JOURNAL OF BACTERIOLOGY, Oct. 1975, p. 212-219
Copyright © 1975 American Society for Microbiology
Vol. 124, No. 1
Printed in U.S.A.

Inhibition of Development in *Dictyostelium discoideum* by Sugars

H. V. RICKENBERG,* H. J. RAHMSDORF, ANNE CAMPBELL, M. J. NORTH, JANET KWASNIAK, AND J. M. ASHWORTH

National Jewish Hospital and Research Center and University of Colorado School of Medicine, Denver, Colorado 80206 and University of Essex, Colchester CO4 3SQ, United Kingdom*

Received for publication 11 June 1975

Sugars such as glucose, maltose, and trehalose, which are metabolized by *Dictyostelium discoideum* and which enhance vegetative growth, inhibit the development of the slime mold at concentrations which stimulate growth maximally. They block the acquisition of aggregation competence as well as aggregation. The same sugars also inhibit the degradation of preformed glycogen, ribonucleic acid, and protein, which is characteristic of development and which occurs when the amoebas are starved by incubation in dilute phosphate buffer.

Adenosine 3',5'-cyclic monophosphate (cAMP) regulates the formation of certain bacterial proteins by a mechanism of positive control (20). The synthesis of such proteins is either blocked or inhibited when growth of the bacteria is supported by an effective source of carbon and occurs at a high rate when the availability of carbon limits the rate of growth. Limitation of carbon stimulates, in a manner not understood at present, the formation of cAMP and leads, eventually, to the release of the cyclic nucleotide into the medium (3, 16). Growth on an effective source of carbon inhibits the formation of cAMP and hence of proteins regulated by catabolite repression (20).

The cellular slime mold, *Dictyostelium discoideum*, grows as a unicellular amoeba under favorable conditions of nutrition. When the food has been exhausted the amoebae form a multicellular aggregate and enter into a developmental cycle which results in the formation of a fruiting body. Starving amoebae synthesize cAMP and release it into their environment where it acts as chemotactic agent and brings about aggregation (1). It has been suggested (15, 18) that intracellular cAMP may play a determinative role in that, depending upon its concentration, it causes some cells to become spores and others to develop into stalk cells.

There appears then a similarity between the effects of starvation in bacteria and in *D. discoideum*: in both organisms starvation leads to an increase in intracellular and extracellular cAMP. In bacteria cAMP permits the expression of specialized functions normally blocked when an effective source of carbon is available. It was of interest therefore to explore the possibility that, in *D. discoideum* also, a utilizable source of carbon might block specialized functions, such as are required for development.

We report here that sugars, which are utilized by the slime molds, severely inhibit normal development, as characterized by aggregation and the formation of the fruiting body when employed at concentrations which stimulate growth maximally. Experiments in progress (H. V. Rickenberg, manuscript in preparation) indicate that the sugars exert this inhibition by interfering with the occurrence of pulses of cAMP, which as Gerisch et al. (G. Gerisch, D. Hülser, D. Malchow, and U. Wick, Trans. R. Soc., in press) have shown, are required for normal development.

MATERIALS AND METHODS

Strain and medium. Strain Ax-2 (ATCC 24397) was grown in axenic medium HL-5 (24) consisting of bacteriological peptone and yeast extract buffered at pH 6.7 with sodium potassium phosphate and, unless otherwise stated, supplemented with 0.1 M maltose. Other sugars, when added, were employed at concentrations indicated for the individual experiments. Incubation was at 22 C on a rotary shaker. The intensity of aeration during growth affected the final yield of amoebae but seemed to have little effect on the growth rate or the ability of the amoebas to undergo development upon subsequent removal from the medium. Amoebe were counted in both a hemocytometer and in a Coulter model B counter.

Development. Development was followed, essentially, as described earlier (22). Amoebae in late exponential phase were harvested by sedimentation, washed twice wih cold distilled water, suspended in pad diluting fluid, and plated at 2 to 5 $\times$ 10^7 amoebae

