose). The interconnections between pools are specified by giving the constant flux (labeled plus unlabeled) between any pair of pools. Flux is expressed as millimolar concentration/min, which is equivalent to micromoles/min/ml of cell volume. It is also possible to specify "external" pools, whose properties include infinite size and constant specific radioactivity. From the input, the program then computes each pool's specific radioactivity as a function of time. A tape of this program and a user's manual are available upon request.

**RESULTS**

Preparation of Spore Cells—A method for the rapid separation of spore cells from stalk cells after incubation with [U-\(^{14}\)C]glucose was necessary in order to compare spore cell metabolism to whole organism metabolism under in vivo conditions. Furthermore, a relatively large spore mass was required in order to have sufficient material for the numerous manipulations needed to obtain pure samples of each metabolite. Shaking of a sorocarp cell suspension at maximum speed on a vortex mixer for 1 min was sufficient to dislodge spore cells from the stalk. Filtration through Whatman No. 114 filter paper by vacuum suction allowed rapid separation of the spore cells from 20 to 30 ml of packed cell volume of D. discoideum. Whatman No. 114 filter paper retained material of 20–25-μm particle size, allowing the smaller spore cells to filter through and retaining the larger stalk cells. In the present study, it was necessary to use organisms at a later stage of differentiation than those described previously (1). A good yield of spores could only be obtained using young sorocarps between 23 and 27 h old, whereas the organisms in the previous investigation were on the average at culmination (20–22 h). Metabolic differences between young and older sorocarp preparations are noted below.

Permeability to Trypan Blue Dye—Fig. 2A shows a typical spore preparation after filtration of sorocarps through Whatman No. 114 paper. The filtrate consisted almost entirely of spore cells. Fig. 2B shows the penetration of trypan blue dye into a few of the larger, irregular shaped stalk cells, but not into the spore cells. Dye penetration also occurred in the stalk cells still associated with the sheathes (not shown).

[\(^{14}\)C]Glucose Labeling of Metabolites—The results from four experiments in which young sorocarps were exposed to tracer [U-\(^{14}\)C]glucose, as described under "Materials and Methods," are presented in Table I. An incubation period of 40 min was chosen for the reasons given previously (1). Metabolites obtained from "mix" cell types represent the isolation of metabolites from whole organisms in which the spore metabolite pools and stalk metabolite pools mix during cell rupture. The specific radioactivity of a compartmented metabolite will equal the total number of counts in both pools divided by the total number of micromoles in both pools.

As stated above, somewhat older sorocarps were used in order to rapidly obtain a clean preparation of spores according to the method described. Apparently stalk metabolism had almost ceased, while the rate of synthesis and accumulation of trehalose was maximal; much higher specific radioactivity of this disaccharide were found in this study as compared to our earlier investigation (1). Whittingham and Raper (10)
demonstrated that stalk cells degenerate and die. During the construction and maturation of sorocarps, changes in metabolite levels and flux are extremely rapid. Glycogen synthase, 5\'-AMP nucleotidase, glycogen, RNA, trehalose, and glucose are no longer detectable as stalk cells mature (11); net glycogen (12) and RNA (13) degradation stop abruptly at this time. The stalk cells become vacuolated and highly permeable (Fig. 2), consistent with the simulated higher rate of exchange of exogenous glucose with stalk as compared to spore glucose (1).

