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

II. Studies with Washed Cells[1]

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The hypothesis that a strain of *Neisseria meningitidis* utilizes the disaccharide, maltose, by a different "metabolic pathway" than that involved in the utilization of its constituent monosaccharide, glucose, was proposed as a result of studies of the growth of the organism (Fitting and Scherp, 1951). Further support for this assumption is presented here with the result of studies of suspensions of washed cells of this meningococcus in the presence of either maltose, glucose, glucose-1-phosphate, or a polysaccharide from a type 1 meningococcus. These systems were explored with respect to rates of oxygen consumption in carbon dioxide-free air and in air buffered with three per cent carbon dioxide, aerobic and anaerobic acid production, and uptake of inorganic phosphate.

With maltose as the substrate, the organism consumed oxygen faster than it did with glucose. Similar observations have been made with a variety of bacteria and have been reported in the literature previously (Hassid and Doudoroff, 1950; Leibowitz and Hestrin, 1945; and Doudoroff, 1940). Oxygen consumption by suspensions of washed cells of this strain of *N. meningitidis* was found, however, to depend upon the history of the culture. For example, by passing the culture through a variety of chemically defined liquid media differing in both the concentration of inorganic phosphate and the type of sugar, populations were obtained which in the presence of glucose consumed oxygen at a rate similar to that observed with maltose. This type of adaptation to glucose utilization is assumed to be one of selection for reasons set forth in the description of studies of growing cultures of this organism (Fitting and Scherp, 1951).

MATERIALS AND METHODS

Cultures. Most of these experiments were done with the strain of *N. meningitidis* studied previously (Fitting and Scherp, 1951). The history of this and of eight other strains of meningococci used was described by Scherp and Fitting (1949).

[1] The data presented in this paper were taken from a thesis submitted in June, 1950, by Charlotte Fitting to the University of Rochester in partial fulfillment of the requirements for the Doctor of Philosophy degree. The study was supported by a contract (N6-ori-126, Task III) between the University of Rochester and the Office of Naval Research. This contract stipulates that "reproduction in whole or in part is permitted for any purpose of the United States Government." Part of the material was presented before the 50th general meeting of the Society of American Bacteriologists at Baltimore, Maryland, May 15, 1950 (Fitting and Scherp, 1950).

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Media. The basic culture medium was that reported by Frantz (1942). Trypticase soy agar (Baltimore Biological Laboratory) was added to the media listed by Fitting and Scherp (1951).

Reagents. Adenosine triphosphate was prepared from rabbit muscle according to the procedure of Dounce *et al.* (1948) resulting in a highly purified product (e.g., 98 to 99 per cent pure).

From a pathogenic strain of *N. meningitidis*, type 1, a purified polysaccharide (no. 33B) was obtained using a variation of the method described by Scherp (1943), in which all reactions were carried out at neutrality.

Analytical methods. In addition to the methods described (Fitting and Scherp, 1951) the following manometric procedures were used:

Oxygen uptake was measured in a Warburg apparatus at 37.9 C. Air, or air and three per cent carbon dioxide, was used as the gas phase. In the latter experiments, the pressure of carbon dioxide was kept constant by using potassium bicarbonate in acid diethanolamine (Eastman Kodak Company) according to the procedure described by Pardee (1949). When air was used as the gas phase, any carbon dioxide produced during the reaction and liberated into the atmosphere was presumably eliminated by the presence of filter paper, saturated with either 20 or 4 per cent potassium hydroxide, in the center well of the Warburg flask.

Carbon dioxide evolved during respiration was measured by the "direct" method (Umbreit, Burris, and Stauffer, 1945).

Acid production during anaerobic metabolism was determined in the conventional way by replacing air with a mixture of 95 per cent nitrogen and 5 per cent carbon dioxide. This gas mixture had previously been freed of residual oxygen by passage through a wash train composed of two towers of chromous potassium sulfate solution and amalgamated zinc (Stone and Beeson, 1936) and one tower containing water. Three ml of the reaction mixture in the Warburg flask were buffered at pH 7.5 by the incorporation of 60 μM of sodium bicarbonate.

Experimental procedures. Washed cells were obtained from a variety of sources which are summarized here:

a. Blood agar plates were inoculated with a drop of a saline suspension of the lyophile culture material and were incubated for 12 to 18 hours at 37 C. At that time the cells (blood agar plate c-1) were washed off with either Frantz-glucose medium or the indicated salt solution. The suspension was filtered through glass wool and finally centrifuged at 2,400 rpm for one to two hours at 4 C. The supernatant was decanted and the cells were resuspended in the respective solution. The final turbidity of the suspensions was expressed as the scale reading on a Klett-Summerson photoelectric colorimeter with filters no. 42 or no. 66. A scale reading of 40 corresponded to a viable count of 3×10^8, on the average.

b. Cells from a blood agar culture (blood agar plate c-1 or c-3) were transferred to a 125 ml Erlenmeyer flask containing 25 ml Frantz-glucose medium. After 18 hours' incubation in a closed container at 37 C, this liquid culture was used to inoculate 100 ml of trypticase soy agar which previously had been layered on the bottom of a 1,000 ml Erlenmeyer flask. These trypticase soy agar flasks were

incubated for 24 hours. The cells were then harvested in the manner described before.

c. In some of the adaptation experiments cells, grown on either Frantz-maltose, no. 4-glucose, or no. 4-maltose media, were used in place of the Frantz-glucose culture described in procedure b. The Frantz and the no. 4 media differed in that the concentration of inorganic phosphate of the latter medium was one-tenth of that found in the Frantz medium.

d. After three successive daily transfers on blood agar plates the cells were collected from the last passage (blood agar plate c-3).

e. Trypticase soy agar plates were used in place of blood agar plates in procedure d.