in a volume of 0.5 ml on dark purple membrane filters (Millipore Corp., average pore size 0.8 μm, diameter 4.7 cm). The filters were placed on membrane cellulose absorbent support pads (Millipore Corp.) in 55-mm plastic petri dishes. The pads were saturated with 1.5 ml of pad diluting fluid which contained in 1 liter of 50 mM sodium potassium phosphate buffer (pH 6.5): 1.5 g of KCl, 0.5 g of $MgCl_2 \cdot 6H_2O$, and 0.5 g of streptomycin sulfate (4). Incubation was at 22 C in the dark. Aggregation under these conditions could first be observed 8 to 10 h after the plating of amoebae harvested from exponentially growing vegetative cultures and mature fruiting bodies appeared 25 to 27 h after plating. Substances which were to be tested for their effects on development were added to the pad diluting fluid. Aggregation competence was also monitored by plating the amoebae directly on plastic petri dishes (13). In this case the amoebae were washed as described above and suspended to approximately 3 to 4 $\times$ 10^6 cells/ml in buffer M (10 mM KCl; 5 mM $MgCl_2$; 10 mM sodium phosphate, pH 6.0; 0.5 g of streptomycin sulfate/liter). Three milliliters of the suspension containing a total of approximately 10^7 amoebae were then pipetted into a 55-mm plastic petri dish (Falcon, 3030 integrid tissue culture dish) and incubated at 22 C in the dark. Aggregation was monitored by observation under an inverted microscope (25- and 300-fold magnification) and occurred approximately 10 to 12 h after the plating of amoebae which had been harvested from an exponentially growing vegetative culture. Substances to be tested for their effects on development were added to medium M.

Amoebae starved by agitation in 0.017 M sodium phosphate buffer, pH 6, also become competent to aggregate (5); it appears that during the first 6 to 8 h of incubation of the amoebae in this buffer physiological events, similar to those that occur during incubation of the amoebae on membrane filters, take place. Hence the early stages of development, particularly competence for aggregation, were studied also in suspensions of amoebae (3 to 6 $\times$ 10^6 cells/ml) shaken at 22 C in 0.017 M sodium phosphate buffer (pH 6) containing 100 μg of streptomycin/ml. After incubation in this buffer the amoebae were harvested by sedimentation, washed twice with cold distilled water, and then plated either directly on plastic petri dishes or, when complete development was to be followed, on membrane filters resting on absorbent support pads.

Glycogen content and turnover. The amoebae were grown for six to eight generations to a final yield of 3 to 5 $\times$ 10^6 cells/ml in medium HL-5 containing uniformly labeled [^{14}C]glucose. The amoebae were washed in cold distilled water and suspended in 0.017 M sodium phosphate buffer, pH 6. Incubation was at 22 C on a rotary shaker and samples were collected at hourly intervals for a period of 6 h. The cells were sedimented at room temperature, and the pellet was drained thoroughly, suspended in 0.5 to 1.0 ml of water, and stored frozen at -15 C. No significant lysis of the amoebae occurred during incubation in the 0.017 M buffer; that lysis did not occur was demonstrated on the basis of both cell counts and deoxyribonucleic acid determinations.

Glycogen was extracted by boiling the cell suspension for 20 min in 30% KOH; it was then precipitated from the cold alkaline extract by the addition of saturated Na_2SO_4 (0.1 ml/1 ml of KOH extract) and 2 volumes of 95% ethanol (23). After being kept for at least 15 min on ice, the glycogen was sedimented (25,000 $\times$ *g*, 10 min, 4 C) and dissolved in distilled water. The precipitation was repeated twice and the glycogen finally was dissolved in 1.2 to 2.0 ml of water. Recovery of the glycogen ranged from 94 to 99%. In experiments in which the turnover of glycogen was measured, extraction was carried out in the presence of 2 mg of oyster glycogen (oyster glycogen, type II, Sigma Chemical Co.) which was added to each sample prior to alkaline hydrolysis. The carbohydrate content of the glycogen was measured as glucose equivalents using the anthrone assay (9). The radioactivity of the glycogen was determined by liquid scintillation counting of samples diluted appropriately in water. The rate of breakdown of the glycogen was calculated from graphs in which the logarithm of the radioactivity of the glycogen was plotted as a function of time; it was assumed that breakdown followed first order kinetics.

Other analytical methods. Deoxyribonucleic acid was determined as described earlier (8); ribonucleic acid (RNA) was measured by the orcinol method of Putnam (19) and protein by the method of Lowry et al. (14).

Chemicals. The chemicals were from commercial sources and used without further purification. The α-methylglucoside contained less than 1% glucose.

RESULTS

Effects of added sugars on growth. Medium HL-5, when not supplemented with a sugar, supported growth to a maximum of approximately 3 $\times$ 10^6 amoebae/ml with a generation time of about 14.5 h. Table 1 shows that glucose, maltose, and trehalose enhanced the final yield and shortened the generation time significantly. Of the three sugars, maltose was slightly, but consistently, more effective in stimulating growth than either glucose or trehalose; all the other sugars tested inhibited growth to a greater or lesser extent. The same held true of a number of amino acids; when added at concentrations of between 0.03 and 0.1 M, L-glutamic acid and L-arginine blocked growth completely (results not shown).

