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EFFECT OF GLUCOSE ON THE BIOSYNTHESIS OF THE MEMBRANES OF *BACILLUS*

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SUMMARY

In the presence of glucose in complex media, the following changes in the characters of the membranes of *Bacillus subtilis* and *Bacillus cereus* were observed. (1) The activity of succinate dehydrogenase and the amount of cytochromes of the membranes were greatly reduced. (2) The ratio of lipid to protein in the membranes was decreased and a membrane subfraction, which had a density of around 1.21 (*B. subtilis*) and 1.24 g/cm^3 (*B. cereus*), was newly formed (*B. subtilis*) or increased (*B. cereus*). (3) The phospholipid and diglyceride contents in the membranes were reduced.

Polyacrylamide gel electrophoresis of proteins of the two types (plus and minus glucose) of the membranes show that the patterns were very different between the two types of membranes, though the lost or newly formed membrane protein components were not observed.

The cytochrome content was not increased when the cells were grown in glucose medium supplemented with haemin, therefore, glucose or its metabolite may not be involved in the inhibition of haem biosynthesis.

INTRODUCTION

It is well known that the respiratory enzymes of *Staphylococcus aureus* [1] and *Salmonella typhymurium* [2] are repressed when these organisms are grown on glucose medium. These bacteria growing on the glucose are known to mainly obtain their energy by glycolysis. In *Bacillus*, the effect of glucose on the synthesis of respiratory enzymes was not known. It is known, however, that the synthesis of cytochromes in *Bacillus cereus* [3] and *Bacillus subtilis* [4] were much reduced when the organisms were grown in anaerobic conditions. Since cytochrome is one of the main protein components of the bacterial membrane, changes in the amount of cytochrome should alter the structure and function of the membrane.

Abbreviations: GCY medium, 1 % glucose, 0.5 % enzymatic hydrolysate of casein, 0.25 % yeast extract and minerals; ACY medium, 1 % sodium acetate, 0.5 % enzymatic hydrolysate of casein, 0.25 % yeast extract and minerals; CY medium, 1 % enzymatic hydrolysate of casein, 0.25 % yeast extract and minerals.

In the course of biochemical studies on the membranes of *Bacilli*, I have found that the activity of the respiratory enzymes and the lipid composition of the membrane were changed by growing on glucose. The present study describes the changes in the characters of the membranes of *B. cereus* and *B. subtilis* grown in the presence and absence of glucose.

MATERIALS AND METHODS

Organisms and growth conditions

B. subtilis W23 (Ade^-, Met^-, Str^R) and *B. cereus* IAM 1656 were used throughout the study. The media used for the cell growth were as follows: (1) 1% glucose, 0.5% enzymatic hydrolysate of casein (Nutritional Biochemicals Co., U.S.A.), 0.25% yeast extract (Difco) and minerals (GCY medium); (2) 1% sodium acetate, 0.5% enzymatic hydrolysate of casein, 0.25% yeast extract and minerals (ACY medium); (3) 1% enzymatic hydrolysate of casein, 0.25% yeast extract and minerals (CY medium). The cells were grown in 500 ml of medium in a 5-l flask (with 3-knods for better agitation) on a rotary shaker operating at about 200 cycles per min at 37 °C. When lipid analyses were performed, the cells were grown in GCY (plus glucose) and CY (minus glucose) medium in the presence of sodium [1-^{14}C]acetate (0.1 μCi of isotope plus 250 μg of sodium acetate per ml). Isotope and carrier were added after cell growth was initiated.