RESULTS AND DISCUSSION

Nonagitated suspensions of washed cells of N. meningitidis (strain 69, type 1) produced more acid in Frantz-maltose than in Frantz-glucose medium, and only in the former system was there a decrease of inorganic phosphate. Growing cells of this organism behaved similarly as we have reported previously (Fitting and Scherp, 1951). In table 1 are summarized the results of one of four experiments in which washed cells were suspended in Frantz medium containing either 0.5 per cent glucose, 1.0 per cent maltose, or no sugar. After 50 hours' incubation at 37 C, a drop of 0.9 pH unit was recorded in the maltose cultures and a drop of only 0.3 pH unit in the glucose cultures. In the absence of sugar an increase of 0.5 pH unit was noted. Fifty-one per cent of inorganic phosphate had disappeared in the maltose cultures and none in the glucose cultures, yet the amount of total reducing substances diminished both in the presence of maltose (27 per cent) and of glucose (21 per cent). On the basis of these observations the differential utilization of the two sugars was investigated further with systems equilibrated more uniformly with their gaseous environment in the Warburg constant volume respirometer. These experiments, however, were complicated by selective phenomena analogous to those previously reported on growth requirements (Fitting and Scherp, 1951 and Scherp and Fitting, 1949).

A selection of variants with an increased capacity to consume oxygen in the presence of glucose and maltose occurred when the organism was cultured from blood agar or trypticase soy agar plates to Frantz-glucose medium and then to trypticase soy agar (table 2). The amplitude of the increased oxygen consumption by cells from trypticase soy agar, which had been inoculated with a liquid culture, was found to depend upon the concentration of inorganic phosphate and upon the type of sugar present in the liquid medium (figure 1). With glucose as the substrate, cells previously cultivated in Frantz media containing high concentrations of inorganic phosphate consumed oxygen at a much higher rate than did cells previously cultivated in Frantz media containing low concentrations of inorganic phosphate. Furthermore, it was observed that, irrespective of the concentration of inorganic phosphate, cells grown in liquid media containing maltose respired glucose approximately two and one-half times as fast as cells previously grown in liquid media containing glucose. In other words, the in-

creased activity of the cells with respect to glucose was not related specifically to the enhanced utilization of that sugar present in the respective medium, but it was related to the concentration of inorganic phosphate and to the heterologous sugar. Since these results are the reverse of those expected of an adaptation, it is suggested that the increase of oxygen consumption by washed cell suspensions of *N. meningitidis* obtained from passage through different media results from

TABLE 1

Washed cells of Neisseria meningitidis in Frantz media with glucose (0.5 per cent), or maltose (1 per cent), or without added sugar

MEDIUM†	FLASK NO.	ANALYSES					
		pH		"Glucose"*		P*	
		Hours incubated					
		28	50	28	50	28	50
				mg/ml		Micromoles/ml	
Frantz-glucose (0.5 per cent)							
Experimental	1	7.2	7.1	4.54	4.20	17.5	17.9
	2	7.2	7.1	4.54	4.20	16.7	17.5
	3	7.2	7.1	4.54	4.20	16.9	17.9
Control	1	7.4	7.4	5.40	5.30	17.2	17.9
Frantz-maltose (1 per cent)							
Experimental	1	6.9	6.5	6.14	5.64	10.6	8.5
	2	6.9	6.4	5.85	5.64	10.6	8.6
	3	6.9	6.4	6.10	5.40	10.7	8.2
Control	1	7.3	7.3	7.15	7.60	17.0	16.7
Frantz without sugar							
Experimental	1	7.7	7.8	—	0.00	17.0	18.0
	2	7.7	7.9	—	0.00	17.0	18.0
Control	1	7.3	7.3	—	0.00	—	18.0

* Each value given is the average of duplicate analyses: "glucose", average deviation = 0.05; P, average deviation = 0.3.

† Fifty ml of the respective medium were dispensed in a 500 ml Erlenmeyer flask. The turbidity of the reaction mixtures was 220 when diluted fivefold.

"the selection of variants which occur under normal conditions in the parent culture" (Dubos, 1945). With maltose as the substrate, an increase of oxygen consumption by the same preparations of washed cells accompanied that described with glucose, but the proportional increase was only one-fourth as great on the average (table 2). In other words, the selection resulted in a much greater increase in the capacity to respire in the presence of glucose than of maltose. Nevertheless, a difference remained in the utilization of the two sugars.

