EFFECT OF METALLIC IONS ON THE GROWTH, MORPHOLOGY, AND METABOLISM OF CLOSTRIDIUM PERFRINGENS

I. MAGNESIUM[1]

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Received for publication October 4, 1954

Magnesium is invariably added to the synthetic media devised for the cultivation of microorganisms, indicating its essentiality. The concentration of Mg^{++} required for growth differs with the type of organism (Webb, 1949) and according to the presence of nutrients and antagonistic ions in the medium (Olson and Johnson, 1949; Abelson and Aldous, 1950; Webb, 1951*a*). Suboptimal levels of Mg^{++} support only limited growth, and in certain microorganisms morphological changes may be observed (Webb, 1949; Rogosa and Mitchell, 1950).

The essential requirement for growth is in general explained by the fact that Mg^{++} acts as an activator for different types of enzymatic reactions: transfer of phosphate (Dixon, 1949), pyruvate metabolism (Korkes *et al.*, 1950), synthesis of citric acid and glutamine (Rudolph and Barron, 1950; Speck, 1949), hydrolysis of peptides (Johnson and Berger, 1942), synthesis of the gram-complex (Henry and Stacey, 1946), and the production of subtilin by *Bacillus subtilis* (Feeney and Garibaldi, 1948). In a few enzymatic reactions (enolase, pyruvic phosphokinase, adenosine polyphosphatase, desoxyribonuclease, dismutation of pyruvate), Mg^{++} can be replaced by Mn^{++}. The irreplaceability of Mg^{++} in the phosphokinases involved in glycolysis indicates its indispensable nature in this process.

Formation of bacterial filaments, often the result of Mg^{++} deficiency (Webb, 1949), is a common phenomenon and is induced by a variety of agents: penicillin, mustard gas, radiations, level of metallic ions, source of nitrogen in medium, dyes (Webb, 1953), and antineoplastic agents (Maxwell and Nickel, 1954). Depending upon the kind and concentration of the agent used, filaments may give rise to normal or degenerate cells (Gates, 1933). Hinshelwood (1946) and Nickerson (1948) considered that the major physiological processes in filamentous and normally dividing cells are the same, and hence the former type of cell is particularly suitable for the study of the cell division mechanism.

The requirement of Mg^{++} for the growth of *Clostridium perfringens* in synthetic and complex media has been reported by several investigators (Webb, 1948; Boyd *et al.*, 1948; Shankar and Bard, 1952). It is only partially replaced by Ca^{++} but not by Mn^{++} (Shankar and Bard, 1952). Webb (1949) reported that Mg^{++} deficient Evans peptone medium supports growth of filamentous cells of certain species of *Clostridium* and *Bacillus* and that the addition of Mg^{++} to such deficient medium restores the formation of normal cells. It was concluded that at low Mg^{++} levels the synthetic activity of cells continues unimpaired but cell division is inhibited. The synthesis of septa or cross-walls requires energy as well as intermediates containing carbon and probably nitrogen. If carbohydrate or protein metabolism is interfered with in some manner, it is likely to influence septum formation. It is logical, therefore, to assume that not all the physiological processes of filamentous and normal cells are the same. Further, factors causing filament production probably affect different sites, presenting a variety of metabolic aspects. Recent biochemical studies of filamentous and normal cells (Nickerson and Sherman, 1952; Webb, 1953) indicate the validity of these arguments.

METHODS

Bacteriological. The preparation of high purity water, heart infusion broth (HIB), metal

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deficient medium A, treatment of glassware, and growth measurements were carried out according to the procedures reported by Shankar and Bard (1952). *Clostridium perfringens* strain BP6K was maintained in HIB by daily transfer. One per cent inoculum from an HIB culture was transferred to a tube containing 10 ml of medium A and incubated in a 37 C water bath for 5 hr. The medium was centrifuged and the supernatant liquid discarded. Cells were suspended in 10 ml of sterile high purity water and centrifuged again. After decanting the top clear liquid, the paste was made into a thick suspension with sterile water and the optical density (OD) adjusted to 0.73 with an Evelyn colorimeter using a 420 mμ filter; 0.05 ml of this suspension was used as the inoculum for 10 ml of experimental medium.