Preparation of membranes

The cells were harvested by centrifugation at the mid-log phase and suspended in 0.4 M sucrose in 0.1 M phosphate buffer (pH 6.8) containing 10 mM of Mg^{2+} and lysozyme (100 μg/ml) and incubated at 37 °C. Cell suspension densities were about 0.6 mg dry weight of cells per ml. After 40–60 min, almost all the cells were converted to spherical protoplast. The protoplast suspension was centrifuged at 20 000 $\times g$ for 20 min. In the case of *B. cereus*, considerable amounts of membranous vesicles (probably mesosomes) were found to be released during protoplast formation [5], even in the presence of Mg^{2+} (in *B. subtilis*, the release was prevented in the presence of Mg^{2+}), and recovered in the supernatant after sedimentation of the protoplast. The released membranous vesicles were recovered in the precipitates when the supernatant was centrifuged at 100 000 $\times g$ for 30 min, after the supernatant was dialysed against distilled water. The protoplast was suspended in 2.5 ml of 0.02 M Tris–HCl buffer (pH 7.4) and poured into 50 ml of the same buffer containing deoxyribonuclease (2 μg/ml) and ribonuclease (10 μg/ml) and homogenized by a Teflon homogenizer. The lysed protoplast suspension was centrifuged at 20 000 $\times g$ for 30 min and the precipitates were collected (protoplast membrane). In the case of *B. cereus*, the protoplast membrane fraction was mixed with the released membranous vesicles, presented above, and suspended in 0.02 M Tris–HCl buffer (pH 7.4). Membrane suspension in Tris–HCl buffer was homogenized and centrifuged at 100 000 $\times g$ for 30 min. This washing step was repeated once more.

Sucrose density gradient centrifugation

The washed membrane was suspended in Tris–HCl buffer (pH 7.4) and was layered onto a linear gradient of 30–55% (w/w) sucrose in Tris–HCl buffer (pH 7.4)

and centrifuged at 216 000 $\times g$ for 3 h in RPS 65T rotor (Hitachi, swinging type). After centrifugation, the contents were fractionated into about 30 fractions. The protein content or turbidity ($A_{650\ nm}$) was estimated with each fraction.

Protein and phosphorus determinations

Protein was estimated by the method of Lowry et al. [6]. The membrane preparations were suspended in 1 M NaOH and boiled a few seconds to solubilize them, and applied to the method. Bovine serum albumin was used as a standard. Lipid phosphorus was determined according to the method of Ames [7].

Enzyme assay

Succinate dehydrogenase was assayed according to the method of Arrigoni and Singer [8]. Cytochromes were determined by difference spectra (reduced by solid $Na_2S_2O_4$ minus oxidized by the bubbling of O_2) at 444 and 428 nm, which correspond to the Soret bands of the *a*- and *b*-type cytochromes of *Bacilli*, respectively. The amount was expressed as the absorbance per 10 mg of protein.

Lipid extraction and analysis

Lipids were extracted from a freeze-thawed cell suspension by the procedure described by Bligh and Dyer [9]. The chloroform phase of the extraction was washed twice with water.

The extracted lipids, labelled with sodium [1-^{14}C]acetate, were chromatographed on a thin-layer plate of silica gel G with a solvent of light petroleum–ethyl ether–acetic acid (80:30:1, by vol.) [10]. The amounts of phospholipid (R_F:0), diglyceride (R_F : 0.36), and free fatty acid (R_F : 0.55) were determined by this method.

The phospholipid composition was estimated in the following manner. The phospholipid and neutral lipid were separated by silicic acid column chromatography as previously described [11] and the phospholipid fraction (methanol eluate) was concentrated in vacuo, then chromatographed on a thin-layer plate of silica gel G with a solvent of chloroform–methanol–acetic acid (70:30:1, by vol.) [12]. The identity of each phospholipid was established by comparing the R_F values of the spots with those of the appropriate standards and also by spraying the plates with ninhydrin and molybdenum reagent. The lipid composition of *Bacilli* reported by Kates [13] and Op den Kamp et al. [14] were also referred for the identification.

In each chromatogram, the spots were detected with iodine vapour or by scanning the plates with a Packard radiochromatogram scanner model 7201. The appropriate areas of silica gel corresponding to each lipid class were scraped into scintillation vials containing 10 ml of toluene scintillation fluid and their radioactivity was counted by a Hitachi–Horiba scintillation spectrometer LS-500. The lipid compositions were shown as the percentage of ^{14}C cpm of each lipid.