The observations reported so far deal with cultures whose passage through a liquid medium increased the rate of oxygen consumption in the presence of malt-

ose or glucose, suggesting that a selection for one substrate also selects for the other substrate. This "multiple" selection, however, did not favor all carbohydrates as can be seen from figure 2 in which a diminution of cellular respiration in the presence of a purified meningococcal polysaccharide is demonstrated following passage through the Frantz medium. This decrease suggests either that the Frantz-glucose culture contained a population well equipped with enzymes needed for the oxidation of both the monosaccharide and the disaccharide but ill equipped for the utilization of the polysaccharide, or that cells obtained from

TABLE 2

Oxygen uptake by washed cells of Neisseria meningitidis in Frantz salts with glucose or maltose

	MICROLITERS OF OXYGEN UPTAKE								
Time in minutes after addition of substrate	20	35	45	55	65	75	85	95	105
Blood agar plate c-3 (d)									
Glucose	6	11	17	20	21	26	28	33	34
Maltose	36	87	124	164	199	234	267	300	326
Endogenous	0	3	6	7	7	7	7	12	12
Trypticase soy agar plate c-3* (e)									
Glucose	3	16	19	20	18	27	31	36	43
Maltose	48	117	164	226	283	358			
Endogenous	15	29	44	40	44	51	58	62	69
Blood agar plate c-1 → Frantz-glucose → Trypticase soy agar plate* (b)									
Glucose	32	99	149	214	271	338			
Maltose	132	269	345						
Endogenous	12	28	33	37	40	48	52	59	64

* Values corrected for minor differences in the density of the cell suspensions.

Content of Warburg vessel: main chamber, 2.0 ml cell suspension; side arm, 0.3 ml sugar solution containing 67 μM, or 0.3 ml Frantz salts; center cup, 0.2 ml 20 per cent KOH.

blood agar present a selected population especially capable of utilizing the meningococcal polysaccharide.

The study of the differential utilization of sugars by this strain of *N. meningitidis*, therefore, was complicated by the occurrence of such selective phenomena as have been described above. By adhering carefully to the determined conditions, i.e., the use of organisms cultivated exclusively on blood agar plates or trypticase soy agar plates according to procedures a, d, or e, it was possible to circumvent these selections allowing further investigation of the carbohydrate metabolism of the organism.

In low concentrations of carbon dioxide, as provided by the Warburg "direct" method, the rates of respiration of washed cells of *N. meningitidis* were found to depend not only upon the presence or absence of either of the two sugars but also

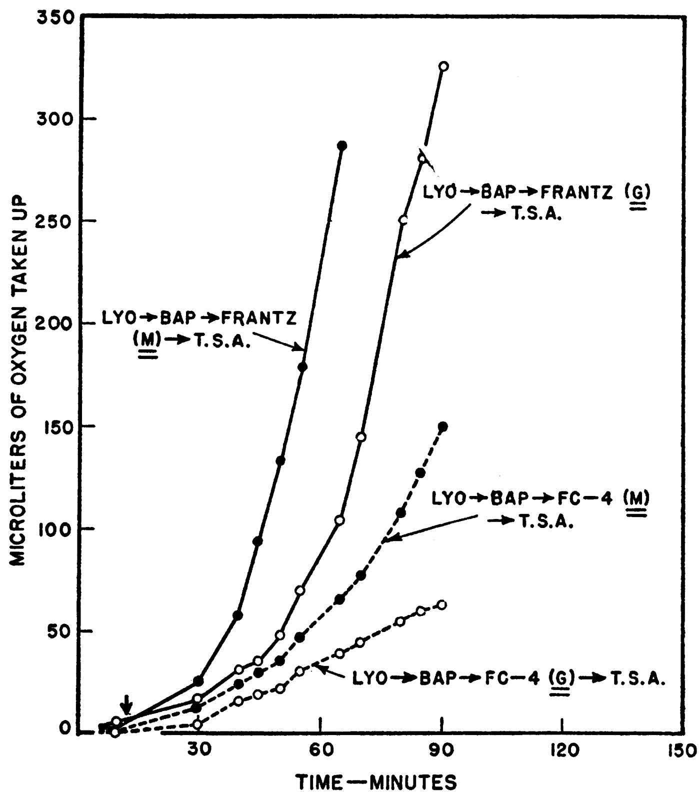

Figure 1. The effect of selective phenomena, which result from passing a culture through liquid media differing in the concentration of inorganic phosphate and in the type of sugar present, upon the capacity of *Neisseria meningitidis* to consume oxygen in the presence of glucose. Content of Warburg vessel: main chamber, 2.0 ml cell suspensions (turbidity of a twentyfold dilution = 164); side arm, 0.3 ml glucose (= 12 mg); center cup, 0.2 ml 20 per cent KOH.

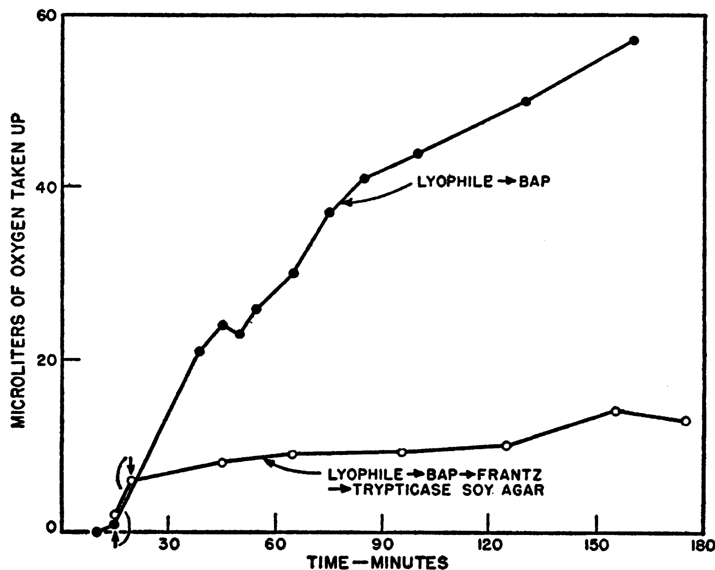

Figure 2. Oxygen uptake by *Neisseria meningitidis* with polysaccharide preparation no. 33B as substrate. Content of Warburg vessel: main chamber, 2.0 ml cell suspension (turbidity of a tenfold dilution = 178); side arm, 0.3 ml polysaccharide (= 12 mg); center cup, 0.2 ml 20 per cent KOH.