Magnesium deficient Evans peptone medium was prepared according to the procedure of Webb (1948): 15 ml of NH_4OH (28 per cent) were added to a solution of 10 g Evans peptone (Evans Medical Supplies, Ltd., Liverpool, England) in 100 ml of distilled water and allowed to stand for 16–20 hr at room temperature. The medium was filtered and the filtrate distilled under reduced pressure; to avoid bumping, air was passed through the boiling medium through a fine glass tube. The pH of the medium was adjusted to 7.2 with dilute NH_4OH (if necessary) and the solution diluted to 350 ml; 7 ml of this concentrated medium are equivalent to 10 ml of 2 per cent peptone solution.

For determining the growth obtained under various conditions, 7 ml quantities of the concentrated Mg^{++} deficient Evans peptone media were pipetted into colorimeter tubes (18 by 150 mm). The required volumes of NaCl solutions (0.4 ml, 12.5 per cent), K_2HPO_4 (0.05 ml, 10 per cent), $MgSO_4$, and other constituents (as required) were added. The tubes were sterilized for 20 min at 120 C; 0.1 ml of a 20 per cent glucose solution (sterilized by passage through an UF sintered glass filter) was added to each tube, and the volume made to 10 ml with sterile high purity water. The medium was heated in an Arnold steamer for 10 min, cooled immediately, and inoculated with 0.05 ml of the standardized inoculum. The tubes were incubated in a 37 C water bath. All salts employed in these experiments were of chemically pure grade (cp) and were in the hydration forms indicated: $MgSO_4 \cdot 7H_2O$; $Na_2HPO_4 \cdot 7H_2O$; K_2HPO_4, NaCl, $NH_4H_2PO_4$, anhydrous.

Filamentous cells were obtained by inoculating Mg^{++} deficient Evans peptone medium with the standardized inoculum. The level of Mg^{++} needed to produce growth of filaments was determined beforehand for each batch of medium. Cultures of filamentous cells were harvested after a 12–14 hr period of growth at 37 C. Normal cells were also cultivated in Mg^{++} deficient Evans peptone to which the optimum level of Mg^{++} (75 μg per 10 ml) was added. After 5 hr growth at 37 C, the cells were collected by centrifugation.

Cell suspensions were prepared by centrifuging the growth medium and washing the thick paste once with 0.85 per cent NaCl (saline). A thick suspension of the cells was made in saline and the OD adjusted in an Evelyn colorimeter (420 mμ filter) so that 0.1 ml of the suspension contained 1.0 mg dry weight of cells. Dried cells were obtained by preparing cell pastes which were dried *in vacuo* over "drierite" at room temperature. For the preparation of cell-free extracts, 6 g of cell paste were made into a thick suspension with 20 ml of 0.03 M $NaHCO_3$ solution. The suspension was subjected to oscillation in a Raytheon 9 kc oscillator for less than 10 min and then centrifuged at 9,000 rpm at 4 C in a Sorvall SS-1 centrifuge for 30 min. The supernatant liquid was decanted and stored at −20 C.

Chemical. Rates of CO_2 and H_2 evolution were measured by conventional Warburg techniques (Umbreit *et al.*, 1949) in an atmosphere of N_2 with 0.04 M phosphate buffer, pH 6.5 at 37 C. Acid production was followed in $NaHCO_3$, pH 6.5, in an atmosphere of 85 per cent N_2 and 15 per cent CO_2.

The amount of glucose fermented by cell suspensions was determined by tipping H_2SO_4 before and after the experiment in different Warburg vessels; the contents were centrifuged separately and glucose determined by the anthrone method (Neish, 1950). Lactic acid was determined in the supernatant liquid of Warburg cup contents by the procedure of Barker and Summerson (1941). The ribonucleic acid (RNA) content of dried cells was estimated according to the method of Ogur and Rosen (1950); the absorption spectrum of the final extract was read at 260 mμ spectrophotometrically and compared to a standard curve drawn from data similarly

obtained with known amounts of ribonucleic acid (Schwarz Laboratories, Inc., N. Y.). The desoxyribonucleic acid (DNA) of dried cells was determined by the diphenyl reagent of Dische (1930) measuring color intensity in an Evelyn colorimeter (660 mμ filter); sperm desoxyribonucleic acid (Nutritional Biochemical Corp., Cleveland, Ohio) was used as the standard for comparison. The protein content of dried cells was estimated by the biuret reaction (Robinson and Hogden, 1940); purified casein was employed as the protein standard.