Polyacrylamide gel electrophoresis

5% acrylamide gels containing 0.1% sodium dodecylsulphate and 1 M urea were prepared as described by Shapiro et al. [15]. The gel column was 15 cm in length with a internal diameter of 5 mm. Samples were dissolved in 0.5% sodium dodecylsulphate and 5 M urea in the presence of 1% 2-mercaptoethanol. An aliquot containing about 150 μg of protein in less than 150 μl was applied to the gel. After

electrophoresis, the gels were stained with 0.25% Coomassie blue in 45% methanol and 9% acetic acid for 3 h and destained in 7.5% acetic acid in 5% methanol. The gels were scanned for absorbance with a Fuji densitometer type FD-A IV (Fuji Riken Co., Tokyo).

RESULTS

Effect of glucose on growth of the cells and physical characteristics of the membranes

The generation times of cells grown in GCY, ACY and CY media were almost the same in both bacteria. In *B. subtilis*, the generation times of the cells grown in GCY, ACY and CY media were as follows: GCY, 32.5; ACY, 31.0; CY, 34.0 min. In *B. cereus*, those of the GCY, ACY and CY media-grown cells were 18.0, 18.0 and 19.5 min, respectively. The maximum yields of the cells were also almost the same in different growth conditions. In *B. subtilis*, the maximum yields in the GCY, ACY and CY media-grown cells were 2.59, 2.26 and 2.15 mg of dry weight of cells per ml of culture, respectively. On the other hand, in *B. cereus* those of the GCY, ACY and CY media-grown cells were 5.16, 5.25 and 5.05 mg of the dry weight of cells per ml of culture, respectively.

The yields of membranes in GCY medium-grown cells were 43.0 (*B. subtilis*) and 35.2 (*B. cereus*) μg of protein per mg of dry weight of cells. On the other hand, those of the membranes in the ACY and CY media-grown cells were 53.6 and 52.6 (*B. subtilis*). 40.6 and 42.2 (*B. cereus*) μg of protein per mg of dry weight of cells, respectively. It is indicated that synthesis of the membranes is reduced in the presence of glucose.

Lysis of the protoplast during and after protoplast formation was determined by counting the cells using a Petroff–Hauser counting chamber and also by turbidity changes during the incubation of the protoplast, but lysis was not detected in each type of the cells. The protoplasts were all stable for several hours when the protoplasts were suspended in greater than 0.15 M sucrose under 37 °C (the lysis was observed in the same rate under 0.12 M sucrose above 40 °C in all kinds of protoplasts). Therefore, the stability of the membranes formed in different growth conditions were the same under the conditions presented above.

Effect of glucose on the formation of respiratory enzymes in the membranes

B. subtilis and *B. cereus* were precultured in GCY, ACY and CY media, then inoculated into the same medium of 500 ml in 5-l flasks and incubated at 37 °C. The membranes were isolated and washed and the amount of cytochromes and the activity of succinate dehydrogenase were estimated. As shown in Table I, the activity of succinate dehydrogenase and the amount of cytochromes were greatly reduced when the cells were grown in GCY medium (plus glucose). The activities were almost the same in the cells grown in ACY and CY media (minus glucose).

Effect of glucose on the profile of sucrose density gradient centrifugation of the membranes and respiratory enzyme activities of the membrane subfractions

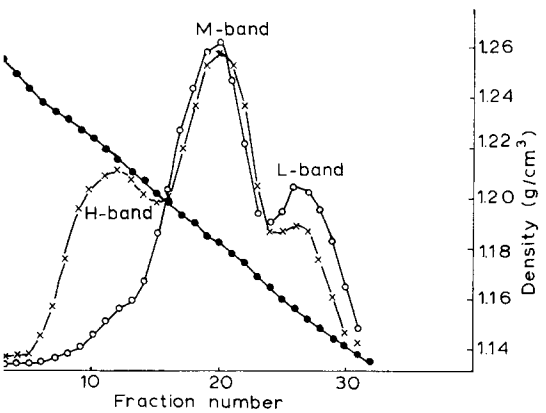
The membranes of the cells which were grown in GCY, ACY and CY media, were isolated and washed. The gross release of the membranous proteins during the membrane isolation and washings was also examined in the following way: the mem-

