Metabolism of the Cellular Slime Mould Dictyostelium discoideum
Grown in Axenic Culture

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1. The DNA, RNA, protein and carbohydrate contents of myxamoebae of
Dictyostelium discoideum strain Ax-2 were measured after growth on bacteria or
in various axenic media. 2. Myxamoebae grown in the different axenic media have
similar DNA, RNA and protein contents, but there are marked differences in the
contents of glycogen and free sugars. The DNA and protein contents of
myxamoebae grown on bacteria are different from those in myxamoebae grown
axenically. 3. Approximately half the DNA found in myxamoebae grown on
bacteria is of bacterial rather than of slime-mould origin. 4. The specific activities
of some enzymes (including UDP-glucose pyrophosphorylase) are higher in
myxamoebae grown axenically than in myxamoebae grown on bacteria. Never-
theless the characteristic increase in the specific activity of UDP-glucose pyro-
phosphorylase occurring during differentiation of cells of the wild-type strain
NC-4 is also found in cells grown axenically. 5. The rate of amino acid oxidation
during axenic growth of the myxamoebae is decreased when the cells are supplied
with glucose.

In the preceding paper (Watts & Ashworth, 1970) a convenient method is described for growing
myxamoebae of the cellular slime mould Dictyo-
stelium discoideum axenically. The present paper
describes an investigation of some aspects of the
growth and metabolism of myxamoebae grown
axenically and on bacteria.

MATERIALS AND METHODS

Materials. Anthrone, ATP, bovine serum albumin
(fraction V), deoxyribose, maltose, NADP*, p-nitrophenyl
N-acetylglucosaminide, ribose, β-amylase (crystalline)
and hexokinase were purchased from Sigma (London)
Chemical Co. Ltd., London S.W.6, U.K. Glucose 6-
phosphate dehydrogenase, glucose phosphate isomerase,
phosphoglucomutase and 6-phosphogluconate dehydro-
genase were obtained from Boehringer Corp. (London)
Ltd., London W.5, U.K., and methyldioctylamine from
Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K.
All other chemicals were of the highest purity available
and were the products of BDH Chemicals Ltd., Poole,
Dorset, U.K., or Fisons Scientific Apparatus Ltd.,
Loughborough, Leics., U.K. Pullulanase was prepared
from the growth medium of Aerobacter aerogenes as
described by Bender & Wallenfels (1966). The final
solution contained 10 mg of protein/ml and was stored at 4°C

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under toluene vapour. The strain of A. aerogenes was the kind gift of Dr C. Mercier, Department of Biochemistry,
University of Florida, Miami, Fla., U.S.A.

Cultures of D. discoideum. Myxamoebae of strain Ax-2
were grown axenically as described by Watts & Ashworth
(1970). Glycerol was added to the medium to give a
concentration of 15.4 mg/ml where necessary. The 700 ml
cultures were grown for 72 h and harvested at about
2×10⁸ cells/ml. After being washed with water, the cells
were resuspended at 2×10⁸ cells/ml in water and stored
at –20°C for future assays of metabolites after addition
of tris–HCl buffer, pH 7.6, to a concentration of 10 mM.
Samples of the cultures were normally plated clonally
with A. aerogenes to check that the cells were all genetically
able to form fruiting bodies and 2.5×10⁷ washed
cells were placed on Millipore supports (Sussman, 1966) to
check that the time-course of differentiation was normal.

Diameter of myxamoebae. Samples of the cultures were
diluted to about 10⁶ cells/ml with water and were left for
15 min. during which time most of the cells became
spherical. The diameters of the swollen cells were
measured by using a Watson image shearer in place of the
eye-piece of a Zeiss microscope with a ×25 objective.
Five samples of each suspension were examined and the first
ten cells seen were measured.