The presence of hexokinase, phosphohexose isomerase, phosphofructokinase, aldolase, and triose phosphate dehydrogenase in cell-free extracts was determined by linking these enzymatic reactions to the reduction of diphosphopyridine nucleotide which was measured in a Beckman spectrophotometer at 340 mμ and room temperature. Diphosphopyridine nucleotide (85 per cent pure) and adenosine triphosphate were obtained from Schwarz Laboratories, Inc., N. Y., and Pabst Laboratories, Milwaukee, Wisconsin, respectively. The sodium salts of glucose-6-phosphate, fructose-6-phosphate, and fructose-1,6-diphosphate were prepared from their respective barium salts which were obtained from Schwarz Laboratories, Inc., N. Y.

RESULTS

Effect of Mg^{++} addition to Mg^{++} deficient Evans peptone medium. The growth and morphology of cells obtained at different levels of Mg^{++} added to Mg^{++} deficient Evans peptone medium are described in table 1. It is evident that filaments are formed only under conditions of Mg^{++} deficiency and when growth is very limited (OD = 0.06 or less). Addition of Mg^{++} improves growth and promotes the formation of normal cells. Certain batches of Evans peptone treated according to Webb (1948) failed to support growth. However, by adding small amounts of Mg^{++}, sufficient to support growth equivalent to OD = 0.06 or less, filaments were produced.

Addition of Mn^{++} in concentrations of 20, 50, 70, 100, and 150 μg per 10 ml of Mg^{++} deficient Evans peptone medium did not result in growth of *C. perfringens*. This finding supports the ob-

TABLE 1

Effect of Mg^{++} on growth and morphology

Added Mg^{++} (μg per 10 ml)	Growth at 24 hr (OD at 660 mμ)	Cell Morphology
0.00	0.053	Filaments
0.25	0.063	Filaments
0.50	0.071	2x to 4x
0.75	0.092	2x
1.00	0.102	2x
1.25	0.122	1x (normal)
1.50	0.143	1x (normal)
12.50	0.168	1x (normal)
25.00	0.215	1x (normal)
75.00	0.280	1x (normal)
Control*	0.215	1x (normal)

* Untreated Evans peptone medium, without added Mg^{++}.

TABLE 2

Filament production in different media treated by Webb's method

Type of Medium	Webb Treatment	Add Mg^{++} (μg per 10 ml)	Growth at 24 hr (OD at 660 mμ)	Cell Morphology
Lactalysate*	+§	1.00	0.058	1x (normal)
	0‖	0.0	0.387	1x (normal)
Myosate*	+	1.50	0.056	1x (normal)
	0	0.0	0.342	1x (normal)
Gelysate*	+	25.0	0.053	1x (normal)
	0	0.0	0.208	1x (normal)
Polypeptone*	+	5.0	0.053	1x (normal)
	0	0.0	0.387	1x (normal)
Phytone*	+	2.7	0.030	1x (normal)
	0	0.0	0.357	1x (normal)
Protone (Bacto)†	+	75.0	0.034	2x to 4x
	0	0.0	0.000	—
Peptone (Bacto)†	+	0.0	0.051	2x to 4x
	0	0.0	0.382	1x (normal)
Yeast extract†	+	1.75	0.076	1x (normal)
	0	0.0	0.392	1x (normal)
Tryptone†	+	1.25	0.075	1x (normal)
	0	0.0	0.387	1x (normal)
Yeast extract plus tryptone†	+	5.0	0.030	1x (normal)
	0	0.0	0.420	1x (normal)
Peptone (Evans)‡	+	1.25	0.046	Filaments (longer than 4x)
	0	0.0	0.260	1x (normal)

* Baltimore Biological Laboratory, Baltimore, Maryland.