of cystine and glutamic acid, and upon the composition of the salt solutions used for suspending the cells. Oxygen uptake by cells suspended in the Frantz medium, i.e., salts containing cystine and glutamic acid, occurred to a similar extent in the presence of glucose and of maltose. However, cells of either strain 69 or of eight other strains (Fitting and Scherp, 1951) suspended in Frantz salts, i.e., in the absence of the amino acids, respired faster in the presence of maltose than of glucose or endogenously (figure 3). As in the previously reported studies of growth

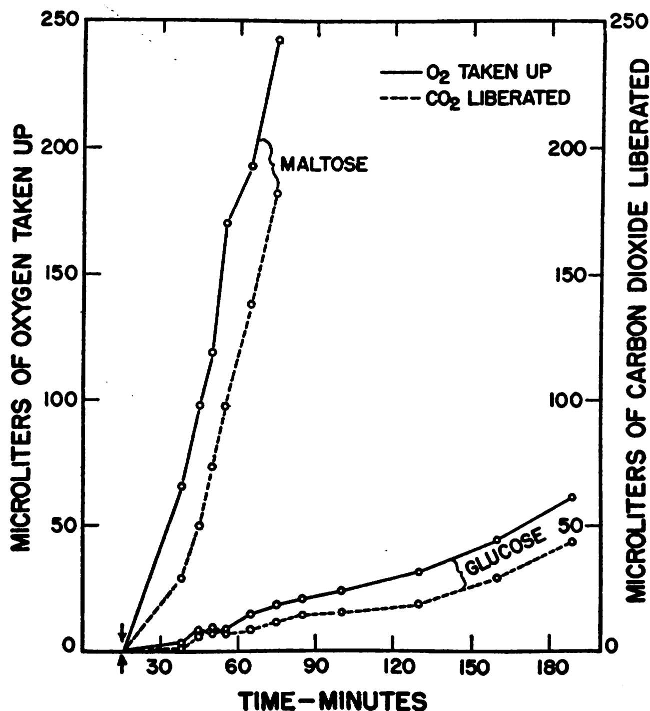

Figure 3. Oxygen uptake and carbon dioxide liberation by *Neisseria meningitidis* with glucose and maltose as substrates. Content of Warburg vessel: main chamber, 2.0 ml cell suspension (turbidity of a tenfold dilution = 178); side arm, 0.3 ml sugar solution (= 12 mg glucose and 24 mg maltose, respectively); center cup, 0.2 ml 20 per cent KOH or 0.2 ml H_2O.

and of acid production, strain 69 showed a greater differential than any of the other cultures and was, therefore, used exclusively for the succeeding experiments. In fact, the respiration of strain 69 with glucose as substrate was often barely greater than the endogenous respiration. On recrystallization of the sugars, the preferential utilization of maltose by the organism did not decrease. Oxygen uptake by cells washed and resuspended in Krebs-Ringer phosphate solution (Umbreit, Burris, and Stauffer, 1945) was completely inhibited regardless of which sugar was added to the reaction mixture. Since this reduction of the oxidative metabolic activities of the organism did not occur with Frantz salts, it is pertinent to point out that the two salt solutions differed not only in the pro-

portions of their constituents but also in that the Krebs-Ringer phosphate contained calcium chloride and the Frantz salts contained ammonium chloride. The role of these inorganic ions in the respiration of this organism has not been investigated further.

