The Influence of Magnesium on Cell Division

1. The Growth of *Clostridium welchii* in Complex Media Deficient in Magnesium

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SUMMARY: The production of filamentous forms of *Clostridium welchii* which occurs in complex media containing certain commercial and chemically treated peptones is due to a deficiency of ionized magnesium. Such filaments revert to cells of normal morphological appearance on subculture in a medium containing free magnesium ions, but the change cannot be brought about by the presence of metallic ions other than magnesium. It is, therefore, suggested that the latter is essential for the activity of the cell-dividing mechanism.

The presence of a growth-inhibitory factor in certain peptones has been established. The active agent appears to be a fatty acid and may be extracted from acidified peptone solutions with ether or chloroform. The presence of the inhibitory substance in peptone markedly decreases the crop yield of *Cl. welchii* but has no direct influence on the production of filaments.

In the development of studies on the autolytic enzyme systems of Gram-positive micro-organisms (Jones, Stacey & Webb, 1948), it was observed that cultures of *Cl. welchii* in 2% Difco Bacto-peptone broth differed considerably in morphological and autolytic characteristics from cultures in the normal growth medium which contained 2% Evans's peptone.* In the former medium, *Cl. welchii* grew in the form of filaments of varying length (Pl. 1, fig. 1) which autolysed less readily than the short and more uniform ('normal') rods (Pl. 1, fig. 2) obtained by the cultivation of the bacillus in Evans's peptone. For example, the change in the Gram-staining reaction which occurs within 24–36 hr. when 'normal' cells are allowed to autolyse at pH 8.0 and 37°, occupied a period of 5 days when a suspension of the filamentous cells was allowed to autolyse under identical conditions. These long filaments reverted to cells of normal morphological appearance on subculture in Evans's peptone broth, indicating that the observed change was a direct response to some environmental stimulus.

On solid media, minute, circular, glistening colonies composed of long thin filaments (Pl. 1, fig. 3) were obtained when the organism was grown anaerobically on Difco peptone-agar plates, whereas cells from the larger colonies on Evans's peptone agar were smaller and more uniform in size (Pl. 1, fig. 4).

The use of other commercial peptones in the growth media for the cultivation of *Cl. welchii* yielded cells which varied in morphology from short rods (Difco Neopeptone), a mixture of short rods and filaments (Difco proteose peptone), to thin filamentous forms (Witte peptonum siccum). The organism failed to grow in a medium which contained 2% Peptonum Siccum (Armour).

* Evans, Sons, Lescher and Webb, Runcorn.

From these results it was considered that the change in morphology associated with growth in the various media was due to differences in the chemical composition of the peptones, filaments being produced in media lacking or deficient in some component essential for normal cell division. Since normal growth and division occurred in media containing Evans's peptone, this was subject to fractionation as follows.

Dialysis of Evans's peptone. A solution of Evans's peptone (40 g.) in distilled water (250 ml.) was dialysed against distilled water (5 l.) for 24 hr. The diffusate was evaporated in an open vessel, finally on a water-bath, and the resulting syrup dissolved in distilled water (50 ml.). The dialysed peptone was diluted to a volume of 2 l. with distilled water containing sodium chloride (10 g.) and glucose (4 g.) and sterilized. The growth of *Cl. welchii* in this medium resulted in the production of filamentous cells which reverted to normal short rods on subculture in the medium containing, in addition, the diffusate at a concentration of 1 ml. in 40 ml.

Fractionation of the diffusate. Fractionation was carried out as shown in the following scheme. A sample of each precipitate was ignited in an open crucible and the inorganic constituent of the residual ash determined by qualitative chemical analysis.

Cl. welchii cultivated in the medium containing the dialysed peptone with the addition of a solution of I or II or of the inorganic constituents of I or II, yielded cells of normal morphological appearance. The reversion of the filamentous forms to cells of uniform normal size was also observed when the dialysed peptone was made up in saturated magnesium phosphate solution or in 0.1 % magnesium sulphate.

It was concluded that the production of filamentous forms of *Cl. welchii* in the dialysed peptone medium was due to magnesium deficiency. In the following section methods are described for the removal of magnesium ions from Evans's peptone. Although such methods are not specific for magnesium, the change in morphology associated with the growth of *Cl. welchii* in such media was reversed only by the addition of recrystallized magnesium salts. It was, therefore, concluded that other positive ions removed with magnesium from the peptone do not play any part in cell division.