† Difco Laboratories, Detroit, Michigan.

‡ Evans Medical Supplies, Ltd., Liverpool, England.

§ Treated by Webb's method.

‖ Medium not treated by Webb's method and no Mg^{++} added.

servations of Shankar and Bard (1952) but does not agree with those of Webb (1951*b*).

Production of filaments in various media. To determine whether Mg^{++} deficiency is the only factor involved in the formation of filamentous cells, a variety of media was made Mg^{++} deficient by Webb's method; the results are presented in table 2. Most of the media failed to support growth after Webb's treatment, and Mg^{++} was added in such cases sufficient to obtain growth equivalent to about 0.06 OD units. With the added traces of Mg^{++} used, filamentous cells were obtained in none of the media tested except Evans peptone.

Synthetic medium consisting of amino acids, vitamins, salts, etc. (Boyd, Logan, and Tytell, 1948), when treated by Webb's method, did not support growth. Addition of Mg^{++} at increasing levels resulted in growth corresponding to 0.06 OD units, but no filaments were observed. These results do not agree with the findings of Webb (1951*b*) who reported that Mg^{++} deficient synthetic medium produces filamentous growth of *C. perfringens*.

Possible factor in Evans peptone responsible for filament formation. The observations described above suggest that there is some factor present in Evans peptone which is responsible for the formation of filaments. At relatively high concentrations of Mg^{++}, the influence of this factor may be assumed to be nullified, permitting the growth of normal cells. Attempts, therefore, were made to search for this factor. It was found that if Evans peptone medium, with or without Webb's treatment, is passed through a column of "permutit H" (Shankar and Bard, 1952), filamentous cells are not produced at the limiting growth of 0.06 OD units. This finding indicates that the factor is removed by permutit H. The column was hence eluted with 0.04 N HCl; the eluate was concentrated by low temperature distillation and adjusted to pH 7.2 with dilute ammonia. The addition of this eluate at various levels to Mg^{++} deficient Evans peptone medium (producing filamentous cells) yielded normal cells, due probably to the presence of Mg^{++} in the eluate extracted from the column by HCl. The eluate was dialyzed in a cellophane sac for 24 hr against high purity water, and the undialyzed portion added to Mg^{++} deficient Evans peptone medium containing added Mg^{++} just sufficient to inhibit the formation of filaments; filamentous cells were not observed. This finding indicates either the absence of the factor in the undialyzed portion, its inactivation, or its diffusion out of the cellophane sac.

To investigate the possibility that the factor might be a neutral compound, the column was eluted with ethyl ether; the ether was distilled away, and the remaining material neutralized and added to medium as described above. The ether extract did not contain the inhibitor of normal cell division. Further attempts to isolate the postulated factor were not made.

Absence of growth inhibitory substances in growth medium yielding filaments. The very poor growth of *C. perfringens* in Mg^{++} deficient peptone medium can also be attributed to the production by filamentous cells of some inhibitory substance(s) which accumulates in the medium. A 12 hr culture of filamentous cells was centrifuged, the supernatant liquid boiled and adjusted to pH 7.2; required amounts of K_2HPO_4 and glucose were added. Such medium yielded filamentous cells without added Mg^{++} and good growth of normal cells with added Mg^{++}. This finding indicates that filamentous cells do not produce a thermostable growth inhibitory substances(s) in the medium.