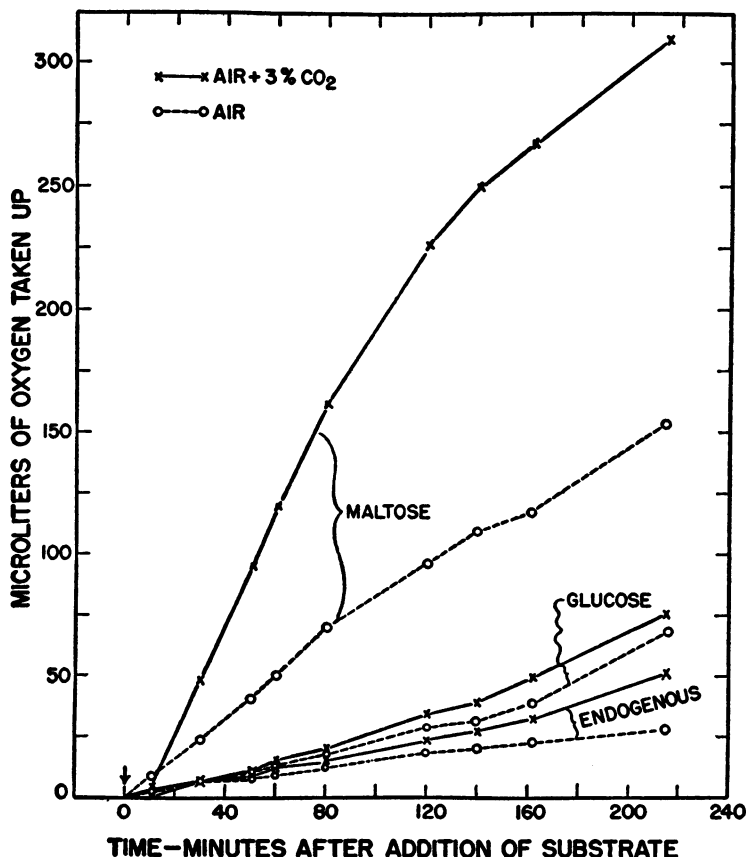

Figure 4. The effect of a three per cent carbon dioxide buffer upon the oxygen uptake of *Neisseria meningitidis* with glucose and maltose as substrates. Content of Warburg vessel: main chamber, 2.0 ml cell suspension (turbidity of a tenfold dilution = 94) and 0.5 ml H_2O; side arm, 0.3 ml sugar solution (= 5.36×10^{-3} M); center cup, 0.2 ml 4 per cent KOH or 0.2 ml diethanolamine reagent.

Oxygen uptake in the presence of carbon dioxide increased in reaction mixtures containing glucose or maltose (figure 4). It is widely acknowledged (Umbreit, Burris, and Stauffer, 1945, and Pardee, 1949) that carbon dioxide may accelerate the rate of oxidation of organic compounds by some microorganisms during growth as well as in the so-called resting state, i.e., in washed cell suspensions. The present experiments clearly demonstrate this effect. Previously, we presented data showing the necessity for carbon dioxide during the growth of this and three other strains of meningococci both in the presence and absence of glucose (Scherp

and Fitting, 1949). Using the method of Pardee (1949), washed cell suspensions of strain 69 oxidized either of the two sugars faster in the presence of three per cent carbon dioxide than in its absence. In view of these findings the respiratory quotients indicated by figure 3 are misleading, since Warburg's "direct" method was used for measuring carbon dioxide liberation. Indeed, these respiratory quotients should diminish the greater the accelerating effect of carbon dioxide upon oxygen uptake. How carbon dioxide exerts this accelerating effect on the cellular metabolism associated with growth or with the resting state of the meningococcus is as yet an unsolved problem.

TABLE 3

Effect of adenosine triphosphate (ATP) upon the oxygen uptake by washed cells of Neisseria meningitidis in Frantz salts with glucose and maltose

	MICROLITERS OF OXYGEN UPTAKE						
Time in minutes after addition of substrate	10	20	25	30	40	50	55
Glucose	0		0		5		11
Glucose + ATP no. 2	0		10		14		19
Glucose + ATP no. 3	0		8		15		22
Endogenous	0		2		6		10
Endogenous + ATP no. 3	0		6		10		19
Maltose*	27	69		121	168	225	254
Maltose + ATP no. 2	27	65		117	167	224	248
Maltose + ATP no. 3	26	76		143	198	260	291
Endogenous*	2	2		6	7	10	12
Endogenous + ATP no. 3	1	5		11	12	17	17

* Cell suspension had been stored in the refrigerator for three hours during the first part of the experiment.

Content of Warburg vessel: main chamber, 2.0 ml cell suspension (turbidity of a tenfold dilution = 100) and 0.3 ml ATP (no. 2 contained 3.9 μM and no. 3 contained 4.2 μM); side arm, 0.3 ml sugar solution containing 67 μM, or 0.3 ml Frantz salts; center cup, 0.2 ml 20 per cent KOH.

Increased respiration in the presence of either glucose or maltose was thus observed on addition of carbon dioxide and also by selecting bacterial populations. Regardless of these conditions, respiration in the presence of maltose was many times as rapid as with glucose but only when the cells were suspended in Frantz salts, i.e., in the absence of glutamic acid and cystine. What then prevents the cells from respiring in the presence of glucose?

In order to eliminate the question of substrate concentration, the amounts of glucose were varied in the reaction mixtures. No significant increase of oxygen consumption, however, was produced by a tenfold increase of the concentration of glucose. Next, we investigated the response of cellular respiration to the addition of adenosine triphosphate preparations. Table 3 records data obtained from

one of six experiments. From these and similar data it was concluded that the addition of adenosine triphosphate did not increase oxygen uptake significantly