Removal of magnesium ion from Evans's peptone

By 'ion-exchange' resin. A solution of Evans's peptone (5 g.) in distilled water (25 ml.) was passed through a column of Zeokarb H.I.P. (Permutit Co. Ltd., London), to remove free positive ions. The resulting acid solution (pH 4.9) was diluted to 250 ml. with distilled water containing sodium chloride (1.25 g.) and glucose (0.5 g.), adjusted to pH 6.2 and sterilized (Medium 47).

Precipitation with ammonium hydroxide. The presence of magnesium and phosphate ions in the diffusate from Evans's peptone suggested that the addition of ammonium hydroxide to a peptone solution would result in the precipitation of magnesium ammonium phosphate. It has previously been shown (Scudder, 1928) that organisms such as *B. alcaligenes* which produce alkalinity in peptone and meat infusion broth cause a rapid precipitation of magnesium ammonium phosphate in the medium.

A solution of Evans's peptone (10 g.) in distilled water (100 ml.) was made strongly alkaline with ammonium hydroxide (15 ml.) when precipitation occurred. After 18 hr. the solution was filtered and ammonia removed from the filtrate by aeration in the apparatus described by Davis & Daish (1913). The solution was concentrated in an open vessel on a boiling water-bath and made up for broth with the addition of 0.05 % K_2HPO_4 (Medium 23). Magnesium, calcium and phosphate were present in the precipitate.

Initially, 18 hr. cultures of *Cl. welchii* in Medium 47 were composed of short filamentous cells and many chains of short rods of normal size, but after two to three subcultures the organism grew as long filaments which were then invariably observed in all subsequent subcultures. Such filaments also resulted when the initial cultures in Medium 47 were incubated for 3 days. These observations indicate that the production of filaments is not due to the temporary alteration in enzyme balance brought about by adaptation of the organism to the new medium (cf. Spray & Lodge, 1943).

In Medium 23, filamentous forms (Pl. 1, fig. 5) were observed in the first and all subsequent subcultures of *Cl. welchii*. The cell deposit was gelatinous in character and aqueous extracts gave strong carbohydrate reactions. When the organism was subcultured every 24 hr. for several weeks in this medium and then subcultured into 2 % Evans's peptone broth the resulting growth was composed entirely of short ('normal') rods.

The effect of the reaction of the medium on the morphology of Clostridium welchii

Since the differences in the pH of the various media used (Table 1) may have contributed to the production of filaments, *Cl. welchii* was cultivated in 2% Evans's peptone broth adjusted to various pH values by the addition of 0.1 N hydrochloric acid or sodium hydroxide. Buffers were not used, as these may produce specific effects (cf. Winslow & Falk, 1923). Control experiments showed that little change occurred in the pH of these solutions on sterilization. *Cl. welchii* failed to grow in all media of pH less than 5, normal cells were observed in cultures of pH 6.0–7.5, while in more alkaline media (pH 8.0–9.3) small oval cells, distorted curved forms and filaments were produced.

Table 1. pH of nutrient media containing glucose (0.2%), NaCl (0.5%) and peptone (2%)

Peptone used in preparation of medium	pH of medium
Evans's	5.6
Difco Bacto	6.88
Dialyzed Evans's	5.0
'Ammonia-precipitated' Evans's	7.35

From these results it was concluded that the differences in pH of the media recorded in Table 1 had little, if any, influence in the production of the filamentous forms of *Cl. welchii*. The variation in morphology, as observed in cultures of pH 8–9, was not due entirely to the unfavourable pH, but to the precipitation of essential ions which occurred in these alkaline solutions. The evidence for this was as follows. Evans's peptone broth (300 ml.) was adjusted to pH 9.64 with N sodium hydroxide and heated at 100° for 30 min. when rapid precipitation occurred. After cooling, the suspension was stirred until homogeneous and 100 ml. removed. This fraction was adjusted to pH 6.2, when the precipitate redissolved, and sterilized (Medium 49). The residual alkaline solution was filtered, the filtrate adjusted to pH 6.1 and sterilized (Medium 50). Cells of normal morphological appearance were obtained when *Cl. welchii* was cultivated in Medium 49, but pleomorphism, with variation from normal rods to long filaments, was observed in cultures of the organism in Medium 50. Qualitative analysis of the precipitated solid revealed the presence of Mg^{++} , Fe^{+++} and $PO_4^{=}$.