Effect of addition of organic substances to Mg^{++} deficient medium. The formation of filaments in Mg^{++} deficient Evans peptone medium and of normal cells in Webb treated synthetic medium (Boyd, Logan, and Tytell, 1948) at the limiting growth level represented by 0.06 OD units suggested the possibility that the nutritional intermediates required for the formation of septa and separation of individual cell units are lacking during growth in Evans peptone medium. This deficiency may not be manifested in the synthetic medium, and the intermediates are synthesized normally in Webb treated Evans peptone medium when higher than critical concentrations of Mg^{++} are provided. To investigate this possibility, amino acids, vitamins, purine and pyrimidine bases, D-ribose, 2-desoxy-D-ribose, etc. were added to the Mg^{++} deficient medium and cell morphology examined. The compounds tested were: amino acids (glycine, DL-alanine, DL-serine, DL-threonine, DL-valine, L-leucine, DL-isoleucine as a group; DL-aspartic and L-glutamic acids; L-arginine, L-lysine, L-cysteine, DL-methionine; DL-phenylalanine and L-tyrosine; L-histidine, L-proline, L-hydroxyproline, and L-trypto-

phan); adenine sulfate, uracil, thymine, guanine, and cytosine as a group; asparagine and glutamine; growth factors (nicotinamide, riboflavin, Ca-D-pantothenate, pyridoxine hydrochloride and biotin as a group); keto acids (pyruvate, oxalacetate, and α-ketoglutarate as sodium salts); pentoses (D-ribose and 2-desoxy-D-ribose); reducing agents (ascorbic and thioglycolic acids). It was observed that in all cases filaments were formed, indicating that the added organic constituents employed are either not deficient in Evans peptone medium (Webb treated) or not involved directly in filament formation.

Cytological study of filamentous and normal cells. Staining of normal and filamentous cells by Giemsa stain (Robinow, 1943–1944) revealed the presence of 2–3 and 4–8 chromatinic bodies in the two types of cells, respectively. Basic fuchsin produced better differentiation of chromatinic bodies than Giemsa stain in filaments. The presence of septa in filaments was observed by staining with the tannic acid-crystal violet technique of Robinow (1943–1944). Shorter filaments have no septa while longer ones have widely spaced septa. Some filaments have septa placed very closely at one of the filaments. These observations agree with those recorded by Webb (1949).

Metabolic studies. The foregoing results indicate that formation of filamentous cells occurs at a critical concentration of Mg^{++} (corresponding to growth of 0.06 OD units or less) in the presence of an unknown factor present in Evans peptone. According to Webb (1953) the synthesis of enzymes responsible for the formation of septa and subsequent separation of units are inhibited in filaments. It is also possible that formation of filaments is not due to the absence of enzymes but to the lack of the necessary intermediates and sufficient energy release. Such deficiencies may affect the nucleic acid and protein content of the filaments. Therefore, a comparative study of filamentous and normal cells was made.

TABLE 3

Anaerobic evolution of H_2 and CO_2 by suspensions of normal and filamentous cells

Type of Cell Suspension	H_2		CO_2	
	Q_{H_2}	μM*	Q_{CO_2}	μM*
Normal	144	2.3	84	1.7
Filamentous	24	2.8	0.6	2.3

Q = μL gas per mg dry wt cells per hr.

* = μM gas per μM glucose fermented.

Protocol. Per Warburg flask: buffer, M/15 phosphate, pH 6.5, 2.0 ml; cell suspension, 5 mg dry wt, 0.5 ml; glucose, 0.025 M, 0.2 ml; KOH, 20 per cent, or H_2O in center well, 0.2 ml; H_2SO_4, 2 N, in side arm, 0.2 ml; total volume with H_2O, 3.2 ml. Atmosphere: N_2. Temperature: 37 C.

Fermentation. The rates of CO_2 and H_2 formation during glucose fermentation by cell suspensions of filamentous cells are considerably lower than those of normal cells (see table 3), but the total quantities of gases produced by the two types of cells are approximately the same. Glucose fermentation by normal cells is complete within 1 hr while filamentous cells require 4–5 hr to ferment the same amount of glucose. There was no evidence for methylglyoxal or pyruvic acid accumulation, 91–96 per cent of the glucose being fermented by both types of cells. Addition of Mg^{++} (0.001 M) or boiled normal juice of *C. perfringens* does not influence Q_{H_2} and Q_{CO_2}. It appears that the very slow rate of fermentation by filamentous cells is due to a deficiency of certain enzyme(s) or coenzyme(s), or the accumulation of some intermediate(s) other than a keto compound type. The supply of energy and intermediates associated with fermentation is therefore very limited in the case of filamentous cells. An attempt to provide energy in the form of adenosine triphosphate was made in which filamentous cells were incubated for 4 hr at room temperature with this compound. No changes in cell morphology were noted. Subsequently, Barnett (1954) reported the occurrence of cell division by filamentous cells of *Escherichia coli* when adenosine triphosphate was added, a finding of considerable interest in view of the marked lability of this compound in the presence of living cells.