The specific radioactivity of Fru-6-P, UDP-Glc, and trehalose (Table I) isolated from spores were similar to those from whole organisms, suggesting that there was only one pool of each of these intermediates and that that pool was present in spore cells. The similarity in specific radioactivity of glycogen isolated from the spore and the whole organism suggests that there was rapid turnover of the spore glycogen only and no turnover of the stalk glycogen and/or that the stalk pool of glycogen was very small compared to the spore pool. On the other hand, mix Glu-6-P, and in two cases mix Glu-1-P obtained from the whole sorocarp were two to three times higher in specific radioactivity than when obtained only from spore cells. This indicated that these two metabolites exist as separate pools in the stalk and in the spore. The exception was Experiment C, where the specific radioactivities of these intermediates in the spore and the whole organism were similar. Older sorocarps were used in Experiment C than in the other experiments and the stalk cells may have been metabolically dead. If so, only spore pools were isolated from the whole organism. Fru-6-P values were always lower than spore Glu-6-P, again with the exception of Experiment C, suggesting that Fru-6-P was cooled by a cold intermediate or precursor (presumably RNA degradation products; see Ref. 3). In the spore, the specific radioactivities of UDP-Glc was similar to spore Glu-1-P indicating that spore Glu-1-P is the precursor of UDP-Glc. Thus, the data in general supported our contention of separate stalk and spore pools of glucose 6-phosphate and glucose 1-phosphate and only one pool each of Fru-6-P, UDP-Glc, and trehalose.

In an ongoing investigation in this laboratory, soluble glycogen isolated after perturbation of \textit{D. discoideum} metabolism with 50 mM \([U-14]C\)glucose yielded an alkali-extractable fraction with a specific radioactivity higher than the acid-extractable glycogen.\(^2\) It was therefore of interest to explore whether glycogen of different specific radioactivity could be obtained with the two methods of extraction from organisms incubated in a nonperturbing concentration of glucose (0.1-0.2 mM).

Table I showed that nearly similar specific radioactivities were obtained with alkali and acid extraction of glycogen.

The specific radioactivity of Fru-6-P, UDP-Glc, and trehalose (Table I) isolated from spores were similar to those from whole organisms, suggesting that there was only one pool of each of these intermediates and that that pool was present in spore cells. The similarity in specific radioactivity of glycogen isolated from the spore and the whole organism suggests that there was rapid turnover of the spore glycogen only and no turnover of the stalk glycogen and/or that the stalk pool of glycogen was very small compared to the spore pool. On the other hand, mix Glu-6-P, and in two cases mix Glu-1-P obtained from the whole sorocarp were two to three times higher in specific radioactivity than when obtained only from spore cells. This indicated that these two metabolites exist as separate pools in the stalk and in the spore. The exception was Experiment C, where the specific radioactivities of these intermediates in the spore and the whole organism were similar. Older sorocarps were used in Experiment C than in the other experiments and the stalk cells may have been metabolically dead. If so, only spore pools were isolated from the whole organism. Fru-6-P values were always lower than spore Glu-6-P, again with the exception of Experiment C, suggesting that Fru-6-P was cooled by a cold intermediate or precursor (presumably RNA degradation products; see Ref. 3). In the spore, the specific radioactivities of UDP-Glc was similar to spore Glu-1-P indicating that spore Glu-1-P is the precursor of UDP-Glc. Thus, the data in general supported our contention of separate stalk and spore pools of glucose 6-phosphate and glucose 1-phosphate and only one pool each of Fru-6-P, UDP-Glc, and trehalose.

In an ongoing investigation in this laboratory, soluble glycogen isolated after perturbation of \textit{D. discoideum} metabolism with 50 mM \([U-14]C\)glucose yielded an alkali-extractable fraction with a specific radioactivity higher than the acid-extractable glycogen.\(^2\) It was therefore of interest to explore whether glycogen of different specific radioactivity could be obtained with the two methods of extraction from organisms incubated in a nonperturbing concentration of glucose (0.1-0.2 mM). Table I showed that nearly similar specific radioactivities were obtained with alkali and acid extraction of glycogen.