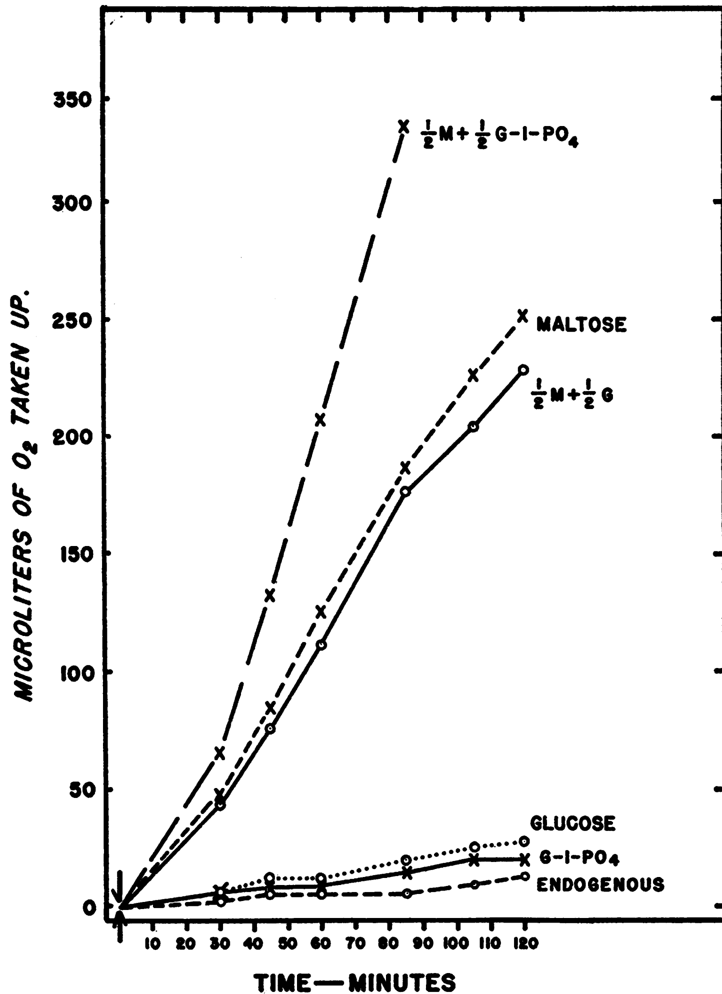

Figure 5. Oxygen uptake by *Neisseria meningitidis* in the presence of adenosine triphosphate (ATP), glucose-1-phosphate, and maltose. Content of Warburg vessel: main chamber, 1.7 ml cell suspension (turbidity of a tenfold dilution = 85), 0.3 ml Frantz salts, and 0.3 ml adenosine triphosphate (= 1.5×10^{-4} M); side arm, 0.1 ml Frantz salts and 0.4 ml of either glucose, maltose, or glucose-1-phosphate (= 16 mg, 16 mg, and 33.8 mg CHO, respectively), or 0.2 ml glucose + 0.2 ml maltose, 0.2 ml maltose + 0.2 ml glucose-1-phosphate, or 0.4 ml Frantz salts; center cup, 0.2 ml 20 per cent KOH.

either in the presence of glucose or maltose. After diluting the respective adenosine triphosphate preparations up to twentyfold and after adding these dilute solutions to the respective reaction mixtures, no further change of the cellular respiration was noted. Furthermore, the rates of respiration of the endogenous

metabolism and that in the presence of glucose-1-phosphate (the two differed little quantitatively) remained unaffected by added adenosine triphosphate. One may assume that similar to yeast (Rothstein and Meier, 1949) the meningococcal

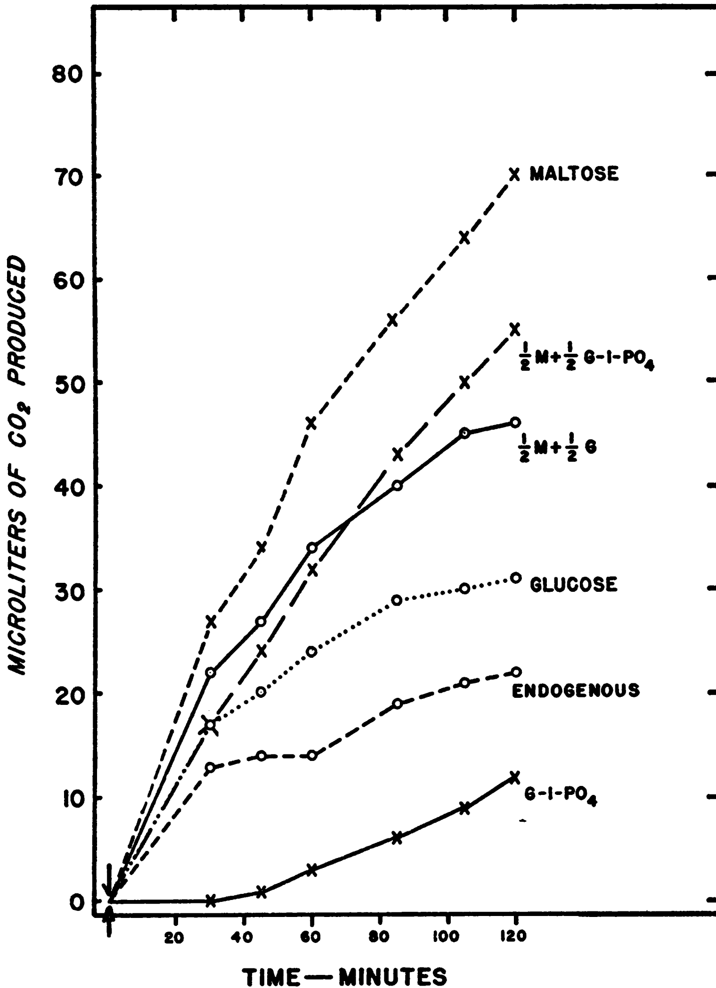

Figure 6. Anaerobic acid production by *Neisseria meningitidis* in the presence of adenosine triphosphate (ATP), glucose-1-phosphate, and maltose. Content of Warburg vessel: 95 per cent N_2 + 5 per cent CO_2; main chamber, same as figure 5 except that 0.5 ml 0.1 M $NaHCO_3$ was substituted for Frantz salts; side arm, 0.1 or 0.5 ml 0.1 M $NaHCO_3$ substituted for Frantz salts.