Enzymatic activities. The slow rate of fermentation by Mg^{++} deficient, filamentous cells suggested that the dissimilatory enzymes involved in fermentation may be limiting factors in the metabolism of such cells. The exact pattern of fermentation in the genus *Clostridium* is not known, but available information indicates the existence of a pathway similar at least in part to the Embden-Meyerhof-Parnas scheme. Bard and Gunsalus (1950) demonstrated the existence of aldolase, triose phosphate isomerase, glyceraldehyde phosphate, and ethanol dehydrogenases in cell-free extracts of *C. perfringens*, hence outlining the fermentation pattern in this organism.

The presence of hexokinase, phosphohexose isomerase, phosphofructokinase, aldolase, glyceraldehyde phosphate dehydrogenase, and possibly triose phosphate isomerase, in cell-free extracts of filamentous and normal cells is indicated in table 4. Since the reduction of diphosphopyridine nucleotide involves these enzymes through a series of reactions and since the rates found for the two types of cell extracts are similar, it appears that Mg^{++} deficiency does not affect the activity of these enzymes.

Nucleic acid and protein. The ribonucleic and desoxyribonucleic acid contents of filamentous and normal cells are approximately the same, but the protein content of filamentous cells is 18 per cent less than found in normal cells (see table 5). It therefore appears that the level of Mg^{++}, present in traces in Mg^{++} deficient Evans peptone, is adequate for the synthesis of nucleic acids but is insufficient for protein synthesis. The significance of this finding is not apparent though it may represent an important difference between filamentous and normal cells.

TABLE 4

Enzymatic activities of normal and filamentous cell extracts with different substrates

Source of Cell Extract	OD Change per Mg Protein per Hr*			
	Glucose	G-6-P	F-6-P	F-1,6-P
Normal cells (5 hr old)	1.06	0.89	1.45	22.2
Filamentous cells (12–14 hr old)	1.18	0.91	1.71	23.4
Additions	**0.02 M**	**0.05 M**	**0.05 M**	**0.05 M**
Veronal buffer, pH 7.2	1.3 ml	1.1 ml	1.1 ml	1.5 ml
Arsenate, 54 mg per ml	0.3	0.3	0.3	0.3
NaF, 0.2 M	0.2	0.2	0.2	0.2
DPN, 1 mg per ml	0.3	0.3	0.3	0.3
Cysteine, 0.04 M	0.3	0.3	0.3	0.3
Cell extract	0.6	0.6	0.6	0.2
Substrate	0.1	0.3	0.3	0.3
ATP, 0.1 M	0.2	0.2	0.2	—
$MgSO_4$, 0.1 M	0.2	0.2	0.2	—
H_2O	—	—	—	—
Total volume	3.5 ml	3.5 ml	3.5 ml	3.5 ml

* Measured as OD at 340 mμ in a Beckman spectrophotometer at room temperature: reduction of diphosphopyridine nucleotide (DPN).

TABLE 5

Protein, ribonucleic and desoxyribonucleic acid content of normal and filamentous cells

Type of Cells	Per Cent Dry Wt of Cells		
	Protein	RNA*	DNA*
Normal	58.6	27.5	3.3
Filamentous	48.0	25.6	3.7

* RNA = ribonucleic acid; DNA = desoxyribonucleic acid.

