OBSERVATIONS ON A STRAIN OF NEISSERIA MENINGITIDIS IN THE PRESENCE OF GLUCOSE AND MALTOSE

III. Cell-free Extracts and the Phosphorolysis of Maltose[1]

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Previous observations on a strain of *Neisseria meningitidis* revealed that inorganic orthophosphate disappeared in the presence of the disaccharide, maltose, but did not do so in the presence of its constituent monosaccharide, glucose (Fitting and Scherp, 1951, 1952). The early studies were made with growing cells and with washed cells. The work to be reported was done with a cell-free extract of this meningococcus and describes an enzymatic phosphorolysis of maltose.

Recent investigations of the differential utilization of disaccharides and their constituent monosaccharides by various microorganisms have showed that cell-free extracts of the organisms catalyze one of three different types of reactions: (a) *hydrolysis* of the disaccharide succeeded by the utilization of one or both of its constituent monoses (Leibowitz and Hestrin, 1945); (b) *transglycosidic exchange* reactions during which a polyose is synthesized in the absence of inorganic phosphate and one of the monoses is liberated (Monod and Torriani, 1950), e.g.,

$$\text{maltose} \xrightleftharpoons[\text{maltase}]{\text{amylo-}} \text{polyose} + \text{glucose}$$

(c) *transglycosidic exchange reaction succeeded by a phosphorolysis* (Doudoroff, Barker, and Hassid, 1947), e.g.,

$$\text{sucrose} \xrightleftharpoons[\text{sidase}]{\text{transgluco-}} \text{glucose-enzyme} + \text{fructose}$$

$$+$$

$$\text{inorganic phosphate}$$

$$\downarrow\uparrow$$

$$\text{glucose-1-phosphate}$$

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The present work was designed to investigate *in vitro* a phosphorylase from a meningococcus which appears to catalyze a reaction of type (c). Studies were directed towards determining which of the characteristics of an enzyme the meningococcal extract manifested and towards an elucidation of the reactive system as a whole.

MATERIALS AND METHODS

Culture. The present work was done with the strain of *N. meningitidis* (strain 69, type I) studied previously (Fitting and Scherp, 1951, 1952). The history of this old laboratory strain was described by Scherp and Fitting (1949).

Media. The media used were those listed by Fitting and Scherp (1951, 1952). "P-salts" is a mixture of the salts of the medium described by Frantz (1942), minus magnesium sulfate, and contains sodium chloride, potassium chloride, ammonium chloride, and also 0.018 M inorganic phosphate.

Reagents. In addition to the compounds mentioned previously (Fitting and Scherp, 1951, 1952), the following were obtained from Eastman Kodak Company: inositol (ash-free), α-methylglucoside, D-galactose, and cellobiose. Other substances tested were: trehalose and cellobiose (Baltimore Biological Laboratory), six per cent depolymerized dextran (East Anglia Chemical Company),[3] isomaltose,[4] type III pneumococcal polysaccharide (prepared according to the method of Heidelberger, Kendall, and Scherp, 1936), and cellobiuronic acid [prepared according to the directions of Goebel (1935) by the hydrolysis of type III pneumococcal polysaccharide]. Two preparations, designated as "meningococcal endotoxin", consisting of the water soluble components of type I meningococci lysed in the presence of chloroform at room temperature, were used. The antisera against two strains of *N. meningitidis* (strain 69 and a virulent type I strain, no. 520) were prepared by repeated injections of washed cells into rabbits.[5]

Analytical methods. In addition to the methods mentioned previously (Fitting and Scherp, 1951, 1952) the following analyses were made: *Absorption spectra* were measured with a Beckman spectrophotometer (model DU). *Partition chromatography* on Whatman no. 1 paper was done with the deproteinized test samples in order to separate their reducing components. The descending technique was used with butanol, pyridine, and distilled water (3:2:1.5) as the solvent front. The papers were sprayed with a solution of 3,5-dinitrosalicylic acid in four per cent sodium hydroxide according to the directions of Jeanes, Wise, and Dimler (1951).

Preparations of washed cells of N. meningitidis (strain 69, type I) were made by methods mentioned previously (Fitting and Scherp, 1952). Those procedures

[3] Dextran solution was obtained through the kindness of Dr. Roger Terry of the Department of Pathology, University of Rochester School of Medicine and Dentistry, Rochester, New York.