OF GLUCOSE ON THE AMOUNTS OF CYTOCHROMES AND SUCCINATE OGENASE ACTIVITY OF THE MEMBRANES OF *BACILLI*

	Media	Cytochromes (Absorbance/10 mg protein per ml)		Succinate dehydrogenase (μmoles/mg protein per min)
		b	*a*	
tilis	GCY	2.05	1.35	0.087
	ACY	14.80	9.10	0.550
	CY	12.50	7.30	0.605
us	GCY	0.303	0.292	0.070
	ACY	0.955	0.740	0.175
	CY	0.985	0.621	0.200

proteins usually have a hydrophobic character and when the proteins in or sucrose solutions were dialysed against distilled water or dilute (under neutral buffer, the proteins were easily aggregated and the solutions became /hen the supernatants of each process of isolation and washing of the mem- ere concentrated (10–20 mg of protein/ml) by ultrafiltration and dialysed istilled water, no water-insoluble proteins were found in the dialysates in all natants. Therefore, it may not be possible that the gross loss of the membra- teins, such as the H-band (see below) in the ACY and CY media-grown cells,

ıe washed membranes were layered onto linear gradients of 30–55% sucrose HCl buffer (pH 7.4) and centrifuged. The patterns of the sucrose density centrifugation of the membranes are shown in Figs 1 and 2. The membranes *tilis* grown in the absence of glucose (ACY or CY media) formed two main densities: (1) 1.155–1.158 g/cm^3 (LS band); (2) 1.181–1.183 g/cm^3 (MS lowever, in the presence of glucose, (GCY medium), an additional broad lensity approx. 1.21 g/cm^3 (HS band) was formed. On the other hand, in the



ttern of sucrose density gradient centrifugation of the membranes of *B. subtilis*. ×–×, s which were isolated from the cells grown in GCY medium (plus glucose). ○–○, mem- ch were isolated from the cells grown in ACY (or CY) medium (minus glucose).

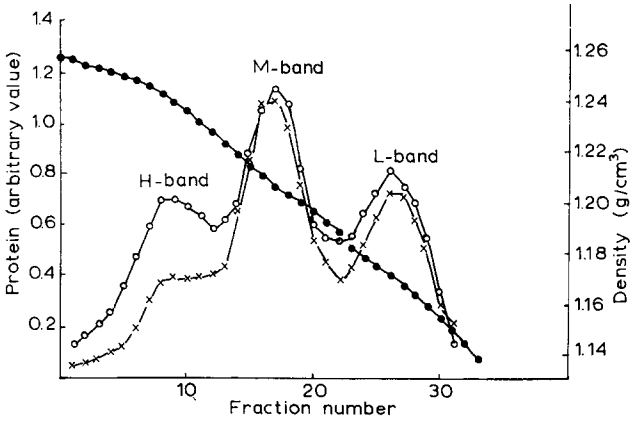


Fig. 2. Pattern of sucrose density gradient centrifugation of the membranes of *B. cereus*. ○–○, membranes which were isolated from the cells grown in GCY medium (plus glucose). ×–×, membranes which were isolated from the cells grown in ACY (or CY) medium (minus glucose).

membranes of *B. cereus* three bands of densities: (1) 1.17 (LC band); (2) 1.206 (MC band); and (3) 1.24 g/cm^3 (HC band) were formed both in the presence and absence of glucose, but the HC band was much increased in the glucose-grown membranes. The LC, MC and HC bands, in the present study, correspond to the membrane subfractions of A40P, L10S and L10P of the previous paper [5], respectively. All the bands were turbid, and the profiles of the sucrose density gradient centrifugation determined by the protein estimation were the same as those determined by the turbidity ($A_{650\ nm}$).

TABLE II

AMOUNT OF CYTOCHROMES AND ACTIVITY OF SUCCINATE DEHYDROGENASE IN THE MEMBRANE SUBFRACTIONS OF *B. SUBTILIS*

The fraction numbers correspond to the numbers in Fig. 1.