Definition of the Models—The data and assumptions on which the models are based may be summarized as follows: (a) the demonstrated reactions depicted in Fig. 1, (b) the initial specific radioactivity and molarity of exogenous \([U-14]C\)glucose associated with the cells, (c) the total cellular concentrations of 7 metabolites, expressed as millimolar concentration cell volume (Table II), (d) the demonstrated intercellular metabolic compartmentation of the glucose (14) and glycogen (11), (e) the predicted compartmentation of the hexose phosphates (1, 3, 15), (f) the assumption and evidence that UDP-Glc and Fru-6-P exist only in the spores (Ref. 1 and Table I), (g) the fluxes of 11 key reactions which have been determined with isotopes in vivo and/or from the rate of metabolite net accumulation or utilization: the rate of glycogen synthesis, turnover and net degradation, the net degra-

| Metabolite          | Concentration of mM packed cell volume of total organism | Data | Experiment A model\(^a\) | Experiment B model\(^a\) |
|---------------------|----------------------------------------------------------|------|--------------------------|--------------------------|
| Exogenous glucose   | ~0.1                                                     | 0.115| 0.098                    |
| UDP-Glu             | 0.1-0.4                                                  | 0.2  | 0.12                     |
| Fru-6-P             | 0.04                                                    | 0.009| 0.009                    |
| Total trehalose     | 1.0-3.0                                                  | 1.45 | 1.80                     |
| Total glycogen      | 17.0                                                    | 10.95| 7.0                      |
| GLY                  | 1.5                                                     | 2.8  |
| GLY2                 | 8.45                                                    | 2.9  |
| STGLY                | 1.0                                                     | 1.3  |
| Total Glu-6-P       | 0.02-0.1                                                | 0.028| 0.033                    |
| Spore Glu-6-P       | 0.024                                                   | 0.029|
| Stalk Glu-6-P       | 0.004                                                   | 0.004|
| Total Glu-1-P       | 0.003-0.02                                             | 0.006| 0.006                    |
| Spore Glu-1-P       | 0.002                                                   | 0.002|
| Stalk Glu-1-P       | 0.004                                                   | 0.004|
| Total glucose       | 0.04-0.1                                                | 0.055| 0.025                    |
| Spore glucose       | 0.005                                                   | 0.005|
| Stalk glucose       | 0.05                                                    | 0.02 |

\(^a\)Taken from Wright et al. (1).

\(^b\)Values were taken from data when available, and were otherwise determined empirically as the best values in the model for matching the specific radioactivity data for all metabolites.

The abbreviations used are: GLY, spore glycogen participating in the glycogen cycle; STGLY, degraded stalk glycogen.

...dation of RNA (represented as ribose 5-phosphate exchanging with Fru-6-P), and the rate of synthesis of trehalose, cellulose, insoluble cell-wall glycogen, and mucopolysaccharide (Table III), and (h) the assumption of metabolic steady state.

Given the range of experimentally determined metabolite levels and reaction rates (Tables II and III), and the spore specific radioactivity data (Table I), the unknown flux rates and metabolite compartmentation ratios were varied to obtain the best fit to the specific radioactivity data set as a whole (Table IV). The models were expected to be more constrained than those described previously, as the specific radioactivities of the spore metabolites were now known.

**Simulation Analysis**—Experiments A and D were simulated using the metabolite concentrations and fluxes shown in Tables II and III. Table IV shows the specific radioactivity output of the models with these parameters. As a consequence of the TFLUX program condition, where flux into a metabolite pool must be equal to flux out, it was necessary to invoke an external pool for the end products that are accumulating, \textit{viz.} cellulose, trehalose, cell wall glycogen, and mucopolysaccharides. To more truly estimate the rate of accumulation of trehalose, the cellular concentration of trehalose was set large (50 mM) which trapped >95% of the radioactivity flowing through the trehalose pool to an external pool. Division of the total counts trapped as trehalose by the specific radioactivity of isolated trehalose in the experiments gave a pool size within the experimental data of 1.0-3.0 \(\mu\)mol/ml packed cell volume (12).