Determination of metabolites. Total carbohydrate was
assayed in cell suspensions by using the anthrone assay
(Hassid & Abraham, 1957), 5 ml of 0.2% (w/v) anthrone
in H₂SO₄ (98%) being added to 2.5 ml of sample. Glucose
solutions were used to construct the standard curve.
Protein was determined by using the method of Lowry,
Rosebrough, Farr & Randall (1951), bovine serum albumin (fraction V) being used for standard solutions. Nucleic acids were separated from 1 ml samples of the cell suspensions by using the method of Schneider (1957) except that the ethanol–ether extractions were omitted. The DNA was finally determined by the indole assay (Banting & Jones, 1957), deoxyribose being used for the standard curve. B. D. Hames & J. M. Ashworth (unpublished work) have shown that, in this assay, the deoxyribose in DNA gives more colour than the same weight of free deoxyribose, so that 1 μg of pure DNA gives the same colour as approx. 1 μg of deoxyribose. RNA was determined with orcinol as described by Schneider (1957). Glucose was determined as described by Engeland & Randle (1967) with hexokinase, glucose 6-phosphate dehydrogenase, ATP and NADP+. Fructose was extracted from cells with 3% (w/v) HClO₄. The perchorlated was removed as insoluble KClO₄ and fructose was determined in the supernatant by the same method as glucose except that 1 unit of glucose phosphate isomerase was also added. The value obtained for fructose also includes any glucose, glucose 6-phosphate or fructose 6-phosphate in the extract. These were determined individually by adding the enzymes in the appropriate order to the cuvette and allowance was made for them in the determination of fructose; the correction was less than 5%. Medium in which ammonia was to be assayed was stored, when necessary, at −20°C after addition of 1 ml of 2 M HCl/7 ml of medium. Before being assayed, the medium was slowly neutralized to pH 6.5 with a known volume of 1 M NaOH and the assays were carried out by the Conway micro diffusion method (Waelsch & Mycek, 1962). Ammonia was also assayed in the initial medium in which the myxamoebae were to be grown, since it contained a significant concentration of ammonia.

Isolation and identification of polysaccharide. A 1 ml portion of a cell suspension containing 10⁶ cells/ml in water was centrifuged to collect the cells. Polysaccharide was then isolated from the cells as described by Cooper & Kornberg (1967) and the polysaccharide was determined with anthrone. A large-scale preparation of the polysaccharide from 20 ml of cell suspension carried out similarly except that Na₂SO₄ was added as co-precipitant and the polysaccharide was precipitated twice from aqueous solution with 66% (v/v) ethanol.

The polysaccharide (5 mg/ml) was hydrolysed in 0.5 M H₂SO₄ at 100°C. The hydrolysates were neutralized with 10% (v/v) methylisocyanate in chloroform (Smith & Page, 1948) and the neutral solutions dried in vacuo. The sugars were then dissolved in water. Chromatography of the hydrolysates was carried out on Whatman no. 1 paper in propan-2-ol–water (4:1, v/v), ethyl acetate–acetic acid–water (14:3:3, by vol.) and ethyl acetate–pyridine–water (12:5:4, by vol.). Reducing sugars were detected by staining with alkaline AgNO₃ (Trevelyan, Procter & Harrison, 1950).

β-Amylolytic activity was carried out as described by Walker & Whelan (1960) and Whelan (1964) with solutions of the polysaccharide (4 mg/ml). Pullulanase (0.1 mg/ml of polysaccharide solution) was included with the β-amylase in some experiments. The total carbohydrate in the solutions was determined with anthrone and maltose with 3,5-dinitrosalicylic acid (Bernfeld, 1955). Chromatography of the digesta was carried out in ethyl acetate–pyridine–water (12:5:4, by vol.), the chromatography paper being pretreated with HgCl₂ at the origin to inactivate the β-amylase.

Assay of enzyme activities. Suspensions of cells were allowed to differentiate on Millipore filters as described by Watts & Ashworth (1970). The cells were harvested in 20 mM-tricine–HCl buffer, pH 8.6, and UDP-glucose pyrophosphorylase was assayed by the method of T. D. Edmundson & J. M. Ashworth (unpublished work). Incubation mixtures contained, in 1.0 ml at pH 8.6, 1.0 μmol of UDP-glucose, 0.85 μmol of NADP⁺, 38 μg of phosphoglucomutase, 19 μg of glucose 6-phosphate dehydrogenase, 56 μg of 6-phosphogluconate dehydrogenase, 5 μmol of MgCl₂, 5 μmol of cysteine, 42 μmol of tricine and cell homogenate. Formation of NADPH was followed spectrophotometrically at 340 nm after addition of 2.0 μmol of Na₂H₄P₂O₇ to start the reaction. One unit of the enzyme catalyses the formation of 1 μmol of glucose 1-phosphate in 1 min. Cells were harvested in water for assay of N-acetylglucosaminidase and the enzyme was assayed with p-nitrophenyl N-acetylglucosaminide as the substrate (Loonan, 1969).