The specific effect of magnesium

The change in morphology which occurred in media from which magnesium had been removed was not due to a low electrolyte concentration, since the growth of *Cl. welchii* in tubes of 'ammonia precipitated' Evans's peptone broth containing respectively 1.5, 2.5 and 3.5% sodium chloride resulted, in each case, in the production of filamentous forms. Furthermore, determination of the relative osmotic pressures of the various culture media by means of membranes of copper ferrocyanide deposited on hardened filter-paper (Austin, Harntung & Willis, 1944), revealed only insignificant differences.

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The addition of metals, such as calcium and copper, normally present in small amount in peptone and which would be coprecipitated with magnesium, to 'ammonia precipitated' Evans's peptone showed that such elements had no effect on cell division.

The addition of ferric iron (1.5 mg. FeCl_3) to 'ammonia precipitated' Evans's peptone (80 ml.) gave a medium which supported an exceedingly poor growth of *Cl. welchii* and some of the filamentous cells showed division into chains (Pl. 1, fig. 6). It was concluded that this latter effect was due to inhibition of the growth of the organism by the iron, the available magnesium present in the medium then being sufficient for division of a greater proportion of the cells.

Table 2. *Magnesium content of certain peptones*

Peptone	Water (%)*	Ash (%)	Magnesium (%)	
			In ash	In peptone
Evans's	7.1	6.1	1.7	0.074
	7.3	5.9	1.8	0.077
Difco Bacto	3.6	3.6	1.8	0.048
	3.0	3.4	1.4	—
Dialysed Evans's	—	2.15	0.76	0.016

* Water (%) as loss in weight at 100°.

In support of this conclusion is the finding (see later) that, when *Cl. welchii* was grown in Difco peptone from which a growth-inhibitory substance had been removed, the resulting filaments were longer than are those which resulted when the bacillus was cultivated in normal Difco peptone broth which contained the growth-inhibitory substance.

Analysis of peptones

The magnesium content of the normal and chemically treated peptones was determined, as the evidence thus far obtained showed that the growth of *Cl. welchii* was influenced by the magnesium concentration of the medium and that inhibition of cell division occurred when this fell below a certain critical value. Samples of the peptones used were dried and ashed. The resulting inorganic residue was boiled for 30 min. with distilled water (100 ml.) containing concentrated sulphuric acid (2.5 ml.) and concentrated nitric acid (2.5 ml.) to convert pyrophosphate to metaphosphate. After cooling, the solution was filtered, the phosphate removed as ferric phosphate and the magnesium precipitated from alkaline solution with 8-hydroxyquinoline according to the method of Kolthoff & Sandell (1948). The washed precipitate was dissolved in dilute hydrochloric acid and, after the addition of a little more of the 8-hydroxyquinoline reagent, reprecipitated by the addition of ammonia. The final precipitate was collected in a sintered glass crucible, washed with dilute ammonia, dried at 110° and weighed. The results (Table 2) show that of the peptones examined, Evans's peptone had the highest magnesium content (0.075 %). Thus, in a medium containing 2% Evans's peptone, the resulting concentration of magnesium ions (*c.* 0.0015 %) is such that normal cell division

occurs throughout the entire growth of *Cl. welchii* that the medium is able to support. Since part of the magnesium present in the medium will be taken up by the organism to form the Gram complex (Henry & Stacey, 1946), it is concluded that the concentration of magnesium ions necessary for the activity of the cell-dividing mechanism is considerably less than this value. In 2% dialysed Evan's peptone broth, which supports a heavy growth of *Cl. welchii*, the ionic magnesium concentration is exceedingly low (c. 0.00008%) and is insufficient for both activation of the cell-division mechanism and the formation of the Gram complex. Consequently, in this medium, the organism grows as long filaments. The magnesium content of a 2% Difco peptone medium is relatively high (c. 0.0009–0.0010%), and it would be expected that this concentration would ensure the normal division of the poor growth that the medium supports. However, in contrast to Evans's peptone, the magnesium in Difco peptone appears to be firmly bound and little is present in an ionic form. That this is the case is shown by the following estimations of ionic magnesium.