[4] Isomaltose was received through the generosity of Dr. Edna M. Montgomery of the Northern Regional Research Laboratory, Peoria, Illinois.

[5] Meningococcal antisera were prepared and kindly donated by Miss Priscilla Fenn, Department of Bacteriology, University of Rochester School of Medicine and Dentistry, Rochester, New York.

were used which "reduced" the selection of cellular populations capable of utilizing glucose, i.e., either blood agar plates or trypticase soy agar plates were inoculated with a drop of saline suspension of the lyophile culture material and were incubated for between 16 and 24 hours at 37 C. The resulting bacterial populations were harvested in the manner described.

Preparations of cell extracts. Washed cells were resuspended in distilled water and then were repeatedly frozen and thawed. The resulting mixture was used *in toto* and was referred to as "cell-brei". Extracts were made from lyophile or acetone-dried cells by suspending them in distilled water or in P-salts, respectively, in a mortar chilled in an ice bath. The mixtures were ground with alundum (Blue Label, R. R. Alundum, 60 mesh) for from five to ten minutes and then were spun for an hour at 2,400 rpm in an angle centrifuge (Sorvall SP) at 4 C. The supernatant extracts were not water clear. It was assumed, therefore, that they contained a high proportion of whole cells or cellular debris. Water clear extracts (referred to as "cell-free" extracts) were made from wet cells. Washed cells of the organism were suspended in distilled water or in P-salts, and either were covered with toluene and incubated at 4 C (unless otherwise specified) for from one to four weeks, or they were repeatedly frozen and thawed in the absence of toluene. Samples of the mixtures were gram stained, and when they showed disintegration of the cellular morphology they were spun at 11,000 rpm in an angle centrifuge (Sorvall Super Speed) at 4 C for one hour and the supernatants were collected. The solution was recentrifuged in the described manner until it was water clear. The extracts were either lyophilized and stored at 0 C or frozen and stored at −25 C. In order to assure the absence of whole cells in these extracts, some of the autolysates were sterilized by filtration through a pyrex glass fritted filter (grade UF).

Experimental procedures. In a typical experiment, ice cold solutions were introduced into pyrex glass test tubes (15 by 125 mm) which had been chilled previously in an ice bath. After stoppering the tubes with rubber stoppers and gently mixing their contents, aliquots were removed for analyses prior to incubation (zero hour analyses). The rubber stoppered test tubes were incubated then in a water bath at 37.9 C (unless otherwise indicated). At intervals the tubes were removed and immediately placed into the ice bath. Once more aliquots were removed for analyses. An attempt was made to keep the samples ice cold at all times, except during the period of incubation, in order to minimize decomposition of possible phosphorylated intermediate reaction products, as well as to avoid excessive denaturation of proteins present in the cell-free extracts.

Dialyses and ultrafiltrations were carried out at 4 C. "Visking cellulose sausage casings" were used as membranes.

Ammonium sulfate fractionations were made by adding the solid salt to the cold extracts. The mixtures were kept at 0 C for several hours, after which the respective precipitates were collected by centrifugation at 11,000 rpm at 4 C. The precipitates were resuspended in P-salts, and the resulting solutions were centrifuged once more at 11,000 rpm in order to remove any insoluble material.

RESULTS AND DISCUSSION

Respiration and fermentation studies on a cell-free extract of *N. meningitidis* (strain 69, type I) yielded the following information: After six hours' incubation in air, which contained relatively low concentrations of carbon dioxide (Warburg's "direct" method), reaction mixtures, containing 0.4 ml of cell-free extract, 20 micromoles of inorganic phosphate, 0.75 micromole of adenosine triphosphate, and 34 micromoles of maltose in a total volume of 2.8 ml, took up 1.8 micromoles of oxygen and 13 micromoles of inorganic phosphate (i.e., 65 per cent of total) and, furthermore, produced enough acid aerobically to result in a pH drop of 0.3 unit. The corresponding endogenous reaction mixtures, as well as those containing 68 micromoles of glucose in place of maltose, produced no measurable change in either pH or phosphate concentration, but did take up oxygen (0.8 micromole and 1.25 micromoles, respectively). In fermentation studies, the amount of the same cell-free extract used was only one-half that used in the respiration studies. The extract in a total volume of 2.8 ml of bicarbonate solution, containing 12 micromoles of inorganic phosphate, 0.75 micromole of adenosine triphosphate, and 33 micromoles of maltose, was placed in a Warburg flask under an atmosphere of 95 per cent nitrogen and 5 per cent carbon dioxide, which held the solution at pH 7.4. After six hours' incubation a decrease of 3.3 micromoles of phosphate (i.e., 28 per cent of the total) was noted, accompanied by the liberation of 0.8 micromole of carbon dioxide. No acid was produced anaerobically, nor was a change of the concentration of inorganic phosphate detected in either the endogenous or the glucose (66 micromoles) reaction mixtures. Similar findings were made with cell-brei and acetone dried or lyophile cells which had been ground with alundum. Since the rates of respiration and of fermentation observed in all these various systems were so low, it was decided to pursue instead the problem of the "phosphorolysis of maltose" by the cell-free extracts.