The results on cell size, and on the content of DNA, RNA, protein and carbohydrate in the cells, relate to three cultures grown on each substrate, the cultures being grown at the same time. Similar results have been obtained from other cultures.

RESULTS

Growth of myxamoebae in axenic culture. The mean time taken by myxamoebae grown axenically to double in number is longer than the 3–4 h reported for myxamoebae grown in various conditions on bacteria (Bonner, 1967). Cells grown with shaking in the axenic medium containing glucose or fructose or in the absence of added sugar have mean doubling times of 8 h, 10 h and 10.5 h respectively, and addition of glycerol to the medium decreases the mean doubling time to 11.5 h. Increases in the size of the cells are associated with decreases in the growth rates so that the mean diameter of myxamoebae of D. discoideum NC-4 grown on bacteria is 11.14 ± 2.05 (s.d.) μm whereas cells of strain Ax-2 grown axenically are at least 13 μm [13.00 ± 2.42 (s.d.) μm and 13.85 ± 2.42 (s.d.) μm for cells grown in the glucose and fructose media respectively]. The distribution of cell diameters is bimodal for myxamoebae grown in the absence of added sugar, and this causes an increase in the mean cell diameters [13.74 ± 2.60 (s.d.) μm and 14.03 ± 3.04 (s.d.) μm for cells grown in the absence of added sugar or in glycerol respectively (Fig. 1)]. When myxamoebae that have been grown axenically in the glucose medium are transferred to agar plates and are grown in association with A. aerogenes, the mean diameter of the myxamoebae immediately returns to that found for myxamoebae of strain NC-4 grown on bacteria [11.14 ± 2.06 (s.d.) and 11.14 ± 2.05 (s.d.) μm (Fig. 1)].
1 and are the same however the cells have been grown.

Protein and carbohydrate contents. The protein content of myxamoebae was similar after growth in all the axenic cultures but was decreased in cells grown on bacteria (Table 1).

The total carbohydrate content of the myxamoebae is given in Table 1. The determinations were carried out by using the anthrone reagent and standard solutions of glucose, and may underestimate the actual carbohydrate contents since the anthrone assay gives a higher colour yield with glucose than with most other hexose and pentose sugars (Ashwell, 1957). The carbohydrate content was high when the cells had been grown in medium containing added sugar (Tables 1 and 3) but when the medium was free of added sugar or contained glycerol tended to be lower than in cells grown on bacteria. When the cells grown in the axenic medium containing glucose were transferred to agar plates and grown in association with A. aerogenes, the carbohydrate content of the cells fell to a value similar to that found in myxamoebae of strain NC-4 grown on bacteria.

Polysaccharide was obtained from the myxamoebae by digesting the cells in 30% (w/v) potassium hydroxide and by precipitation with 66% ethanol (v/v). Polysaccharide was found to account for the major part of the carbohydrate within the myxamoebae if they had been grown in the presence of glucose, maltose or mannose (Tables 2 and 3), but cells grown in the presence of fructose contained a high concentration of free fructose. However, polysaccharide may account for a much higher percentage of the total carbohydrate in the myxamoebae grown in the fructose medium if the cells are harvested at a density above 5 x 10⁶ cells/ml. Comparison of the results in Tables 1, 2 and 3 would also suggest that the polysaccharide and total carbohydrate content of the cells grown in the unsupplemented medium increases if the cells are grown without shaking.

Nature of the polysaccharide. Polysaccharide was isolated on a large scale from cells grown on glucose. The material was 85% carbohydrate (as glucose) after being twice precipitated from aqueous solution with 66% ethanol, determinations being made with the anthrone reagent and standard solutions of glucose. After dialysis against water and drying in vacuo with phosphorus pentoxide, the material was assayed as 100% carbohydrate. Protein accounted for less than 0.5% of the material.