Effects of sugars on development. It is known that a complete medium, such as HL-5, blocks the development of the amoebae. As pointed out in the introduction, certain sugars block the formation of a number of proteins in bacteria. It was of evident interest therefore to test the hypothesis that, in

TABLE 1. *Effect of sugars on growth*

Sugar	Amount (M)	Generation time (approx h)	Maximum yield ($\times 10^6$)
None		14.5	3.1
Glucose	0.03	9	11
Glucose	0.1	8.5	33
Maltose	0.03	8.5	19
Maltose	0.1	8	23
α-Methylglucoside	0.1	18	2.1
Trehalose	0.03	9.5	20
Trehalose	0.1	9.5	22
Ribose	0.03	15.5	3.5
Ribose	0.1	22	3.1
Arabinose	0.03	15	2.5
Arabinose	0.1	30.5	0.7
Galactose	0.03	36	2.4
Galactose	0.1	35	0.9
Lactose	0.03	17	2.2
Lactose	0.1	22	1.3
KCl	0.03	12.5	3.1
KCl	0.1	12.5	2.9

the case of *D. discoideum* also, the utilization of an effective source of carbon would suffice to block development, even in the absence of the other ingredients of the medium required for vegetative growth.

Amoebae were grown and then plated on membrane filters (Millipore Corp.) as described above. We found in several experiments that glucose, maltose, and trehalose, when added to the cellulose pads, severely inhibited development. The severity of the inhibition, however, varied from experiment to experiment; the appearance of mature fruiting bodies were delayed 6 to 12 h by these sugars when they were employed at a concentration of 0.1 M. Of the other sugars tested, α-methylglucoside, arabinose, and ribose delayed development by 2 to 6 h and the effects of lactose were highly variable; galactose seemed to be toxic since in a majority of experiments it blocked development altogether. KCl and NaCl, when employed at a concentration of 0.05 M (additional to the KCl already in the buffer), delayed development by 2 to 6 h.

Clearly those sugars which supported vegetative growth (glucose, maltose, trehalose) also inhibited development most severely; the variability of the effect between individual experiments was significant however and, conceivably, reflected the fact that the sugars had to diffuse from the cellulose pad into the filter. It was decided therefore to examine the effects of sugars by plating the amoebae directly onto plastic petri dishes where an early stage of development, i.e., aggregation, occurred.

Amoebae after growth in medium HL-5 containing 0.1 M maltose were washed, suspended in buffer M, and pipetted into 55-mm plastic petri dishes; 10^7 amoebae in a total volume of 3 ml were added to each dish. The amoebae settled out and attached to the bottom of the dish within minutes. Substances to be tested for their effect on aggregation were added to buffer M and were present during incubation. The effects of a number of compounds on aggregation are presented diagramatically in Fig. 1. Mature aggregates, consisting of an estimated 5×10^3 to 2×10^4 amoebae each, floated in the buffer, whereas nonaggregating amoebae continued to adhere to the bottom of the petri dish. Glucose, maltose, and trehalose, as well as unsupplemented medium HL-5, blocked aggregation indefinitely; other sugars and also KCl and NaCl delayed but did not block aggregation.

The fact that aggregates were not formed in the presence of utilizable sugars showed that these blocked an early stage of development, i.e., aggregation. The experiment did not differentiate, however, between an effect of the sugars on the steps leading to aggregation competence and an effect on aggregation itself. The development of aggregation competence and aggregation can be separated by the incubation of the amoebae in buffer under conditions where they become aggregation competent but do not aggregate. Amoebae become competent to ag-

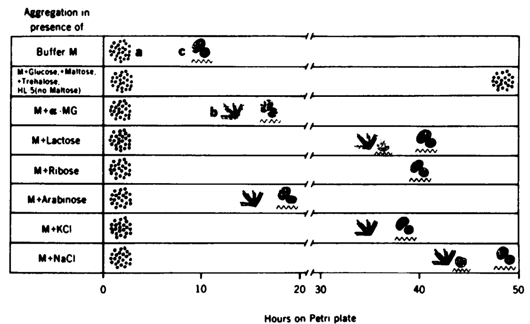

FIG. 1. *Effect of sugars, KCl, and NaCl on aggregation. Amoebae were grown in medium HL-5-maltose to a density of 5 × 10^6 cells/ml, washed, and resuspended to 3 × 10^6 cells/ml in either medium HL-5 or in buffer M containing substances as indicated. The final concentration of the sugars was 0.1 M and of the salts 0.05 M. Cells (9 × 10^6) in a volume of 3 ml were plated in duplicate in 55-mm plastic petri dishes. Incubation was at 22 C in the dark and development was followed by observation with an inverted stage microscope. α-MG, α-Methylglucoside. (a) Single amoebae attached to bottom of petri plate; (b) aggregation streams; (c) mature, floating aggregates.*