In the presence of maltose and of inorganic phosphate, cell-free extracts of this meningococcus exhibited the following characteristics which generally are associated with enzymes.

A protein fraction. Heat lability was demonstrated by the loss of the phosphorolytic ability when the cell-free extract was heated for three minutes at 98 C. Activity was related to the protein portion of the extracts (tables 1, 2, and 3 and figure 1). Table 1 shows the results obtained with extracts prepared under different conditions from the same suspension of cells. Activity was associated with a nondialyzable substance which did not dissociate readily. Using aliquots of the same extract, the activity of the untreated, the dialyzed, and the ultrafiltered material was determined in the presence of similar amounts of maltose and of inorganic phosphate. The data of one of four such experiments are given in table 2. These findings suggested that a readily dissociable cofactor, e.g., adenosine triphosphate or magnesium ions, was not an essential component of the reaction. In order to ensure the absence of intact bacterial cells, extracts were sterilized by filtration through a pyrex glass sintered filter (UF) without loss of activity. During this process considerable amounts of the yellow material that was always pres-

ent in the extracts were removed from the solution by adsorption. When the cell-free extract was analyzed in the ultracentrifuge, a heterogeneous pattern was noted in which the intense yellow color was associated with the fast moving front. The fast moving front was separated from the slow moving front and tests showed both solutions to be active. This finding indicated that the active material is not necessarily associated with the yellow color. Activity was concentrated by fractional precipitation with ammonium sulfate. Data illustrating this finding are presented in table 3. Similar fractionations were made in which no activity was

TABLE 1

A comparison of the phosphorolytic activity of cell-free meningococcal extracts prepared under different conditions

EXTRACTS			SUBSTRATE	PHOSPHATE* PRESENT AFTER 2 HRS' INCUBATION AT 37.9 C	PHOSPHATE DECREASE
Preparation		Protein nitrogen*			
Temp	Time				
	hr	*mg/ml*		*micromoles/ml*	*per cent*
4 C	61	0.74	None	17.8	
			Glucose	17.4	
			Maltose	11.0	38
Room	61	1.30	None	17.2	
			Glucose	16.2	
			Maltose	10.0	42
37 C	61	0.42	None	18.2	
			Glucose	19.8	
			Maltose	14.4	21
37 C	13		None	17.2	
			Glucose	17.2	
			Maltose	11.8	31

* Each value given is the average of duplicate analyses: phosphate, average deviation = 0.05; protein nitrogen, average deviation = 0.005.

Composition of test systems: 0.5 ml extract, 0.5 ml P-salts, and 0.5 ml sugar solution (= 5 mg in P-salts) or P-salts. The extracts were prepared by the lysis of the cells under toluene.

found in the fractions precipitating between zero and three-tenths and between eight-tenths and fully saturated ammonium sulfate. It was concluded, therefore, that the active material is precipitated in the range from between three-tenths and eight-tenths saturated ammonium sulfate. Absorption spectra of the ammonium sulfate fractionated extracts showed a peak at 260 mμ which diminished almost completely after precipitation with acetic acid at pH 4.0. Whether or not the nucleoproteins *per se* play a role in this phosphorolysis is a problem still to be investigated.