Evans's peptone. Evans's peptone (10.0042 g.) in distilled water (100 ml.) was treated with ammonium hydroxide as before. After 30 min. the precipitate was collected in a sintered glass crucible, washed with dilute ammonia and dried at 110°. The solid (0.1024 g.) was dissolved in 5 N acetic acid (10 ml.) and the solution diluted to 250 ml. with distilled water. Quantitative determination of the ions detected by qualitative analysis, gave the following results: magnesium (precipitation with 8-hydroxyquinoline) 8.96%; calcium (as CaSO_4) 8.37%; ammonium (microKjeldahl) 2.7%; P_2O_5 (by PbMoO_4 method) 55.0%. The magnesium thus precipitated corresponds to 93.9% of the total magnesium present in the peptone.

Difco peptone. The above procedure was repeated with Difco peptone. 9.9777 g. peptone yielded 0.0165 g. of precipitate which contained, in addition to some organic material, 29.8% P_2O_5 , but no magnesium precipitable with 8-hydroxyquinoline.

An increased amount of cell division was observed in cultures of *Cl. welchii* in 2% Difco peptone broth which contained 0.1% magnesium sulphate. The addition of magnesium ions to the culture medium, however, failed to increase the crop yield, and the cells retained their distorted appearance (Pl. 1, fig. 7).

*The magnesium content of Clostridium welchii when grown
in Evans's and Difco peptone broth*

Cultures of the organism (18 hr. old) were centrifuged (Sharples super-centrifuge) and the cells washed with water (twice), precipitated with ethanol and dried with ethanol and ether. The dry cells were ashed and the inorganic residue analysed as previously described. The results (Table 3) revealed that the cells from the Evans's peptone medium contained more than twice the amount of magnesium contained by the cells from the Difco peptone medium. The results of Table 2 and 3 show that the growth of *Cl. welchii* in Evans's peptone medium utilized 11.1% of the total magnesium present in the culture. The apparent anomaly that the magnesium removed from the Difco peptone medium by the growth of *Cl. welchii* only accounts for 2.5% of the total available, may be explained on the assumption that only a small percentage

of the magnesium of the peptone is in the ionic form. Such a conclusion would be in agreement with observations that magnesium is not precipitated from a solution of Difco peptone by the addition of ammonium hydroxide.

Table 3. *Magnesium content of Clostridium welchii grown in Evans's and Difco peptone media*

Peptone used	Yield dry cells/l. culture media (g.)	Ash of cells (Mean value) (%)	Magnesium (mean %)	
			In ash	In cells
Evans's	0.5	7.3	5.4	0.35
Difco Bacto	0.15	6.5	2.5	0.16

Growth of Clostridium welchii in 4 % Difco peptone broth

In experiments undertaken before it was established that much of the magnesium present in Difco peptone is not in the ionic form, *Cl. welchii* was inoculated into tubes of 4 % Difco peptone broth under a liquid paraffin seal, as such a medium would have a magnesium content similar to that of 2 % Evans's peptone broth (cf. Table 2). After 16 hr. at 37° the poor growth was subcultured into a second tube of the same medium. No growth ensued and the culture was apparently sterile, since subcultures in 2 % Evans's peptone broth remained completely clear. Stained smears prepared from the first subculture in 4 % Difco peptone broth showed short curved rods which, in contrast to cultures of similar age in the normal medium, were consistently Gram-negative. It was therefore concluded that there was present in Difco peptone a certain concentration of a bactericidal substance which in 2 % Difco peptone broth caused partial inhibition, and in 4 % Difco peptone broth complete inhibition of the growth of *Cl. welchii*. The observation that the cells from the first subculture in the 4 % peptone medium were Gram-negative suggested that this substance was surface active (compare the use of surface active agents in the extraction of the Gram complex; Henry & Stacey, 1946) possibly a lipid or a fatty acid. Such a bactericidal substance was indeed isolated by extracting acidified solutions of the peptone with ether and other fat solvents.

Ether extraction of Difco peptone

A solution of Difco peptone (114 g.) in distilled water (500 ml.) was extracted three times with ether (which itself left no residue on evaporation). The combined extracts were dried (CaCl_2) and evaporated to give 0.01 g. of a yellow oil which was insoluble in water. The bactericidal substance was not removed by this procedure, for when *Cl. welchii* was inoculated into a medium containing 2 % of this peptone the resulting growth was poor and was composed of distorted filamentous cells. The remaining aqueous solution was therefore adjusted to pH 3.5-4.0 with hydrochloric acid and re-extracted with ether (three times). Evaporation of the ether gave 0.14 g. of a brown, water-insoluble glass which was soluble in alkali and was precipitated as a brown oil on acidification of the alkaline solution. A solution of the sodium salt was 'salted out' on the addition of sodium chloride and gave a flocculent

precipitate with calcium chloride. Fractions which possessed similar properties were also obtained by extracting the acidified peptone solution with chloroform.