Fraction	Cytochromes (Absorbance/10 mg protein per ml)		Succinate dehydrogenase (μmoles/mg protein per min)
	b	*a*	
(A) GCY medium			
6–10	0.02	0.01	0.020
11–14	0.05	0.02	0.037
15–18	1.57	0.75	0.087
19–22	4.24	2.71	0.095
23–26	2.94	1.53	0.090
27–30	2.46	1.36	0.091
(B) ACY medium			
6–10	0.00	0.00	0.000
11–14	0.30	0.20	0.378
15–18	1.59	0.68	0.498
19–22	15.85	9.01	1.076
23–26	14.21	8.55	0.634
27–30	10.40	6.24	0.411

TABLE III

AMOUNT OF CYTOCHROMES AND ACTIVITY OF SUCCINATE DEHYDROGENASE IN THE MEMBRANE SUBFRACTIONS OF *B. CEREUS*

The fraction numbers correspond to the numbers in Fig. 2.

Fraction	Cytochromes (Absorbance/10 mg protein per ml)		Succinate dehydrogenase (μmoles/mg protein per min)
	b	*a*	
(A) GCY medium			
4–7	0.025	0.022	0.012
8–11	0.085	0.066	0.025
12–15	0.202	0.217	0.080
16–19	0.380	0.386	0.229
20–23	0.322	0.350	0.328
24–27	0.253	0.305	0.173
28–31	0.188	0.201	0.096
(B) ACY medium			
4–7	0.038	0.026	0.038
8–11	0.430	0.170	0.155
12–15	0.576	0.360	0.260
16–19	1.951	1.165	0.755
20–23	1.970	1.081	0.615
24–27	0.935	0.645	0.240
28–31	0.725	0.590	0.076

If the activities of the respiratory enzymes of the HS and HC bands were low, the reduced activities of the glucose-grown membranes might possibly be due to the increased or newly formed HC and HS bands in the presence of glucose. Therefore, the respiratory enzyme activities were estimated with each membrane subfraction. As shown in Tables II and III, the activity of succinate dehydrogenase and the amount of cytochromes were much reduced in all of the membrane subfractions of glucose-grown membranes. The reduced activities in the glucose-grown membranes, therefore, were not due to the formation of the H band (HS and HC).

Lipid content and composition

Table IV shows the phospholipid content of the cells and the ratio of phospholipid to protein of the membranes of the cells which were grown in GCY and CY

TABLE IV

EFFECT OF GLUCOSE ON THE PHOSPHOLIPID SYNTHESIS IN *BACILLI*

Organism	Condition medium	Phospholipid (μg/mg dry weight of cells)	Phospholipid/protein in the membranes (μg/mg of protein)
B. subtilis	GCY	24.3	564
	ACY	34.0	634
	CY	34.8	658
B. cereus	GCY	21.7	616
	ACY	26.7	657
	CY	28.1	665

media. As shown in the table, the phospholipid content of the GCY medium-grown cells was much reduced. In *B. subtilis*, the phospholipid content of GCY medium-grown cells was about two-third of that of the ACY and CY media-grown ones. Since there is no difference in the ratio of phospholipid to protein of L (LC and LS) and M (MC and MS) bands between GCY and CY (or ACY) media-grown cells, the reduced phospholipid content in GCY medium-grown cells might mainly be due to the formation or increase of the H (HC and HS) band in the presence of glucose.

TABLE V

EFFECT OF GLUCOSE ON THE LIPID COMPOSITION OF *BACILLI*

Organism	Growth condition medium		Lipids*		
			Phospholipid	diglyceride	free fatty acid
B. subtilis	GCY	1**	88.43	11.35	0.22
	GCY	2	85.28	12.27	2.45
	CY	1	78.70	18.52	2.78
	CY	2	78.24	19.20	2.56
B. cereus	GCY	1	87.30	12.45	0.35
	GCY	2	89.64	10.24	0.12
	CY	1	78.53	21.29	0.18
	CY	2	78.97	20.91	0.12

* Expressed as percentage of total lipid.
** Experiment number.

The lipid composition of the GCY and CY media-grown cells was examined next. As shown in Table V, the lipid composition of the two types of cells was quite different. About twice as much diglyceride was found in CY medium-grown cells as in GCY medium-grown cells. Since the phospholipid content was higher in CY than in GCY media-grown membranes, the total lipid content in CY medium-grown cells was much higher than that of GCY medium-grown cells.