Samples of the polysaccharide were hydrolysed for various times in 0.5M-sulphuric acid at 100°C. After 1h 86% of the polysaccharide was hydrolysed to glucose (determined by enzymic assay), and chromatography in ethyl acetate–pyridine–water or propan-2-ol–water showed that three other

Fig. 1. Distribution of cell diameters of myxamoebae grown in various conditions. The number of cells measured was 160 when the myxamoebae had been grown on bacteria or 150 when they had been grown axenically. The arrows indicate the mean cell diameters. (a) D. discoideum strain NC-4 grown in association with Aerobacter aerogenes; (b) D. discoideum strain Ax-2 grown in medium containing 86 mM-glucose; (c) D. discoideum strain Ax-2 grown in medium with no added sugar; (d) D. discoideum strain Ax-2 grown on A. aerogenes after previous growth in the axenic medium containing glucose.

DNA and RNA contents. The DNA content of cells of D. discoideum Ax-2 grown axenically is less than that of cells of strain NC-4 grown on bacteria (Table 1). It is known that at least 89% of the myxamoebae grown on bacteria are haploid (Sackin & Ashworth, 1970) and this, together with the finding that the DNA content rises when myxamoebae of strain Ax-2 are grown on bacteria, suggests that the myxamoebae of both strains have the same DNA content but contain considerable quantities of bacterial DNA or its polynucleotide degradation products when they have been grown with A. aerogenes.

The RNA contents of the cells are given in Table

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Each polymer was determined in suspensions of cells from each of three cultures. DNA and RNA were determined against standards of deoxyribose and ribose respectively, protein against bovine serum albumin, and total carbohydrate against glucose. Results are given as means ± S.E.M.

<table>
<thead>
<tr>
<th>Strain of D. discoideum</th>
<th>Culture conditions</th>
<th>DNA (µg of deoxy-ribose/10⁸ cells)</th>
<th>RNA (µg of ribose/10⁸ cells)</th>
<th>Protein (µg/10⁸ cells)</th>
<th>Total carbohydrate (mg of glucose/10⁸ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ax-2</td>
<td>Glucose medium*</td>
<td>22.1 ± 0.7</td>
<td>328 ± 21</td>
<td>11.0 ± 0.3</td>
<td>2.15 ± 0.22</td>
</tr>
<tr>
<td>Ax-2</td>
<td>Fructose medium*</td>
<td>22.5 ± 3.1</td>
<td>313 ± 83</td>
<td>11.5 ± 2.1</td>
<td>1.42 ± 0.24</td>
</tr>
<tr>
<td>Ax-2</td>
<td>Medium with no added sugar*</td>
<td>22.7 ± 2.2</td>
<td>302 ± 38</td>
<td>10.8 ± 1.2</td>
<td>0.29 ± 0.03</td>
</tr>
<tr>
<td>Ax-2</td>
<td>Glycerol medium*</td>
<td>23.7 ± 1.3</td>
<td>302 ± 55</td>
<td>11.7 ± 1.6</td>
<td>0.35 ± 0.06</td>
</tr>
<tr>
<td>NC-4</td>
<td>On bacteria†</td>
<td>49.0 ± 4.1</td>
<td>298 ± 19</td>
<td>7.8 ± 0.6</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td>Ax-2</td>
<td>Transferred from glucose medium to bacteria†</td>
<td>46.1 ± 11.2</td>
<td>248 ± 35</td>
<td>6.7 ± 0.5</td>
<td>0.46 ± 0.05</td>
</tr>
</tbody>
</table>

* 700 ml cultures grown at 22°C with shaking and harvested when growing exponentially at 1-3×10⁶ cells/ml.
† Grown in association with A. aerogenes on agar plates (Sussman, 1966).

Table 2. Polysaccharide content of myxamoeba of strain Ax-2

The myxamoebae were grown as described in Table 1. Results are given as means ± S.E.M.