DISCUSSION

The results presented above indicate that magnesium deficiency is not the sole factor involved in the growth of *C. perfringens* as filamentous cells. A variety of media, capable of supporting growth of this organism, did not yield filamentous cells when magnesium was removed from these media. Of the media studied, only Evans peptone medium possesses this capacity. When magnesium was removed in the former instances, either no growth occurred or the slight growth obtained consisted of normal or elongated cells. A factor inhibiting cell division, operative in the absence of an adequate magnesium concentration, is hence suggested as being present in Evans peptone medium. Further evaluation of this hypothesis seems warranted but was not the primary objective of this investigation. Availability of filamentous cells was achieved with Mg^{++} deficient Evans peptone medium as described originally by Webb (1948) who clearly demonstrated the relationship between magnesium deficiency and filament production by *C. perfringens*.

The limited fermentative capacity of filamentous cells of *C. perfringens* is paralleled by the reduced oxidative capacity of *Bacillus cereus* obtained as filamentous cells by growth in Mg^{++} deficient medium (Nickerson and Sherman, 1952). The reduced rates of energy production and supply of intermediates necessary for synthetic activity hence appear to be characteristic of filamentous cells. Gale and Mitchell (1949) have demonstrated the energy requirement for amino acid assimilation in *Micrococcus aureus*. Reduction in the rate of such a process may contribute to the decreased rate of cell division leading to filament formation. The enzymatic studies reported above were designed to reveal the sites of biochemical insufficiency of filamentous

cells, but no marked differences were noted between normal and filamentous cells with respect to the enzymes involved in the initial steps of carbohydrate breakdown. Which enzymes are functioning at a reduced rate remains to be described, but it may be assumed, in light of present knowledge, that these sites are those linking energy release to assimilation and biosynthesis. Evidence for this assumption is found in the somewhat lowered protein content of filamentous cells as compared to normal cells.

ACKNOWLEDGMENT

The authors are grateful to Miss N. Canamucio of Smith, Kline and French Laboratories for her valuable assistance in the preparation of the manuscript.

SUMMARY

A variety of complex media was rendered magnesium deficient, but only Evans peptone medium so treated supported growth of filamentous cells of *Clostridium perfringens*. The possibility that this medium contains a cell division inhibitor, active in the absence of magnesium, is suggested. Filamentous cells ferment glucose at a greatly reduced rate. However, both normal and filamentous cells demonstrate comparable hexokinase, phosphohexose isomerase, phosphofructokinase, aldolase, and triose phosphate dehydrogenase activities. Likewise, the levels of ribonucleic and desoxyribonucleic acids are similar although filamentous cells contain less protein than normal cells on a dry weight basis.

REFERENCES

ABELSON, P. H., AND ALDOUS, E. 1950 Ion antagonisms in microorganisms: Interference of normal magnesium metabolism by nickel, cobalt, cadmium, zinc and manganese. J. Bacteriol., **60**, 401–413.

BARD, R. C., AND GUNSALUS, I. C. 1950 Glucose metabolism of *Clostridium perfringens:* Existence of metallo-aldolase. J. Bacteriol., **59**, 387–400.

BARKER, S. B., AND SUMMERSON, W. H. 1941 The colorimetric determination of lactic acid in biological material. J. Biol. Chem., **138**, 535–554.

BARNETT, R. C. 1954 Cell division of *E. coli*. Federation Proc., **13**, 7.

BOYD, M. J., LOGAN, M. A., AND TYTELL, A. A. 1948 The growth requirements of *Clostridium perfringens* (*welchii*) BP6K. J. Biol. Chem., **174**, 1013–1025.

DISCHE, A. 1930 Über einige neue charakteristische Farbreactionen der Thymonukleinsäure und eine Mikromethode zur Bestimmung derselben in tierischem Organen mit Hilfe dieser Reaktionen. Mikrochemie, **8**, 4–32.

DIXON, M. 1949 *Multi-enzyme systems*. Cambridge Univ. Press, London.

FEENEY, R. E., AND GARIBALDI, J. A. 1948 Studies on the mineral nutrition of the subtilin-producing strains of *Bacillus subtilis*. Arch. Biochem., **17**, 447–458.

GALE, E. F., AND MITCHELL, P. D. 1949 The assimilation of amino acids by bacteria. 8. Trace metals in glutamic acid assimilation and their inactivation by 8-hydroxyquinoline. J. Gen. Microbiol., **3**, 369–384.