gregate during aerobic incubation in 0.017 M sodium phosphate buffer (pH 6) (5); if incubated for 8 to 10 h with agitation and then plated on membrane filters at an appropriate density, the amoebae aggregate within 2 h and form mature fruiting bodies within 18 to 20 h. The effects of maltose and α-methylglucoside, when present during preincubation, on the subsequent ability of the amoebae to aggregate and form fruiting bodies on membrane filters were studied. As may be seen from Fig. 2, the presence of 0.1 M maltose during preincubation delayed the aggregation of the cells by approximately 8 h, whereas α-methylglucoside at the same concentration retarded it by only 1 to 2 h.

This experiment shows that maltose inhibited reactions required for rendering the amoebas aggregation competent. Aggregation itself, including the elongation of the individual amoebae preceding aggregation and the formation of aggregation streams, is best observed (with an inverted microscope) on amoebae plated on plastic petri dishes. When amoebae were harvested from the growth medium, washed, and plated without prior starvation, they elongated and formed aggregation streams within 7 to 10 h of plating. By contrast, if they were starved by incubation with shaking in phosphate buffer for 10 h and were then plated, they arrived at the same stage of development within a mere 1 to 2 h of plating (Fig. 3). The presence of maltose or glucose during preincubation blocked the development of aggregation competence completely and, in fact, amoebae preincubated with maltose were retarded in their ability to aggregate by comparison with amoebae harvested from the complete medium. α-Methylglucoside and arabinose affected the emergence of aggregation competence only slightly, whereas the other sugars and salts had intermediate effects; trehalose frequently was as inhibitory as glucose.

A comparison of Fig. 1 and 3 shows that those compounds which affected aggregation most severely also had the most pronounced effect on the development of aggregation competence. This observation might suggest that sugars affect the latter process exclusively. That this is not so, however, is demonstrated by the finding (not shown) that the addition of maltose to amoebae which were already aggregation competent blocked aggregation completely; i.e., maltose exerted an effect not only on the acquisition of competence for aggregation but also on the formation of aggregates. In other experiments it was observed that, when amoebae were preincubated in buffer for 10 h and then suspended for 2 h in either buffer containing maltose or medium HL-5 containing maltose, they retained aggregation competence

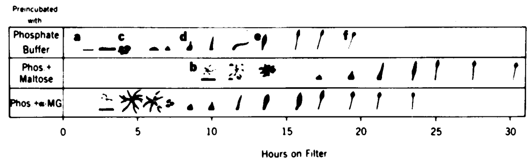

FIG. 2. *Development on membrane filters after preincubation in phosphate buffer. Amoebae were grown in medium HL-5-maltose to a density of 3 × 10⁶ cells/ml and washed. They were then resuspended to a density of 3 × 10⁶ cells/ml in phosphate buffer, in buffer containing 0.1 M maltose, and in buffer containing 0.1 M α-methylglucoside (α-MG), respectively. The suspensions were then shaken for 8 h at 22 C, harvested, washed, and resuspended in pad diluting fluid to a density of 5 × 10⁷ cells/ml. Amoebae (2.5 × 10⁷ per membrane filter) were plated in duplicate and incubated at 22 C in the dark. Development was followed by observation with a binocular microscope. (a) Rippling; (b) aggregation streams; (c) aggregates; (d) fingers; (e) immature fruiting bodies; (f) mature fruiting bodies.*