<table>
<thead>
<tr>
<th>Sugar added to axenic medium</th>
<th>Total carbohydrate (mg of glucose/10⁸ cells)</th>
<th>Total polysaccharide (mg of glucose/10⁸ cells)</th>
<th>Free fructose (mg/10⁸ cells)</th>
<th>% of carbohydrate as polysaccharide</th>
<th>% of carbohydrate as fructose</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.32 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td>31 ± 5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>2.18 ± 0.60</td>
<td>2.04 ± 0.63</td>
<td>94 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose</td>
<td>2.01 ± 0.08</td>
<td>0.84 ± 0.06</td>
<td>0.99 ± 0.01</td>
<td>42 ± 2</td>
<td>49 ± 2</td>
</tr>
</tbody>
</table>

Table 3. Carbohydrate contents of myxamoeba of strain Ax-2 grown in axenic media containing various sugars

The myxamoebae were grown in 60 ml cultures without shaking. Sugars were at a concentration of 16 mg/ml. Results are given as means ± S.E.M.

<table>
<thead>
<tr>
<th>Sugar added to axenic medium</th>
<th>Total carbohydrate (mg of glucose/10⁸ cells)</th>
<th>% of carbohydrate as polysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribose</td>
<td>0.63 ± 0.06*</td>
<td>81 ± 12</td>
</tr>
<tr>
<td>Lactose</td>
<td>1.42 ± 0.38</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.04 ± 0.96</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Mannose</td>
<td>1.48 ± 0.24</td>
<td>83 ± 8</td>
</tr>
<tr>
<td>Maltose</td>
<td>1.38 ± 0.18</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>None</td>
<td>0.47 ± 0.13</td>
<td>63 ± 4</td>
</tr>
</tbody>
</table>

* The carbohydrate in cells grown on ribose may be greatly underestimated since the anthrone reacts poorly with ribose.

sugars were also present. One had the same RP as maltose in both solvent systems, and the other two sugars ran more slowly. After hydrolysis for 3 or 5 h one of the slower-running sugars, which was probably isomaltose or maltotriose, was still just detectable in the hydrolysate, and glucose was the only other sugar present. The glucose then accounted for 95% of the carbohydrate present in the hydrolysates, none of the carbohydrate having been destroyed during the hydrolysates.

These results show that the polysaccharide contains only glucose and that some of the glucose units are linked α-(1→4). The nature of the linkages was further investigated by degrading the
polysaccharide with enzymes known to hydrolyse specific glucosidic bonds. β-Amylase hydrolyses only α-(1→4)-linkages and attacks its polysaccharide substrates from the non-reducing ends, liberating maltose (Whelan, 1964), whereas pullulanase catalyses the hydrolysis only of α-(1→6)-linkages (Bender & Wallenfels, 1961; Brown, Illingworth & Kornfeld, 1965). After incubation of the slime mould polysaccharide with β-amylase for 24 h maltose accounted for 48% of the total carbohydrate and there was no further release of maltose in the next 24 h. Chromatography showed that maltose and glucose were the only low-molecular-weight products of the digestion. Since glucose reacts in the 3,5-dinitrosalicylic assay method for maltose, the estimate of the yield of maltose by the 3,5-dinitrosalicylic acid assay is too high, determination of the glucose suggesting that the maltose was overestimated by about 10%. A combination of pullulanase and β-amylase increased the yield of maltose to 91% (again overestimated by about 10%) in 24 h and little further increase occurred in the next 24 h. No sugars could be detected by chromatography in an incubation of the polysaccharide with pullulanase alone, which shows that the preparation of pullulanase was not contaminated with β-amylase. The increase in the hydrolysis of the polysaccharide by β-amylase in the presence of pullulanase must thus result from hydrolysis by the pullulanase of α-(1→6)-glucosidic linkages that would otherwise prevent further action of the β-amylase.

The polysaccharide is thus a glucose polymer containing α-(1→4)- and α-(1→6)-glucosidic bonds. It gave a reddish-brown colour with a solution of iodine in potassium iodide although the colour was paler than that given by an equal concentration of rabbit liver glycogen. The polymer is probably similar to the one isolated by White & Sussman (1963) from myxamoebae grown on bacteria, although their polymer was not shown definitely to contain α-(1→6)-linkages and was more susceptible to hydrolysis in 1 M-hydrochloric acid than the polysaccharide isolated from myxamoebae grown axenically.