Activity was dependent upon incubation temperature. Table 4 gives the observa-

tions from one of three similar experiments in which the activity decreased from 38 to 8 per cent when the reaction temperature was decreased from 37.9 C to 4 C. Activity was not appreciably affected by lyophilizing an aliquot of a cell-free

TABLE 2

Phosphorolysis in the presence of maltose and untreated, ultrafiltered, or dialyzed cell-free meningococcal extract

TEST SOLUTION	PROTEIN NITROGEN	SUBSTRATE	PHOSPHATE ANALYSES AFTER 3 HRS' INCUBATION AT 37.9 C	
	mg/ml		*micromoles/ml**	*per cent decrease*
Ultrafiltrate	0.00	None	11.0	
		Maltose	10.9	0
Ultrafiltrate + Residue	0.24	None	11.0	
		Maltose	8.2	25
Ultrafiltration Residue	0.24	None	9.9	
		Maltose	7.4	25
Dialysis Residue	0.24	None	10.0	
		Maltose	7.4	26
Extract	0.15	None	11.0	
		Maltose	7.7	30

* Each value given is the average of duplicate analyses; average deviation = 0.05.

Composition of test system: 0.5 ml test solution, 0.5 ml of 0.0125 M aqueous maltose solution or water, and 1.0 ml P-salts.

TABLE 3

Precipitation of the phosphorolytic activity of a cell-free meningococcal extract by ammonium sulfate

FRACTION PRECIPITATED WITH $(NH_4)_2SO_4$ SATURATED		INORGANIC PHOSPHATE*			pH	
		0 hr	4.5 hr	Decrease	0 hr	4.5 hr
		micromoles/ml		*per cent*		
Experiment I	0.0	16.3	13.1	20	7.5	7.4
	0.7	19.6	12.4	36	7.4	7.0
Experiment II	0.7	18.9	14.8	21	7.3	7.2
	0.5	18.8	13.1	30	7.3	7.1

* Each value given is the average of duplicate analyses; average deviation = 0.08.

Experiment I: 1.0 ml extract fraction + 1.0 ml 0.3 M maltose + 3.0 ml P-salts.

Experiment II: 0.5 ml extract fraction + 1.0 ml 0.3 M maltose + 3.5 ml P-salts.

extract. Figure 1 illustrates the observations of one of three studies in which the phosphorolytic activity of a freshly prepared cell-free extract was compared with that of its lyophile aliquot. In addition, the activity was dependent upon the extent of the incubation period, a fact also given in this graph.

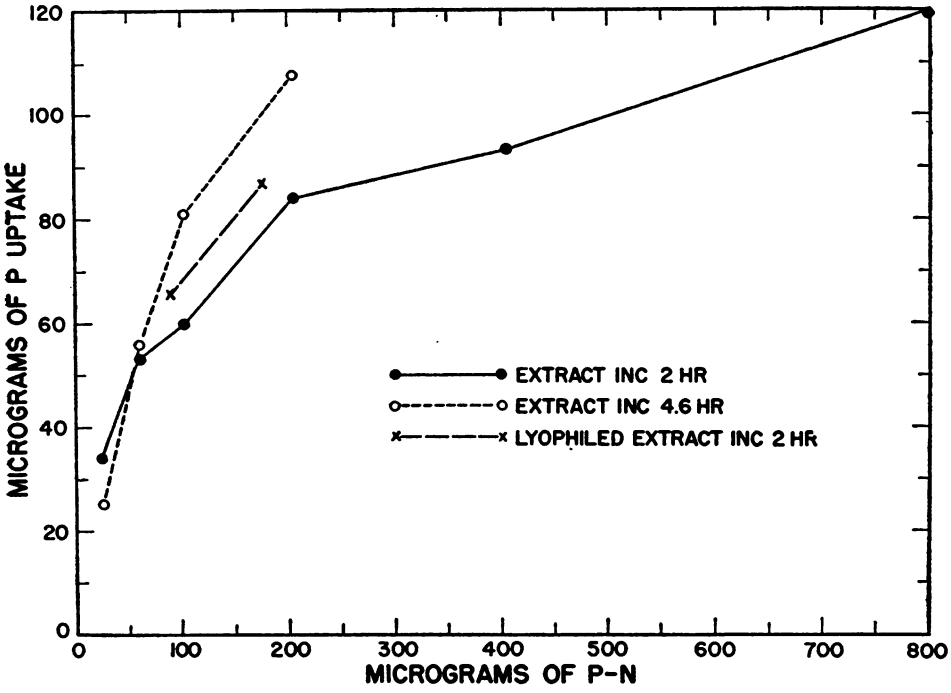

Figure 1. Relationships between the rate of disappearance of inorganic phosphate, reaction time, and dilution (indicated as amount of protein nitrogen) of either fresh or lyophilized meningococcal extract (0.5 ml) in the presence of maltose (5 mg in 1 ml of P-salts).