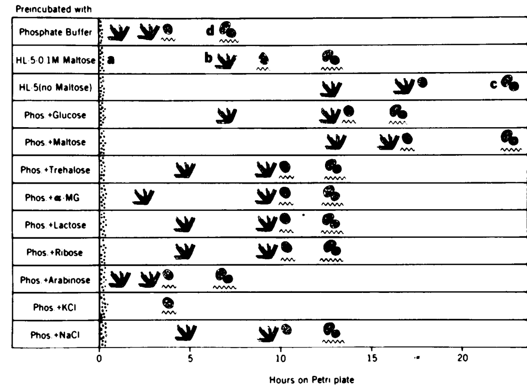

FIG. 3. *Aggregation on petri dishes after preincubation in phosphate buffer. Amoebae were grown on medium HL-5-maltose to 5 × 10⁶ cells/ml, washed, and resuspended in either medium HL-5 or in phosphate buffer containing substances as indicated. The sugars were employed at a final concentration of 0.1 M and the salts at 0.05 M. The suspensions were shaken at 22 C for 10 h; a control culture was incubated in medium HL-5-maltose for the same period of time. The amoebae were then harvested, washed, and resuspended in buffer M to a density of 3 × 10⁶ cells/ml. Cells (9 × 10⁶) in a volume of 3 ml were plated in duplicate in 55-mm plastic petri dishes. Incubation was at 22 C in the dark and development was followed by observation with an inverted stage microscope. (a) Single amoebae attached to bottom of petri plate; (b) aggregation streams; (c) immature aggregates attached to bottom of petri plate; (d) mature, floating aggregates.*

when plated in the absence of the sugar or complete medium. If, however, the amoebae after preincubation were exposed for 9 h to either the buffer containing maltose or medium HL-5 with maltose, and then tested for aggregation competence, they had lost aggregation competence.

Effect of sugars on turnover of preformed glycogen. Hames and Ashworth (7) have shown that strain Ax-2 grown on medium HL-5 containing 0.1 M glucose had considerably more glycogen than did the amoebae after growth in medium HL-5 unsupplemented with glucose; the concentration of glycogen varied with the growth state of the cells. It is known that amoebae grown in the medium supplemented with glucose (and hence containing relatively high concentrations of glycogen) develop and give rise to fruiting bodies in about the same period of time as do amoebae grown in the medium unsupplemented with glucose (and containing only little glycogen; 4). It appears therefore that the derivatives of glucose which are formed intracellularly as a result of the breakdown of glycogen are not equivalent to extracellular glucose with respect to their ability to inhibit development. The possibility that glycogen would be broken down only when more readily available sources of carbon, such as glucose, had disappeared from the medium was considered and tested as described above. In five independent experiments it was found that the presence of 0.1 M glucose in the 0.017 M phosphate buffer severely inhibited the breakdown of glycogen; the breakdown in the presence of glucose was 0.7 ± 0.5%/h compared with 11.2 ± 1.5%/h in the absence of glucose. The data from one of the experiments are presented graphically in Fig. 4, which also shows that the addition of glucose at 1.7 h after the onset of starvation arrests the further degradation of glycogen. α-Methylglucoside added at 0.1 M concentration at time 0 had no effect on the breakdown of glycogen (not shown). The addition of 250 μg of cycloheximide per ml to the buffer, which inhibited the synthesis of protein (incorporation of [^{14}C]leucine) by more than 95% (16), had no effect on the rate of the breakdown of the glycogen in either the presence or absence of glucose. This indicates that the breakdown of glycogen, which occurs under conditions of starvation for carbon, does not require the de novo synthesis of protein.

Effect of sugars on breakdown of preformed RNA and protein. Development in *D. discoideum* is accompanied by a massive breakdown of RNA and protein (7). It was of interest therefore to determine the effect of the presence of a sugar on the breakdown in amoebae incubated in buffer. Figure 5 shows that maltose completely inhibited the breakdown of RNA; α-methylglucoside had no significant effect on the hydrolysis of RNA (not shown). The addition of cycloheximide did not prevent the inhibition by maltose; the antibiotic did slow the rate of loss of RNA in the absence of maltose. A study of the breakdown of protein (North and Ashworth, manuscript in preparation) showed that the rate of loss of protein in buffer was much less than the rate observed after the amoebae were deposited on membrane filters. The presence of glucose in the buffer, however,