Fig. 3 shows the general shape of the labeling patterns for the metabolites isolated in Experiment A as simulated by the best fit computer kinetic model over a period of 40 min. In our previous analysis it was not possible to deduce directly the specific radioactivity of stalk Glu-6-P and stalk Glu-1-P. This simulation indicates that, compared to spore Glu-6-P and Glu-1-P, stalk Glu-6-P and stalk Glu-1-P became more radioactive in less than 2 min after incubation in \([U-14]C\) glucose, reflecting the greater permeability of the stalk cells. Stalk Glu-1-P had a different labeling pattern from spore

\(^2\)P. Befumo and B. E. Wright, unpublished observations.
A comparison of reaction rates determined experimentally with values used in models of Experiments A and B

| Reaction                  | Data* | Experiment A model* | Experiment B model* |
|---------------------------|-------|---------------------|---------------------|
| EXGLU → GLU              | 0.007 | 0.01                |
| EXGLU → STGLU            | 0.05  | 0.05                |
| Glu-1-P → Glu-6-P        | 0.11  | 0.105               |
| Fru-6-P → ribose-5-P     | 0.005 | 0.005               |
| Glu-1-P → Glu-6-P        | 0.03  | 0.09                |
| Glu-1-P → UDP-Glu        | ~0.16 | 0.12                |
| Glu-6-P → Fru-6-P        | 0.04  | 0.035               |
| Glu-6-P → Glu-1-P        | 0.08  | 0.16                |
| Glu-6-P → Glu-6-P        | 0.03  | 0.03                |
| Glu-6-P → TRE            | 0.01-0.03 | 0.02                | 0.04               |
| GLY → Glu-1-P            | ~0.13 | 0.17                | 0.0                |
| GLY2 → Glu-1-P           | 0.05  | 0.01                | 0.05               |
| Ribose-5-P → Fru-6-P     | 0.02  | 0.095               | 0.115              |
| UDP-Glu → Cellulose      | 0.03  | 0.025               | 0.02               |
| UDP-Glu → GLY            | ~0.16 | 0.17                | 0.05               |
| UDP-Glu → IN-GLY         | ~0.02 | 0.015               | 0.01               |
| UDP-Glu → TRE            | 0.01-0.03 | 0.02                | 0.04               |
| STGLP → STG6P            | 0.009 | 0.002               |
| STGLU → STG6P            | 0.03  | 0.03                |
| STGLY → STG1P            | 0.05  | 0.015               | 0.03               |

*Values were taken from data when available, and were otherwise determined empirically as the best values in the model for matching the specific radioactivity data for all metabolites.

The abbreviations used are: EXGLU, exogenous radioactive glucose; STGLU, stalk glucose; GLU, spore glucose; TRE, trehalose; STG1, stalk glucose 1-phosphate; STG6P, stalk glucose 6-phosphate; STGLY, degraded stalk glycogen; IN-GLY, insoluble cell wall glycogen; MUCO-P, mucopolysaccharide; GLY, spore glycogen participating in the glycogen cycle.

Evidence for Compartmentation—The penetration of trypan blue dye into stalk cells but not spore cells demonstrated the greater permeability of stalk cells which had been postulated (1). Results presented in Table I also supported our earlier predictions (1) that glucose 6-phosphate, glucose 1-phosphate, and hence glucose exist in two metabolically independent compartments: a spore compartment of low specific radioactivity and a stalk compartment of high specific radioactivity. Furthermore, the similar values of Fru-6-P and UDP-Glc isolated from spores and from the whole organism argued for one pool in the spores, as did their low specific radioactivities. The best fit computer simulation of Experiment A gave values of 86.0 × 10⁴ and 32.6 × 10⁴ cpm/μmol for stalk Glu-6-P and stalk Glu-1-P, respectively. The existence of a stalk pool of UDP-Glc would have led to the isolation of higher specific radioactivity mix UDP-Glc. Furthermore, the specific radioactivity of spore Glu-1-P was similar to that of UDP-Glc (see Fig. 3), consistent with a precursor-product relationship. The comparable specific radioactivities of trehalose and of glycogen isolated from spores as compared to the whole organism pointed to the spore as the site of synthesis, and indicated that the pool sizes of these compounds, if present in sorocarp stalk cells, must be small.