The phospholipid composition has been known to change depending on the growth phases in bacteria [16], therefore, care had been taken to harvest the cells at precisely the same turbidity for both GCY and CY media-grown cells ($T = 300$ in Experiment 1, $T = 400$ in Experiment 2). As shown in Table VI, the phospholipid composition changed significantly in *B. subtilis* during the log-phase of growth, but in *B. cereus*, the composition was not much changed during the log-phase. When the phospholipid patterns of GCY and CY media-grown cells were compared, the increase in phosphatidylglycerol and the decrease in phosphatidylethanolamine in the absence of glucose were the most significant changes in the phospholipid composition. The increase in the amount of phosphatidylglycerol appeared to be counterbalanced for the greater part by a decrease in the amount of phosphatidylethanolamine.

Polyacrylamide gel electrophoresis

The patterns obtained with the membranes of GCY, ACY and CY media-grown cells are shown in Figs 3 and 4. Marked differences were found in the patterns

TABLE VI

EFFECT OF GLUCOSE ON THE PHOSPHOLIPID COMPOSITION OF *B. SUBTILIS* AND *B. CEREUS*

PE, phosphatidylethanolamine; PG, phosphatidylglycerol; CL, caldiolipin; LPG, lysylphosphatidylglycerol; PS, phosphatidylserine. Each phospholipid was expressed as a percentage of the total phospholipid.

Organism	Growth condition medium		Phospholipid			
			PE	PG	CL	LPG
B. subtilis	GCY	1	24.12	61.31	4.41	9.16
	GCY	2	23.15	59.28	7.23	10.34
	CY	1	20.86	64.30	5.26	9.58
	CY	2	21.27	62.50	7.02	8.20
			PE	PG	CL	PS
B. cereus	GCY	1	64.42	26.38	4.29	4.91
	GCY	2	64.47	25.78	4.08	5.61
	CY	1	58.89	30.29	5.64	5.18
	CY	2	58.15	29.70	5.76	4.43

Fig. 3. Pattern of polyacrylamide gel electrophoresis of the membranes of *B. subtilis*. —, membranes which were isolated from the cells grown in GCY medium (plus glucose); - - -, membranes which were isolated from the cells grown in ACY (or CY) medium (minus glucose).

of protein between the two types of membranes, but no lost or deficient protein components were found between the two types of membranes. The differences were striking in the membranes of *B. subtilis*.

Effect of haemin on the formation of cytochromes in the membranes grown in glucose

It has been known that the cytochrome content of *Staphylococcus epidermis* is decreased when the cells were grown anaerobically but when the cells were grown anaerobically in the medium supplemented with haemin, a marked increase in the

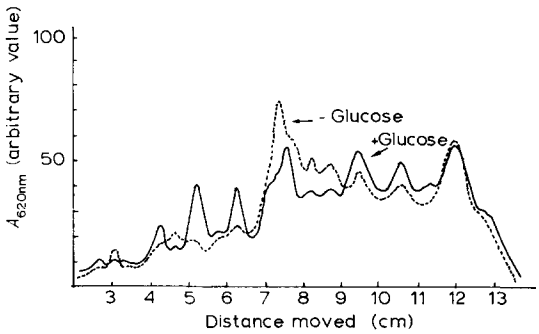


Fig. 4. Pattern of polyacrylamide gel electrophoresis of the membranes of *B. cereus*. —, membranes which were isolated from the cells grown in GCY medium (plus glucose); - - -, membranes which were isolated from the cells grown in ACY (or CY) medium (minus glucose).

formation of cytochromes was observed [17]. In the present study, if the reduced content of cytochromes in *Bacilli* was due to the limitation of the production of haem in the presence of glucose, the content should be increased when haemin was supplied to the medium, if haemin was taken up by the cells of *Bacilli*, as *Staphylococcal* cells.

B. subtilis and *B. cereus* were grown in GCY medium containing haemin (0.5 μg/ml), and the cells were harvested in the mid-log phase and the membranes were isolated. The amount of cytochromes and the activity of the succinate dehydrogenase were estimated with the membrane preparations. As shown in Table VII, the cytochromes and succinate dehydrogenase were not increased in the presence of haemin.