**Carbohydrate and protein metabolism.** Results given in Table 4 show the relationship between carbohydrate and protein metabolism in myxamoebae grown axenically. The measurements of glucose uptake were made with the highly sensitive assay with hexokinase and glucose 6-phosphate dehydrogenase, but the uptakes were nevertheless extremely difficult to determine and were highly variable when the myxamoebae had been grown in medium containing 86 mM-glucose. Results of experiments not included in Table 4 also indicated that the uptake of glucose was about 1 mg/ml of culture. No glucose uptake could be detected when the glucose concentration was 16 mM and the low rate of uptake is reflected by the low carbohydrate content found within the cells.

**Specific activities of enzymes.** The specific activities of a number of enzymes were determined in myxamoebae of strain NC-4 grown on bacteria and in myxamoebae of strain Ax-2 grown axenically. The specific activities of UDP-glucose pyrophosphorylase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, N-acetylglucosaminidase, ribonuclease and proteinase were higher in the axenic strain whereas the specific activities of amylase and acid phosphatase were the same in both strains. Although there is a five- to ten-fold increase in the specific activity of N-acetylglucosaminidase, the increases in those of the other enzymes were usually twofold.

The specific activity of UDP-glucose pyrophosphorylase is higher in the axenic strain than in myxamoebae of strain NC-4, but an increase in specific activity still occurs during differentiation as in cells of strain NC-4 (Fig. 2).

### Table 4. Protein and carbohydrate metabolism in myxamoebae grown axenically

<table>
<thead>
<tr>
<th>Sugar added to medium</th>
<th>Ammonia production (μmol/ml of culture)</th>
<th>Glucose uptake (μg/ml of culture)</th>
<th>Carbohydrate uptake (μg of glucose equivalents/ml of culture)</th>
<th>Carbohydrate in cells (μg of glucose equivalents/ml of culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>86 mM-Glucose</td>
<td>3.28 ± 0.03</td>
<td>956</td>
<td>127 ± 35</td>
<td>53 ± 4</td>
</tr>
<tr>
<td>None</td>
<td>8.70 ± 0.67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16 mM-Glucose</td>
<td>6.44 ± 0.06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>9.32 ± 0.22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
between myxamoebae of *D. discoideum* NC-4 grown on bacteria and myxamoebae of strain Ax-2 grown axenically. These differences appear to fall into three categories. First, there are differences that depend on cells of strain Ax-2 being grown axenically and that disappear immediately the axenically grown cells are transferred to agar plates and allowed to grow on bacteria. These differences include the DNA, protein and carbohydrate contents and the size of the myxamoebae. Secondly, there are differences that disappear more slowly when myxamoebae grown axenically are transferred to growth on bacteria. Thus the myxamoebae have to be subcultured on bacteria several times before the specific activities of the enzymes UDP-glucose pyrophosphorylase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase return from high values to the lower values typical of myxamoebae of strain NC-4 grown on bacteria (T. D. Edmundson, personal communication). The morphology of the fruiting bodies also fails to revert to that typical of strain NC-4 until after a long period of growth on bacteria (Watts & Ashworth, 1970). So far only one difference has been detected that falls into the third category. This is the unique ability of myxamoebae of strain Ax-2 to grow in a simple axenic medium, and the myxamoebae retain the ability to do this even after months of continuous growth on bacteria.

These results suggest that most of the differences between the two strains result from adaptation of the axenic cells to growth in the axenic medium. However, the finding that myxamoebae of strain Ax-2 will still grow in the axenic medium after months of growth on bacteria (Watts & Ashworth, 1970) indicates that strain Ax-2 was not gained merely as a result of a gradual adaptation to growth in the axenic medium but also as a result of a stable mutation. The nature of the mutation is at present unknown, but there seems to be no reason to believe that it has caused any major change in the sequence of events taking place during differentiation.

**DISCUSSION**

**N-Acetylglucosaminidase** is different since no increase in specific activity was detected during differentiation of cells of strain Ax-2. If myxamoebae of strain Ax-2 are grown on bacteria, however, the specific activity of the enzyme decreases to a value similar to that found in strain NC-4 and then increases during differentiation (J. M. Ashworth, unpublished work).

**DISCUSSION**

**Differences between myxamoebae of strains Ax-2 and NC-4.** Many differences have been detected...
amongst eucaryotic cells. Thus the increased size of axenically grown myxamoebae is probably a reflection of a decreased growth rate rather than of an increased number of aneploids. It is curious, however, that, although in procaryotes the faster a cell grows the larger it is, the converse appears to be true in *D. discoideum*.