TABLE 4

A comparison of the phosphorolytic activity of a cell-free meningococcal extract at 37.9 C and 4 C in the presence of maltose, glucose, and dextran

SUBSTRATE	ANALYSES AFTER 4 HRS' INCUBATION AT					
	37.9 C			4 C		
	pH	Inorganic phosphate*		pH	Inorganic phosphate	
		micro-moles/ml	*per cent decrease*		*micro-moles/ml*	*per cent decrease*
Maltose	7.2	7.9	38	7.2	11.9	8
Dextran	7.4	13.0	0	7.2	12.9	0
Glucose	7.4	12.9	0	7.2	12.9	0
None	7.4	12.8	0	7.2	12.9	0

* Each value given is the average of duplicate analyses with an average deviation of 0.09; theoretical value for the concentration of inorganic phosphate per ml at 0 hr = 12.9 micromoles.

Composition of test systems: 0.5 ml extract + 0.5 ml of 0.2 M carbohydrate in P-salts + 0.5 ml of 0.9 per cent NaCl (except 0.5 ml of 6 per cent depolymerized dextran in 0.9 per cent NaCl) + P-salts to make a total volume of 3.5 ml.

The pH optimum of the phosphorolysis of maltose in the presence of cell-free extract was approximately pH 6.5 (figure 2). The average pH during the reaction

was plotted against per cent phosphate uptake since these determinations were made in phosphate buffer, i.e., during the reactions a decrease of pH occurred paralleling the extent of phosphate esterification. In a similar experiment the optimum pH was determined to be pH 6.7.

Activity was dependent upon substrate concentration. When the concentration of maltose was varied from 10 to 60 micromoles per ml of reaction mixture, the decrease of inorganic phosphate ranged from 2.0 to 3.8 micromoles, respectively (i.e., from 11 to 21 per cent of total). These observations when presented graphically result in an asymptotic curve.

So far the characterization of the active material of the cell-free extract had centered around its protein-like nature. Thus, it was found to be a heat labile,

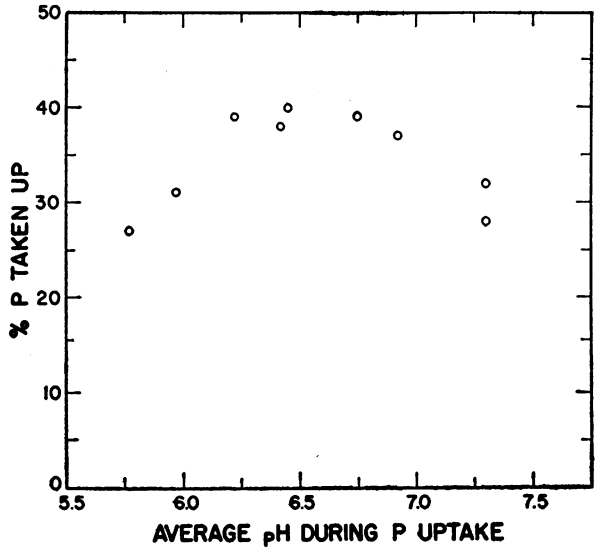

Figure 2. Effect of pH upon the rate of disappearance of inorganic phosphate in mixtures of cell-free meningococcal extract (0.5 ml) and 0.025 M maltose in P-salts solution (1 ml). Incubated for 3 hours at 37.9 C.

nondialyzable, and nonultrafilterable substance which could be preserved by desiccation from the frozen state and could be concentrated by fractional precipitation with ammonium sulfate; its phosphorolytic activity was related to the protein portion of the extract and to time, temperature, pH, and substrate concentration. Another definitive characteristic of biocatalysts is their relatively specific substrate affinity.

Substrate specificity. With the exception of the disaccharide, maltose, no substrate has yet been found with which there is an uptake of inorganic phosphate in the presence of the meningococcal extract. Using a procedure similar to the one which has been described previously, the following substances were tested. They represent primarily various differences in the stereochemical configuration of maltose(4-[α-D-glucopyranosyl]-D-glucose):

Monoses,

D-glucose, D-galactose

Glycosides.