TABLE VII

EFFECT OF HAEM ON THE BIOSYNTHESIS OF CYTOCHROMES AND SUCCINATE DEHYDROGENASE IN *B. SUBTILIS*

Growth condition medium	Cytochromes (Absorbance/10 mg of protein per ml)		Succinate dehydrogenase (μmoles/mg protein per min)
	b	*a*	
GCY+haemin	1.88	0.89	0.158
GCY−haemin	2.53	1.41	0.192

DISCUSSION

It is well known that the cytochromes and succinate dehydrogenase are the important protein components of the bacterial membranes. Changes in the amount of these enzymes, therefore, may induce the alteration of the structure and function of the membranes.

It has been shown here that the *a*- and *b*-type of cytochromes and succinate dehydrogenase were much reduced in the membranes of *B. subtilis* and *B. cereus* when these organisms were grown in the presence of glucose. Sucrose density gradient centrifugation of the membranes showed that a membrane subfraction, H band (HC and HS), which was reduced or deficient in the membranes formed in the absence of

glucose, was formed in the glucose-grown cells. The activities of the respiratory enzymes of the H band were extremely low, however, and the reduced activities of the respiratory enzymes in the glucose-grown membranes was not due to the newly formed H band. The activities of the L (LC and LS) and M (MC and MS) bands of the glucose-grown membranes were also much reduced. These results may indicate that glucose or its metabolite inhibited the synthesis of respiratory enzymes in some unknown mechanisms.

Recently, Jacobs and Conti [17] reported that cytochromes in anaerobically grown *S. epidermis* were increased when the cells were grown in haemin-containing medium. If glucose inhibited haem biosynthesis in *Bacilli*, then one could expect an increase in the amount of cytochromes when the cells were grown in haemin-containing GCY (plus glucose) medium (if the haemin was taken up by the cells of *Bacilli*). The results presented in Table VII, show that the cytochromes were not increased when the cells were grown in the presence of haemin. Therefore, glucose or its metabolite might not be involved in the regulation of the biosynthesis of haem in *Bacilli*.

Facultative anaerobes, such as *S. aureus* and *S. typhimurium*, could obtain their energy by glycolysis when glucose was present, and have a decreased amount of cytochromes [18]. However, in *Bacilli*, obligate aerobes, cells could not be grown in anaerobic conditions and could not obtain their energy by glycolysis. Therefore, in *Bacilli*, there must be some other mechanisms, which are involved in the inhibition of the cytochrome synthesis.

The results presented in Figs 1 and 2 show that the high density H band is always accumulated in the membranes grown in glucose. The reason why the H band was accumulated in glucose-grown membranes was not answered at present. As reported in the previous paper [5], the H band of *B. cereus* corresponded to the membrane subfraction L10P. L10P was found to consist of "membrane precausor protein" and contained apoprotein of cytochromes. It was also found that the H band of *B. subtilis* contained apoprotein of cytochromes (unpublished). The H band might, therefore, be considered as a "membrane precausor protein" accumulated in the presence of glucose.

The distinct difference with regard to the lipid composition between the two types of membranes was the difference in their diglyceride content. About twice as much diglyceride was found in the CY medium-grown (minus glucose) as in the GCY medium-grown (plus glucose) cells. Since diglyceride is one of the main products of the hydrolysis of phospholipid by phospholipase C, an abundance of diglyceride in the CY medium-grown membranes would indicate that more intracellular phospholipase C was formed in the CY medium- than in the GCY medium-grown cells. The estimation of the activity in these two kinds of cells has, however, found that more activity was found rather in GCY medium- than CY medium-grown cells. Therefore, it appears unlikely that diglyceride simply represented the degradation product of membrane phospholipid.

The difference in the profile of polyacrylamide gel electrophoresis between GCY and ACY or CY media-grown membranes reflected the difference in the enzyme activity of membrane preparations. No differences were found between the protein components which constitute the membranes. However, the amount of each component was changed significantly with changes in the growth conditions. It, therefore,

appears that when some components were reduced in the membrane, some other components were increased and counterbalanced to maintain the structure and function of the membrane.

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