The DNA content of myxamoebae grown on bacteria is anomalously high (Table 1). Although such myxamoebae have been well washed and are not grossly contaminated with viable bacteria, they contain partially degraded bacteria in their food vacuoles (Ashworth, Duncan & Rowe, 1969), and the excess of approximately 25\(\mu\)g of deoxyribose/10\(^8\) bacterially grown cells over that present in axenically grown cells presumably reflects this fact. Thus in myxamoebae grown on bacteria at least half the DNA in the cells is of bacterial rather than of myxamoebal origin. This may complicate interpretation of experiments in which it has been assumed that, after growth of myxamoebae on bacteria containing DNA labelled with \(^3\)H]-thymidine, all the intracellular tritium is in myxamoebal DNA (see, e.g. Takeuchi, 1969).

Protein and carbohydrate metabolism. Baumann & Wright (1968) concluded from studies on myxamoebae of strain NC-4 growing on bacteria that ‘in the amoeboid form the slime mold grows mainly at the expense of proteins and amino acids’. Our studies of the relative importance of glucose and amino acid oxidation in providing energy for growth of the axenic strain suggest that this view is not necessarily correct.

Amino acids, in order to provide suitable substrates for energy production, must first be deaminated. Hence the rate of ammonia production during growth is a measure of the rate of amino acid oxidation. In Table 4 it can be seen that provision of a high concentration of glucose (86\(\text{mM}\)) in the medium leads to a decrease in the rate of ammonia production. Thus glucose spares amino acid oxidation during growth and, in agreement with this, comparison of the figures for glucose uptake and carbohydrate accumulation suggest that 90% of the glucose is metabolized to non-carbohydrate products. When glucose is provided at a low concentration (16\(\text{mM}\)) such that there is little detectable carbohydrate uptake or accumulation there is still a significant effect on ammonia production, reinforcing the conclusion that, when offered a choice of oxidizing glucose or amino acids the slime mould myxamoebae prefer to oxidize glucose.

When the myxamoebae were grown in medium containing 86\(\text{mM}\)-glucose, glycogen accounted for virtually all the carbohydrate material within the cells. When fructose was present in the medium, however, a high intracellular concentration of fructose was found. Thus phosphorylation appears to limit the rate of fructose utilization whereas glucose metabolism is limited by the rate at which glucose can cross the plasma membranes of the myxamoebae. The plasma membranes would appear to be somewhat impermeable to sugars, since high concentrations of fructose, and probably also of lactose and sucrose, could be maintained within myxamoebae despite the cells being kept in water for at least 30min while being washed (Tables 2 and 3).

Some preliminary estimates have been made of the activities of enzymes phosphorylating glucose and fructose. About 15munits/mg of protein were found for an enzyme phosphorylating glucose, but activity of an enzyme phosphorylating fructose was barely detectable. This correlates well with the observation that fructose metabolism is limited by phosphorylation but glucose metabolism by the rate of glucose entry into the cells. The \(K_p\) of the enzyme phosphorylating glucose is about 100\(\mu\)M, so it could work at close to \(V_{\text{max}}\), in the myxamoebae even if there were only a low intracellular glucose concentration, and an activity of 15munits/mg of myxamoebal protein could then account for the rates of glucose phosphorylation detected during growth of the myxamoebae.

Maltose and mannose can both stimulate the growth of myxamoebae cultured axenically (Watts & Ashworth, 1970) and cause accumulation of high concentrations of polysaccharide within the amoebae (Table 3). This suggests that these two sugars can also be metabolized by the slime moulds.

The growth yield of myxamoebae is increased by the presence of 86\(\text{mM}\)-glucose or fructose in the axenic medium. Cleland & Coe (1969) have shown that cells of *D. discoideum* do not carry out glucogenesis from amino acids during cell differentiation, and our results suggest that glucogenesis does not occur to a great extent during vegetative growth either. Thus, if the myxamoebae could carry out glucogenesis from amino acids, the growth yield should be independent of the glucose concentration in the medium since the high intracellular content of protein in the axenically grown myxamoebae (Table 1) shows that the myxamoebae have a plentiful supply of protein from which carbohydrate could be formed.

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**REFERENCES**