1-α-substitution

α-methyl glucoside

1-α-(D-glucopyranosyl)-D-glucopyranose = trehalose

1-β-substitution

1,3-cellobiuronic acid linkages = type III pneumococcus polysaccharide

4-β-substitution

4-β-(D-glucopyranosyl)-D-glucose = cellobiose

4-β-glucuronosido-glucose = cellobiuronic acid

6-α-substitution

6-α-(D-glucopyranosyl)-D-glucose = isomaltose

1,6-α-(D-glucopyranoside)-linkages = dextran

6-β-substitution

6-β-(D-glucopyranosyl)-D-glucose = gentiobiose

Miscellaneous:

inositol, polysaccharide from type I meningococcus

From these observations it is concluded that a 1,4-α-glucopyranoside linkage is required for the phosphorolysis catalyzed by the cell-free extract of this strain of *N. meningitidis.*

Characterization of the reactive system. In order that one could reasonably conclude that a maltase was not involved prior to a phosphorylation, the following protocol was carried out: a cell-free extract was freed of inorganic phosphate by dialysis against distilled water; phosphorolytic activity in the presence of maltose and P-salts was not lost. Test mixtures containing phosphate-free extract and 0.12 M, 0.06 M, 0.03 M, 0.01 M, 0.004 M, and no maltose in 0.02 M borate buffer at pH 7.25 were incubated for from 0 to 4½ hours at 37.9 C. At intervals aliquots were removed and immediately deproteinized and analyzed for total reducing values according to the method of Schales and Schales (1945). No increase of reducing substances was found (the experimental error was ±5 per cent) indicating that under the stated experimental conditions an active maltase was absent. Further support for this conclusion comes indirectly from the evidence that D-glucose is not a substrate for a phosphorylation catalyzed by the cell-free extract (table 1). It is inferred, therefore, that the specificity of the described reaction does not involve a specific hydrolase, i.e., maltase, but that the specificity may be related to the presence of either a specific transglucosidase or a phosphorylase, both of which may be present in the crude meningococcal extract.

The identification of the end products of the phosphorolysis of maltose was difficult since their chemical isolation could not be accomplished with the available quantities of cell-free extract. By a process of elimination we have come to the conclusion that one of the end products of this reaction is glucose-1-phosphate and the other is a reducing substance which has the same chromatographic partition coefficient as glucose.

Since reaction-type *a* was eliminated (see previously), we next investigated the possibility of reaction-type *b*, during which there should be an accumulation of a polymer of one of the constituent monoses of the disaccharide. Monod and Torriani (1950) observed that in a test system containing maltose and a cell-free extract of a strain of *Escherichia coli* a polymer was formed which gave an iodine reaction. With the meningococcal extract it has not been possible so far to demonstrate the formation of a polymer which gives either an iodine reaction or forms a precipitate in the presence of type I meningococcal or type II pneumococcal antisera. It is well known that the addition of a trace of the polysaccharide catalyzes its enzymatic synthesis by this type of reaction. As an additional test for

TABLE 5

Incorporation of inorganic phosphate into an organic phosphate fraction formed by a cell-free meningococcal extract with either maltose or α-D-glucose-1-phosphate and glucose as substrates

SUBSTRATE	MALTOSE			α-D-GLUCOSE-1-PHOSPHATE + GLUCOSE		
Analysis	Inorganic phosphate*			Inorganic phosphate		
	0 hr	4.5 hr	Change	0 hr	4.5 hr	Change
	micromoles/ml	*micromoles/ml*	*per cent*	*micromoles/ml*	*micromoles/ml*	*per cent*
Material analyzed:						
Reaction mixture						
Before hydrolysis†	19.7	10.5	−46.7	20.6	20.6	0.0
After hydrolysis	22.1	22.2	+0.5	62.6	63.3	+1.1
Magnesia mixture precipitate‡						
Before hydrolysis	19.7	10.0	−49.2	21.5	20.6	−4.2
After hydrolysis	19.5	11.2	−42.5		20.8	

* Each value given is the average of duplicate analyses with an average deviation of 0.10.

† Hydrolysis in 1.0 N HCl for 10 minutes at 100 C.