membrane is impermeable to the Cori ester, and, thus, the organism is unable to utilize this compound. Since Leahy, Stokinger, and Carpenter (1940) demonstrated an active phosphatase in two strains of the meningococcus, it may be that the added adenosine triphosphate is first dephosphorylated by this phosphatase before its diffusion through the cell membrane. Inconclusive attempts

were made to poison the phosphatase with ammonium molybdate as Rothstein and Meier (1949) had succeeded in doing with yeast. In this connection it was rather startling to find that in the presence of adenosine triphosphate, but not in its absence, the addition of glucose-1-phosphate increased oxygen consumption with maltose (figure 5); anaerobic acid production, on the other hand, remained very low regardless of the presence or absence of glucose-1-phosphate (figure 6). These results may constitute support for a hypothesis that an enzyme, e.g., this assumed phosphatase, can be inactivated temporarily by saturation with one of its substrates (either glucose-1-phosphate or adenosine triphosphate) allowing the other substrate to be utilized by a different reaction sequence. From

TABLE 4

Effect of iodoacetate (IAc) upon the oxygen uptake and anaerobic acid production by washed cells of Neisseria meningitidis in Frantz salts with glucose and maltose

	MICROLITERS OF OXYGEN UPTAKE*						MICROLITERS OF CARBON DIOXIDE LIBERATION†					
Time in minutes after addition of substrate	15	40	45	60	75	90	15	40	45	60	75	90
Glucose	2	27	33	42	55	63	9	13	12	17	19	19
Glucose + IAc‡	8	24	25	30	35	39	11	12	12	18	18	18
Maltose	70	287	327				19	36	37	44	54	59
Maltose + IAc‡	24	124	147	200	253	302	12	32	33	44	53	59
Endogenous	0	11	11	11	17	20	7	10	10	13	15	15
Endogenous + IAc‡	1	10	8	10	15	15	8	11	11	15	15	15

Content of Warburg vessel: main chamber, 1.0 ml cell suspension (turbidity of a twenty-fold dilution = 95),* 0.3 ml H_2O and either 1.0 ml IAc or 1.0 ml Frantz salts, † 0.5 ml 0.1 M $NaHCO_3$ and either 1.0 ml IAc or 1.0 ml Frantz salts; center cup, * 0.2 ml 20 per cent KOH; side arm, * 0.4 ml sugar solution containing 16 mg and 0.1 ml H_2O or 0.5 ml H_2O, † 0.4 ml sugar solution and 0.1 ml 0.1 M $NaHCO_3$ or 0.4 ml H_2O and 0.1 ml 0.1 M $NaHCO_3$.

† Under 95 per cent N_2 + 5 per cent CO_2.

‡ 3.6×10^{-5} M.

these observations it is inferred that added adenosine triphosphate has neither an accelerating effect on the oxygen consumption of the organism in the presence of glucose, maltose, or the Cori ester, nor does it influence the endogenous reactions, and that any adenosine triphosphate required for these reactions is present in sufficient quantity inside the cell.

A comparison of anaerobic acid production with oxygen uptake by washed cells of this strain of the meningococcus showed that even with maltose as the substrate a relatively low rate of glycolysis occurred (table 4). Since washed cell suspensions decreased the concentration of inorganic phosphate of the suspending medium (table 1) only in the presence of maltose, and since we also observed these low rates of glycolysis, it became of interest to determine whether or not the organism utilized the sugars according to the conventional Embden, Meyerhof, and Parnas scheme. The disappearance of inorganic phosphate from the

suspending medium was assumed to represent an esterification, and if that be so and if this scheme was involved, the addition of iodoacetate should inhibit both

TABLE 5

A summary of the effects of varying concentrations of iodoacetate (IAc) upon phosphate esterification, oxygen consumption, and anaerobic acid production by washed cells of Neisseria meningitidis

GAS PHASE	SUBSTRATES		AIR		AIR + 3 PER CENT CO_2		95 PER CENT N_2 + 5 PER CENT CO_2	
			O_2 uptake	Decreased phosphate*	O_2 uptake	Decreased phosphate*	CO_2 liberated	Decreased phosphate*
		min	*μl*	*per cent*	*μl*	*per cent*	*μl*	*per cent*
Exp. 1	Glucose	215	71	−4	75	0		
	+ 1.9 × 10^{-3} M IAc	215	9	0	44	0		
	Maltose	215	156	−22	310	−12		
	+ 1.9 × 10^{-3} M IAc	215	25	−21	49	−6		
Exp. 2	Glucose	165	88	0				
	+ 3.6 × 10^{-4} M IAc	165	8	−5				
	Maltose	165	284†	−2				
	+ 3.6 × 10^{-4} M IAc	165	101	−15				
Exp. 3	Glucose	160	96				16	0
	+ 3.6 × 10^{-5} M IAc	160	38				12	−6
	Maltose	160	327†	−8			69	−12
	+ 3.6 × 10^{-5} M IAc	160	302†	−21			71	−24

* Phosphate analyses are averages of duplicate flasks on each of which duplicate determinations were made.

† Measurement stopped before total incubation time elapsed.