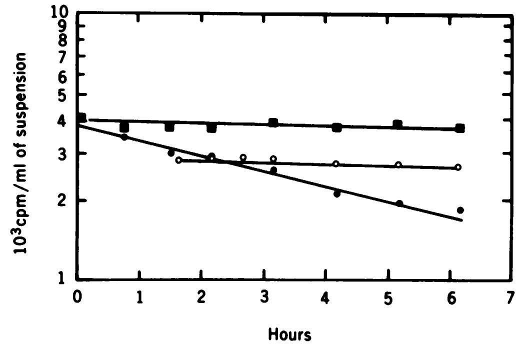

FIG. 4. *Effect of glucose on degradation of preformed glycogen during incubation in phosphate buffer. The amoebae were grown in medium HL-5-glucose containing 390 μCi of [U-^{14}C]glucose/liter for approximately eight generations and to a final density of 3.5 × 10^6 cells/ml. Initially the amoebae contained approximately 1 mg of glycogen (anthrone-positive material) per 10^8 cells. The amoebae were harvested, washed, and resuspended in 0.017 M phosphate buffer to a density of 5 × 10^6 cells/ml. They were then incubated at 22 C with shaking. 2-ml samples were taken at hourly intervals, the cellular glycogen was extracted in the presence of 2 mg of oyster glycogen/sample, and the radioactivity was determined. Symbols: ●, no addition; ■, glucose added to final concentration of 0.1 M at 0 time; ○, glucose added to final concentration of 0.1 M at 1.7 h after resuspension of the amoebae. Note: In other experiments in which glycogen extraction was performed in the absence of carrier glycogen, the total glycogen (as contrasted with preformed glycogen) in the suspension incubated with glucose from 0 time on increased 2.2-fold over a period of 6 h, whereas that in the suspension incubated without glucose decreased approximately twofold over the same period (data not shown). Over the same period the specific radioactivity of the glycogen decreased by about one-half in cells incubated in the presence of glucose but remained unchanged in cells incubated without glucose.*

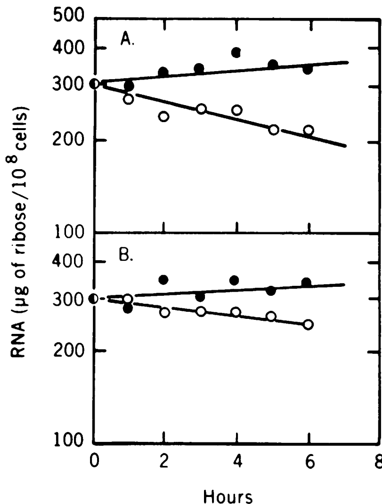

FIG. 5. *Effect of maltose on degradation of amoebal RNA. The amoebae were grown in medium HL-5-maltose to a density of 3 × 10⁶ cells/ml, washed, and suspended in phosphate buffer or in phosphate buffer containing 0.1 M maltose to a density of 5.3 × 10⁶ cells/ml. Incubation was at 22 C with shaking. Duplicate samples were taken at hourly intervals and the RNA was determined on cellular trichloroacetic acid-precipitable material according to the method of Putnam (19). (A) Incubation in the absence of cycloheximide: ○, no maltose added; ●, 0.1 M maltose. (B) Incubation in presence of 250 μg of cycloheximide per ml: ○, no maltose added; ●, 0.1 M maltose.*

caused a 50% reduction in the rate of protein breakdown (North and Ashworth, manuscript in preparation).

DISCUSSION

Development in *D. discoideum* entails both catabolic and anabolic reactions and occurs in the absence of added nutrients (1). It is characterized, in the first 6 h after removal of the nutrients, by marked changes in cellular morphology, the antigenic composition of the cell surface (2), and a number of other biochemical parameters. The findings presented here indicate that sugars which enhance vegetative growth of *D. discoideum* block the aggregation and subsequent development of the amoebae when employed at concentrations which stimulate growth maximally. Sugars which are not metabolized by *D. discoideum*, as well as NaCl and KCl, inhibit development but do not block it, and normal fruiting bodies are formed eventually in the presence of the nonmetabolized sugars or salts. α-Methylglucoside which resembles maltose closely in structure, but which is not utilized for growth, inhibits development only minimally. The nature of the inhibition by the nonmetabolized compounds is not understood; it may reflect osmotic effects of the high concentrations at which they were employed; sugars were used at a concentration at which they stimulated growth significantly. Krichevsky and Wright (12) reported earlier that glucose, at concentrations lower then those used in the present work, stimulated morphogenesis. The conditions for testing development as well as the fact that a nonaxenic strain was employed in the earlier experiments make it difficult to compare the findings.

There is extensive degradation of RNA and glycogen during development, irrespective of whether this occurs on filters or in suspension in phosphate buffer. The degradation of glycogen and RNA seems to follow first-order kinetics (Fig. 4 and 5; 7). There is much less breakdown of protein in amoebae suspended in buffer than in amoebae deposited on filters, and the degradation shows complex kinetics (North and Ashworth, manuscript in preparation).

The increase in the catabolism of glycogen concomitant with starvation does not require the de novo synthesis of protein; this is indicated by the insensitivity of the degradation of glycogen to cycloheximide. It appears that at least two enzymes, an amylase with a pH optimum of 4.8 (25) and a glycogen phosphorylase with a pH optimum of 6.9 (10), catalyze the breakdown of glycogen in *D. discoideum*.

The hydrolysis of RNA during starvation is at least partially resistant to cycloheximide. It appears then that the effect of starvation on catabolic reactions is mediated primarily by the activation of preexisting enzymes.