Trehalose Synthesis—As mentioned earlier, the sorocarps used in the present experiments were a few hours older than those used previously (1). This age difference is consistent with the lower total levels of all three hexose phosphates and glycogen in the present models compared to our earlier models.
shown. B (see text A, shows the pathways of glycogen metabolism proposed for Experiment 111) showed that rapid synthesis and/or turnover of glycogen was necessary to model glycogen metabolism differently in than those in Experiment A (24 h), yet the isolated glycogen in the former was higher in specific radioactivity (Table I), indicating active glycogen turnover late into the sorocarp development stage. In our computer simulation analysis, it was necessary to model glycogen metabolism differently in Experiments A and B in order to match the model specific radioactivity output with the data (Fig. 4). The metabolic configuration for Experiment A (Model A) closely resembles that proposed for culmination cells (1), with net glycogen decreasing at a rate of 0.01 mM/min. The turnover glycogen pool remained small at 1.5 mM. However, data from Experiment B could only be matched with Model B configuration shown in Fig. 4. Here the spore glycogen participating in the glycogen cycle pool accumulates (represented by flux to an external pool) at a rate of 0.05 mM/min, equaling the rate of GLY2 degradation. Thus the total glycogen in the spore remains constant at a concentration of 5.7 mM in the model (this amount was arbitrarily divided between the two pools).

Glycogen Metabolism—Our analysis of Experiment A (Table III) showed that rapid synthesis and/or turnover of glycogen were occurring during sorocarp formation, although there is a net decrease in glycogen content during this period of differentiation (12). The total amount of glycogen falls from 30 mM at 21 h into differentiation to 11 mM at 26 h. Thereafter glycogen content remains constant (12). In our experiments, cells used in Experiment B were older (27 h) than those in Experiment A (24 h), yet the isolated glycogen in the former was higher in specific radioactivity (Table I), indicating active glycogen turnover late into the sorocarp development stage. In our computer simulation analysis, it was necessary to model glycogen metabolism differently in Experiments A and B in order to match the model specific radioactivity output with the data (Fig. 4). The metabolic configuration for Experiment A (Model A) closely resembles that proposed for culmination cells (1), with net glycogen decreasing at a rate of 0.01 mM/min. The turnover glycogen pool remained small at 1.5 mM. However, data from Experiment B could only be matched with Model B configuration shown in Fig. 4. Here the spore glycogen participating in the glycogen cycle pool accumulates (represented by flux to an external pool) at a rate of 0.05 mM/min, equaling the rate of GLY2 degradation. Thus the total glycogen in the spore remains constant at a concentration of 5.7 mM in the model (this amount was arbitrarily divided between the two pools).

Not much is known concerning the intracellular organization and metabolic dynamics of glycogen synthesis and degradation. However, it is known that glycogen is present as two physically separable pools, one in the cytoplasmic matrix, and the other in vesicles (16), and that these two pools of glycogen may have different functions with respect to storage and degradation. Our models may depict an actual in vivo change in glycogen metabolism in older sorocarps, as glycogen is synthesized for storage in the spores entering the final stages of dormancy. It was not possible to use configuration B (Fig. 4) to simulate the data for Experiment A, as the specific radioactivity of Glu-1-P and UDP-Glc were too low compared to the data.

Model Constraints—In order to determine the precision with which a given model value must be specified, a series of values were substituted for the one in the standard model, which had been selected for best fit to all the data (1, 17). Output from this series of models was then examined with respect to the effect on the specific radioactivities of all metabolites at 40 min. In these earlier models, compensatory relationships between the sizes and specific radioactivities of spore and stalk pools were possible in attempts to fit the experimental data. This is no longer the case; the present kinetic models are almost totally constrained. In the present model, the available data on the specific radioactivity of spore Glu-6-P and especially Glu-1-P, as well as the mix Glu-6-P and Glu-1-P, together with the total (spore plus stalk) concentrations of each hexose phosphate fixed their relative concentrations in the two cell types. Consequently, the specific radioactivity of stalk Glu-6-P and Glu-1-P are also determined. These data in turn highly constrain the rates of glycogen turnover and degradation of spore and stalk glycogen, as well as their pool sizes. The rates of glycogen turnover, spore glycogen degradation, and stalk glycogen degradation were varied in a range encompassing the value in the standard

**FIG. 4. Steady state model of glycogen metabolism.** Model A shows the pathways of glycogen metabolism proposed for Experiment A, and Model B shows glycogen metabolism proposed for Experiment B (see text for discussion). Fluxes used in the simulations are also shown.