GATES, F. L. 1933 The reaction of individual bacteria to irradiation with ultra-violet light. Science, **77**, 350.

HENRY, H., AND STACEY, M. 1946 Histochemistry of gram-staining reaction of microorganisms. Proc. Roy. Soc. (London), B, **133**, 391–406.

HINSHELWOOD, C. N. 1946 *Chemical kinetics of the bacterial cell*. Clarendon Press, Oxford.

JOHNSON, M. J., AND BERGER, J. 1942 The enzymatic properties of peptidases. Advances in Enzymol., **2**, 69–89.

KORKES, S., STERN, J. R., GUNSALUS, I. C., AND OCHOA, S. 1950 Enzymatic synthesis of citrate from pyruvate and oxalacetate. Nature, **166**, 439–440.

MAXWELL, R. E., AND NICKEL, V. S. 1954 Filament production in *E. coli* induced by azaserine and other antineoplastic agents. Science, **120**, 270–271.

NEISH, A. C. 1950 *Analytical methods for bacterial fermentations*. Natl. Research Council Can., Saskatoon.

NICKERSON, W. J. 1948 Enzymatic control of cell division in microorganisms. Nature, **162**, 241–245.

NICKERSON, W. J., AND SHERMAN, F. G. 1952 Metabolic aspects of bacterial growth in the absence of cell division. II. Respiration of normal and filamentous cells of *Bacillus cereus*. J. Bacteriol., **64**, 667–678.

OGUR, M., AND ROSEN, G. 1950 The nucleic acids of plant tissues. 1. The extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid. Arch. Biochem., **25**, 262–276.

OLSON, B. H., AND JOHNSON, M. J. 1949 Factors producing high yeast yields in synthetic media. J. Bacteriol., **57**, 235–246.

ROBINOW, C. F. 1943–44 Cytological observations on *Bacterium coli*, *Proteus vulgaris* and various aerobic spore-forming bacteria with special reference to the nuclear structures. J. Hyg., **43**, 413–423.

ROBINSON, H. W., AND HOGDEN, C. G. 1940 The biuret reaction in the determination of serum proteins. 1. A study of the conditions necessary for the production of a stable color which bears a quantitative relationship to the protein concentration. J. Biol. Chem., **135**, 707–725.

ROGOSA, M., AND MITCHELL, J. A. 1950 The indispensability of magnesium for *Lactobacillus helveticus* and its inavailability in a magnesium-azide complex. Bacteriol. Proc., **1950**, 130.

RUDOLPH, G. G., AND BARRON, E. S. G. 1950 Citric acid synthesis by the condensation of acetate and oxalacetate in rabbit kidney. Biochim. et Biophys. Acta, **5**, 59–65.

SHANKAR, K., AND BARD, R. C. 1952 The effect of metallic ions on the growth and morphology of *Clostridium perfringens*. J. Bacteriol., **63**, 279–290.

SPECK, J. H. 1949 The enzymatic synthesis of glutamine, a reaction utilizing adenosine triphosphate. J. Biol. Chem., **179**, 1405–1426.

UMBREIT, W. W., BURRIS, R. H., AND STAUFFER, J. F. 1949 *Manometric techniques and tissue metabolism*. Burgess Publishing Co., Minneapolis, Minn.

WEBB, M. 1948 The influence of magnesium on cell division. 1. The growth of *Clostridium welchii* in complex media deficient in magnesium. J. Gen. Microbiol., **2**, 275–287.

WEBB, M. 1949 The influence of magnesium on cell divisions. 2. The effect of magnesium on the growth and cell division of various bacterial species in complex media. J. Gen. Microbiol., **3**, 410–417.

WEBB, M. 1951*a* The influence of magnesium on cell division. 4. The specificity of magnesium. J. Gen. Microbiol., **5**, 480–484.

WEBB, M. 1951*b* The influence of magnesium on cell division. 5. The effect of magnesium on the growth of bacteria in chemically-defined media of varying complexity. J. Gen. Microbiol., **5**, 484–495.

WEBB, M. 1953 Effect of magnesium on cellular division in bacteria. Science, **118**, 607–611.