‡ Results calculated back to the original volume.

Composition of test systems: 5.0 ml P-salts + 2.0 ml cell-free extract (which previously had been precipitated successively with 0.7 and 0.5 saturated ammonium sulfate) + either 3.0 ml 0.3 M maltose or 2.0 ml 0.2 M glucose-1-phosphate + 1.0 ml 0.3 M glucose; incubated at 37.9 C for 4.5 hours.

the participation of polyose in the reaction, therefore, we tested the effect of adding meningococcal polysaccharide to test systems containing as substrates glucose, maltose, and isomaltose, respectively. No change in the uptake of inorganic phosphate was detected. Similar results were obtained on the addition of two preparations of meningococcal "endotoxin". Another evidence of polyose formation would be an increase of the viscosity of the reacting system. Such an increase, however, was not demonstrable. The data, therefore, do not indicate that maltose is first polymerized prior to a phosphorolysis by the meningococcal cell-free extract.

A sugar phosphate. Evidence is presented in table 5, which records the results

of one of four similar experiments, that in the presence of the enzyme, maltose and inorganic phosphate yield a sugar phosphate that is hydrolyzed completely within 10 minutes at 100 C in the presence of 1.0 N hydrochloric acid. The substance containing the labile phosphate was not precipitable by magnesia mixture, which did, however, remove the inorganic phosphate quantitatively. No phosphate was released when the reaction mixture was heated in 1.0 N sodium hydroxide for as long as 30 minutes. From these findings it may be inferred that an esterification of maltose by phosphate occurs in the one-alpha position. Two reactions may, therefore, be postulated which are catalyzed by this cell-free extract:

I. Maltose + inorganic phosphate $\rightleftharpoons$ maltose-1-phosphate + water
II. Maltose + inorganic phosphate $\rightleftharpoons$ glucose-1-phosphate + glucose

In order for reaction I to occur a source of energy must be supplied. We have already shown that dialysis as well as ultrafiltration does not separate an essential cofactor from the extract (table 3). In fact, the addition of adenosine triphosphate or of magnesium ions, or of both, to the cell-free extract did not influence the rate of phosphate esterification in the presence of maltose. Unless the energy donor is tightly bound to the active substance, i.e., it is bound so tightly that it does not dissociate on dialysis or ultrafiltration, reaction I remains improbable on a thermodynamic basis. Furthermore, when aliquots of the reaction mixtures were taken before and after incubation and analyzed by paper partition chromatography after deproteinization, it became evident also that a reducing substance having the same partition coefficient as glucose was formed by the reaction of maltose and inorganic phosphate in the presence of the cell-free extract. The reverse reaction, however, starting with α-D-glucose-1-phosphate and glucose, could not be demonstrated.[6]

The data support the conclusion that the utilization of maltose takes place according to reaction II. It is as yet not possible to say whether this reaction of type c is catalyzed by only one or by more than one enzyme from this strain of *N. meningitidis*. It does appear that a similar reaction is promoted, whether it be during the growth of this organism or by washed cells or by a cell-free extract (Fitting and Scherp, 1951, 1952). The enzyme, or enzyme system, seems to differ from similar ones described previously by being "constitutive", i.e., it is formed by the organism during growth in the absence of the specific substrate. Since the media used for cultivation of the organism (trypticase soy agar or blood agar) probably contained small amounts of one of the products of the reaction, namely, glucose, this view is susceptible of amplification (Monod, 1947).

SUMMARY

Cell-free extracts of a strain of *Neisseria meningitidis* (strain 69, type I) were found to exhibit an enzyme-like phosphorolytic activity in the presence of inor-

[6] Since the preparation of the present manuscript, Fitting and Doudoroff (1952) have shown that this paradox derives from the fact that the phosphorylated product is β-D-glucose-1-phosphate and have demonstrated the reverse reaction by starting with mixtures of this ester and glucose.

ganic phosphate and maltose. The activity depended upon a heat labile, nondialyzable and nonultrafilterable substance, which could be concentrated by fractional precipitation with ammonium sulfate and was related to the protein portion of the extract. The phosphorolytic activity depended also upon the time and temperature of incubation, pH, and concentration of substrate. With the exception of the disaccharide, maltose, no effective substrate has as yet been found. The end products of the phosphorolysis appear to be glucose-1-phosphate and glucose.

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