**TABLE V**

| Experiment | Glu-6-P | Glu-1-P |
|------------|---------|---------|
|            | Spore   | Stalk   | Mix    | Spore   | Stalk   | Mix    |
| Data       | 8.3     | 8.6     | 19.6   | 12.0    | 12.0    | 26.0   |
| Model      | 8.6     | 86.0    | 19.6   | 12.0    | 32.6    | 26.0   |

**Rate of GLY turnover**

- UDP-Glu → GLY → Glu-1-P
- Glu-1-P → UDP-Glc (0.11 → 0.36)

**Rate of GLY2 degradation** (0.0 → 0.06)

- 9.1 → 7.1
- 86.1 → 86.3
- 20.1 → 18.3
- 13.9 → 6.7
- 32.6 → 32.4
- 26.7 → 24.2

**Rate of STGLY degradation** (0.0 → 0.08)

- 21.3 → 6.5
- 323.2 → 55.0
- 64.4 → 15.0
- 21.1 → 10.3
- 327.9 → 5.6
- 230.5 → 7.1

*The abbreviations used are: GLY, spore glycogen participating in the glycogen cycle; GLY 2, degraded spore glycogen; STGLY, degraded stalk glycogen.
*Fluxes are in millimolar/min.
model (Table V). Changes in these parameters gave specific radioactivities of spore Glu-1-P incompatible with the experimental data (Table V). No compensatory relationships were possible.

Finally, compared to the earlier model (1), it was necessary in the present models to invoke a higher rate of flux from ribose 5-phosphate in order to obtain the specific radioactivity of Fru-6-P observed and the required flux of precursor materials for end product synthesis (Table III). This could suggest that at the terminal stages of sorocarp development the precursors of end product syntheses came primarily from RNA degradation products.

REFERENCES
1. Wright, B. E., Thomas, D. A., and Ingalls, D. J. (1982) J. Biol. Chem. 257, 7587-7594
2. Kelly, P. J., Kelleher, J. K., and Wright, B. E. (1979) Biochem. J. 184, 589-597
3. Wright, B. E., and Kelly, P. J. (1981) Curr. Top. Cell. Regul. 19, 103-158
4. Liddel, G. M., and Wright, B. E. (1961) Dev. Biol. 3, 265-267
5. Thompson, J. (1979) J. Bacteriol. 140, 774-785
6. Killick, K. A., and Wright, B. E. (1972) J. Biol. Chem. 247, 2967-2969
7. Herbert, D., Phipps, P. J., and Strange, R. E. (1971) Methods Microbiol. 5B, 209-344
8. Pannbacker, E. G. (1967) Biochemistry 6, 1283-1286
9. Sherwood, P., Kelly, P. J., Kelleher, J. K., and Wright, B. E. (1979) Comput. Programs Biomed. 10, 66-74
10. Whittingham, W. F., and Raper, K. B. (1960) Proc. Natl. Acad. Sci. U. S. A. 46, 642-649
11. Rutherford, C. L., Taylor, R. D., Merkle, R. K., and Frame, L. T. (1982) Trends Biochem. Sci. 7, 108-111
12. Rosness, P. A., and Wright, B. E. (1974) Arch. Biochem. Biophys. 164, 60-72
13. Walsh, J. W., and Wright, B. E. (1978) J. Gen. Microbiol. 108, 57-62
14. Wilson, J. B., and Rutherford, C. L. (1978) J. Cell Physiol. 94, 37-45
15. Wright, B. E., Tay, A., and Killick, K. A. (1977) Eur. J. Biochem. 74, 217-225
16. Muller, U., and Hohl, H. R. (1975) Protoplasma 85, 199-207
17. Wright, B. E. (1984) J. Theor. Biol. 110, 445-460