We find that catabolism of glycogen and RNA which occurs during development is blocked completely by a metabolizable source of carbon (Fig. 4 and 5). It appears that the degradation of protein is also inhibited (North and Ashworth, manuscript in preparation).

Work in progress (H. V. Rickenberg, manuscript in preparation) shows that metabolized sugars also block anabolic reactions required for development. Thus maltose inhibits the synthesis of cellular and extracellular cAMP phosphodiesterase and the formation of the "A" sites (2) involved in the aggregation of the amoebae. Furthermore we find that maltose inhibits not only reactions required for aggregation competence, but also aggregation per se. The possibility that metabolized sugars interfere with later stages of development as well is not excluded and will be examined.

Certain analogies between the phenomenon of

catabolite repression in bacteria and its reversal and development in *D. discoideum* were pointed out in the introductory section. In both cases starvation permits the expression of functions which are blocked in the presence of an effective source of carbon. *D. discoideum* requires a complex mixture of amino acids for growth. These amino acids serve as source of carbon as well as of nitrogen; hence the possibility could not be tested that in the case of the amoebae it is also starvation for carbon, specifically, which acts as trigger for development. That a utilized source of carbon inhibits development is clear however.

In *Escherichia coli* starvation for carbon leads to an increase in both cellular and extracellular cAMP. The increase in intracellular cAMP permits the transcription of genes controlled by catabolite repression. The extracellular cAMP exerts no obvious effect under laboratory conditions of cultivation. Starvation of *D. discoideum* also brings about the synthesis and release of cAMP (11, 17). The extracellular cyclic nucleotide then acts as chemotactic agent and causes aggregation by a mechanism not understood at present. Little is known about the intracellular effects of an increase in the cellular concentration of cAMP in *D. discoideum*.

Work in progress (H. V. Rickenberg, manuscript in preparation) shows that amoebae starved in 0.017 M sodium phosphate buffer have, at certain periods during starvation (approximately at 8 to 10 h after removal from the growth medium), significantly higher levels of cAMP than do amoebae incubated in buffer supplemented with 0.1 M maltose or amoebae at the onset of starvation. In aggreement with the findings of Gerisch (personal communication), we observed that the cellular concentration of cAMP oscillated sharply. Our preliminary findings indicate that the amplitude of the cAMP oscillations in the starving amoebae at 8 to 10 h of starvation is at least twice that of amoebae furnished with maltose (or of amoebae at the onset of starvation). The fact that maltose inhibits the increase in cAMP suggests that it is indeed starvation for a source of energy which brings about the increase in cAMP, much as it does in the bacterium *E. coli* (1).

It is tempting to speculate that the enhancement of glycogenolysis and of the hydrolysis of RNA which occurs during starvation relates in some manner to the increase in cellular cAMP. Rosness and co-workers (21) found that cAMP stimulated the in vivo glycogenolysis in *D. discoideum*. To our knowledge, an effect of cAMP on the hydrolysis of RNA has not been reported. Attempts in our laboratory to overcome the inhibition by sugars of development, in general, and of glycogenolysis, in particular, by either exogenous cAMP or by agents thought to stimulate the endogenous production of cAMP gave variable results. The problem of the interaction of exogenously administered cAMP and endogenous energy metabolism will evidently have to be reexamined in view of the recent finding of Gerisch and Hess (6) and our unpublished observation (H. V. Rickenberg, manuscript in preparation) that development in *D. discoidum* seems to be controlled by oscillations rather than by long-lasting changes in cAMP concentrations.

ACKNOWLEDGMENTS

We thank Louise Louden, Eva Spitz, and Marianne Tecklenburg for their excellent technical assistance. H. J. Rahmsdorf is a fellow of the Deutsche Forschungsgemeinschaft and M. J. North is a Science Research Council Postdoctoral Fellow.

This research was supported in part by Public Health Service grant no. AM 11046 from the National Institutes of Arthritis, Metabolism, and Digestive Diseases, and by the Science Research Council of Great Britain.