Experiment 1: Content of Warburg vessel: main chamber, 2.0 ml cell suspension (turbidity of a tenfold dilution = 94) and either 0.5 ml H_2O or IAc; side arm, 0.3 ml sugar solution (2.7 mg glucose or 5.4 mg maltose); center cup, 0.2 ml 4 per cent KOH or diethanolamine reagent.

Experiment 2: Content of Warburg vessel: main chamber, 1.0 ml cell suspension (turbidity of a tenfold dilution = 90), 0.3 ml H_2O, and 1.0 ml of either IAc or Frantz salts; side arm, 0.5 ml sugar solution (= 2 mg); center cup, 0.2 ml 20 per cent KOH.

Experiment 3: Content of Warburg vessel: main chamber, 1.0 ml cell suspension (turbidity of a twentyfold dilution = 95), 0.3 ml H_2O or 0.5 ml 0.1 M $NaHCO_3$, and 1.0 ml of either IAc or Frantz salts; side arm, 0.4 ml sugar solution (= 16 mg) and either 0.1 ml H_2O or 0.1 M $NaHCO_3$; center cup, 0.2 ml 20 per cent KOH or empty.

oxygen consumption and anaerobic acid production in the presence of either glucose or maltose. It is well known that iodoacetate inhibits the action of triosephosphate dehydrogenase by complexing with the sulfhydryl group of the enzyme. On addition of iodoacetate giving a final concentration of 3.6 × 10^{-5} M, oxygen consumption decreased approximately 50 per cent, whereas no change was

noted in the amount of acid produced anaerobically by test systems containing either glucose or maltose (table 4). When the concentration of iodoacetate was increased as much as 50 times, the inhibitory effect on oxygen uptake increased five- to sixfold. The lack of inhibition of carbon dioxide liberation from bicarbonate buffer on the addition of iodoacetate (table 4) is puzzling and might be considered from this point of view: anaerobic acid production in the endogenous system was similar to that of the glucose system, and both test systems liberated very small quantities of acid. With maltose, however, the acid liberation may result from the formation of a sugar phosphate which has a higher pK than phosphoric acid. The energy for the assumed sugar ester could be obtained from the transglycosidic bond of maltose, making the formation of the sugar phosphate independent of adenosine triphosphate regenerated from oxidations and thus also of the iodoacetate-sensitive triosephosphate dehydrogenase system. The fate of inorganic phosphate was, therefore, investigated during the respiration of the organism in the presence and absence of iodoacetate (table 5). Allowing an error of ± 5 per cent in the phosphate analyses, we have concluded that in the presence of glucose, washed cells of this strain of *N. meningitidis* did not take up inorganic phosphate in such quantities as we could measure regardless of the addition of varying amounts of iodoacetate and of the gas phase in which the reaction took place. A measurable "trapping" of inorganic phosphate by the accumulation of a phosphorylated intermediate would not be expected, since the regeneration of adenosine triphosphate (and consequently the phosphorylation of glucose) would be inhibited by the iodoacetate. The amount of phosphate that would disappear owing to the endogenous adenosine triphosphate would probably be less than the error of the analytical procedure. In the presence of maltose, however, inorganic phosphate was esterified anaerobically and aerobically, and the addition of relatively small quantities of iodoacetate "trapped" esterified phosphate. These findings suggest that the meningococcus possesses an active enzyme system whose poisoning by iodoacetate prevents the dephosphorylation and with it the oxidation of an intermediate sugar ester which is formed from maltose and not from glucose.

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SUMMARY

Observations on washed cells of *Neisseria meningitidis* (strain 69, type 1) are presented with the view that they supplement our previous findings made with growing cells. In either test system the differential utilization of the disaccharide, maltose, and of its constituent monosaccharide, glucose, appears to be founded on a phosphorylation reaction which occurs only in the presence of maltose.

Similarly to growing cultures, washed cell suspensions decreased the amount of total reducing substances with either maltose or glucose and produced more acid from the former. Respiration in the presence of maltose was many times as rapid as with glucose but only when the cells were suspended in an appropriate menstruum (e.g., the salts of the Frantz medium). There was no differential when the amino acids of the Frantz medium were present. Respiration was completely inhibited in the Krebs-Ringer phosphate solution. With either sugar, the rates of oxygen uptake increased when the reaction mixtures were buffered with three per cent carbon dioxide. On addition of adenosine triphosphate there was neither a significant increase in the rate of respiration with either glucose or maltose, nor a utilization of glucose-1-phosphate. If, however, adenosine triphosphate and glucose-1-phosphate were added to maltose, an increased oxygen consumption was noted without an increased anaerobic acid production. Incorporating varying amounts of iodoacetate into glucose test systems resulted in a decrease of oxygen uptake without affecting either the minimal anaerobic acid production or the nonmeasurable phosphate esterification. Maltose test systems, on the other hand, to which varying amounts of iodoacetate were added, responded similarly to the glucose systems in that oxygen uptake was decreased, but differed from the latter in that even relatively small quantities of iodoacetate "trapped" esterified phosphate.

A selection of variants with an increased capacity to consume oxygen in the presence of either glucose or maltose occurred when the organism was transferred through chemically defined liquid media containing different concentrations of inorganic phosphate and either of the two sugars. The result was not specific, e.g., the greatest increase in respiration with glucose was noted with cells that had been cultured in the presence of maltose and higher concentrations of inorganic phosphate.

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