H. V. R. is the Ida and Cecil Green Investigator in Developmental Biochemistry.

LITERATURE CITED

1. Ashworth, J. W. 1974. The development of the cellular slime moulds, p. 7-34. *In* J. Paul (ed.), Biochemistry of cell differentiation, MTP international review of science, biochemistry series one, vol. 8. Butterworths, London.
2. Beug, H., F. E. Katz, and G. Gerisch. 1973. Dynamics of antigenic membrane sites relating to cell aggregation in *Dictyostelium discoideum*. J. Cell Biol. **56**:647-658.
3. Buettner, M. J., E. Spitz, and H. V. Rickenberg. 1973. Cyclic adenosine 3',5'-monophosphate in *Escherichia coli*. J. Bacteriol. **114**:1068-1073.
4. Garrod, D. R., and J. M. Ashworth. 1972. Effect of growth conditions on development of the cellular slime mould, *Dictyostelium discoideum*. J. Embryol. Exp. Morphol.- **28**:463-479.
5. Gerisch, G. 1959. Ein Submerskulturverfahren fur entwicklungsphysiologische Untersuchungen an Dictyostelium discoidum. Naturwissenschaften **46**:654-656.
6. Gerisch, G., and B. Hess. 1974. Cyclic-AMP-controlled oscillations in suspended *Dictyostelium* cells: their relation to morphogenetic cell interactions. Proc. Natl. Acad. Sci. U.S.A. **71**:2118-2122.
7. Hames, B. D., and J. M. Ashworth. 1974. The metabolism of macromolecules during the differentiation of myxamoebae of the cellular slime mould *Dictyostelium discoideum* containing different amounts of glycogen. Biochem. J. **142**:301-315.
8. Hames, B. D., G. Weeks, and J. M. Ashworth. 1972. Glycogen synthetase and the control of glycogen synthesis in the cellular slime mould *Dictyostelium discoideum* during cell differentiation. Biochem. J. **126**:627-633.
9. Hassid, W. Z., and S. Abraham. 1957. Chemical procedures for analysis of polysaccharides, p. 34-50. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press Inc., New York.

10. Jones, T. H. D., and B. E. Wright. 1970. Partial purification and characterization of glycogen phosphorylase from *Dictyostelium discoideum*. J. Bacteriol. 104:754-761.
11. Konijn, T. M., Y. Y. Chang, and J. T. Bonner. 1969. Synthesis of cyclic AMP in *Dictyostelium discoideum* and *Polysphondylium pallidum*. Nature (London) **224**:1211-1212.
12. Krichevsky, M. I., and B. E. Wright. 1963. Environmental control of the course of development in *Dictyostelium discoideum*. J. Gen. Microbiol. **32**:195-207.
13. Lee, K.-C. 1972. Permeability of *Dictyostelium discoideum* towards amino acids and inulin: a possible relationship between initiation of differentiation and loss of 'pool' metabolites. J. Gen. Microbiol. **72**:457-471.
14. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
15. Maeda, Y., and M. Maeda. 1974. Heterogeneity of the cell population of the cellular slime mold *Dictyostelium discoideum* before aggregation, and the subsequent locations of the cells. Exp. Cell Res. **84**:88-94.
16. Makman, R. S., and E. W. Sutherland. 1965. Adenosine 3',5'-phosphate in *Escherichia coli*. J. Biol. Chem. **240**:1309-1314.
17. Malkinson, A. M., and J. M. Ashworth. 1973. Adenosine 3':5'-cyclic monophosphate concentrations and phosphodiesterase activities during axenic growth and differentiation of cells of the cellular slime mould *Dictyostelium discoideum*. Biochem. J. **134**:311-319.
18. Pan, P., J. T. Bonner, H. J. Wedner, and C. W. Parker. 1974. Immunofluorescence evidence for the distribution of cyclic AMP in cells and cell masses of the cellular slime molds. Proc. Natl. Acad. Sci. U.S.A. **71**:1623-1625.
19. Putnam, E. C. 1957. Paper chromatography of sugars, p. 62-72. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 3. Academic Press Inc., New York.
20. Rickenberg, H. V. 1974. Cyclic AMP in prokaryotes. Annu. Rev. Microbiol. **28**:353-369.
21. Rosness, P. A., G. Gustafson, and B. E. Wright. 1971. Effects of adenosine 3',5'-monophosphate and adenosine 5'-monophosphate on glycogen degradation and synthesis in *Dictyostelium discoideum*. J. Bacteriol. **108**:1329-1337.
22. Sussman, M. 1966. Biochemical and genetic methods in the study of cellular slime mold development, p. 397. *In* D. Prescott (ed.), Methods in cell physiology, vol. 2. Academic Press Inc., New York.
23. Van Handel, E. 1965. Estimation of glycogen in small amounts of tissue. Anal. Biochem. **11**:256-265.
24. Watts, D. J., and J. M. Ashworth. 1970. Growth of myxamoebae of the cellular slime mould *Dictyostelium discoideum* in axenic culture. Biochem. J. **119**:171-174.
25. Wiener, E., and J. M. Ashworth. 1970. The isolation and characterization of lysosomal particles from myxamoebae of the cellular slime mould *Dictyostelium discoideum*. Biochem. J. **118**:505-512.