The acid peptone solution after ether extraction was adjusted to pH 7.0 with *N* sodium hydroxide and then made up for broth in the usual way. Growth of *Cl. welchii* in this medium resulted in a greater crop yield (0.27 g./l.) compared with the yield in the unextracted Difco peptone broth (0.15 g./l., Table 3). These cells were filamentous in form (Pl. 1, fig. 8) and appeared longer than those from cultures in the unextracted medium (Pl. 1, fig. 1). Thus the presence of the antibacterial substance did not itself play any part in the production of the filaments. The cells contained 4.85% ash which had a magnesium content of 3.62%, corresponding to a concentration of this element of 0.17% in the original cells. From the data of Table 2 it was calculated that, in this case, the magnesium removed from the ether-extracted medium by the growth of the organism amounts to 5.1% of the total magnesium.

Cultures of *Cl. welchii* in 2% ether-extracted Difco peptone media containing magnesium sulphate (50 mg./80 ml.) consisted of short ('normal') cells (Pl. 1, fig. 9), and a profuse growth of short, stout rods (Pl. 1, fig. 10) occurred in a medium which contained 4% of the extracted peptone.

Cl. welchii failed to grow when inoculated into the 2% ether-extracted peptone medium which contained a solution of the growth-inhibitory substance at a concentration corresponding to that present in the original unextracted peptone medium. As poor growth of the organism occurred in a medium containing 2% Difco peptone which contained the antibacterial substance, together with the fact that this substance cannot be extracted before acidification of the peptone solution, it is suggested that the fatty material is rendered less active by combination with other constituents of the peptone. In Evans's peptone broth containing 0.002% (w/v) of the active substance the growth of *Cl. welchii* was poor and the cells were distorted in appearance; a concentration of 0.004% (w/v) of the extract completely inhibited growth.

The bacteriostatic activity (serial dilution method) of the ether-soluble fraction against *Staphylococcus aureus* in Evans's peptone broth was 1:1000 after incubation at 37° for either 24 or 48 hr. It had no activity against the Gram-negative organism *Bact. lactis aerogenes*.

Extraction of other peptones

Acidified solutions of other commercial peptones were extracted with ether as above. The ethereal extracts were washed with water, dried (MgSO_4) and evaporated to dryness under reduced pressure. The properties of these fractions are recorded in Table 4.

The presence of an actively bacteriostatic substance in Peptonum Siccum (Armour) explains why *Cl. welchii* failed to grow in a medium containing this peptone. It would appear that the bacillus is exceptionally sensitive to these growth-inhibitory fatty acids, for both Peptonum Siccum and Difco peptone were used for the cultivation of strains of staphylococci, micrococci, and streptococci without any untoward effect.

The effect of subsequent addition of magnesium to young cultures of Clostridium welchii in magnesium-deficient media

The fact that when *Cl. welchii* is subcultured from a medium deficient in magnesium to one containing free magnesium ions the filamentous inoculum gives rise to a population in which normal cell division occurs, suggested the possibility of these ions causing the division of pre-existing filaments. In one series of experiments, sterile magnesium sulphate (1 ml. of 3 %) was added to

Table 4. *Comparison of the bacteriostatic activities of the ether-extractable substances from peptones*

Peptone	• Ether extract yield g./100 g. peptone	Bacteriostatic activity against <i>Staph. aureus</i> after 48 hr. inhibitory dilution
Lab Lemco	0.135	1:500
Peptonum Siccum (Armour and Co.)	0.075	1:2000
Evans's	0.015	Inactive
British Drug Houses	0.030	Inactive

7 hr. cultures of *Cl. welchii* in 'ammonia precipitated' Evans's peptone broth and the cultures then incubated for a further 18 hr. At the end of this period, cells from control cultures containing no added magnesium were in the form of filaments and chains, whereas the growth in the experimental series contained filaments and shorter cells. Such observations show that if normal cell division occurs in a culture after the addition of magnesium at some time during the logarithmic growth phase, this addition does not cause the division of existing filaments. Furthermore, no detectable change in morphology occurred when filaments from an 11 hr. culture in the above medium were incubated with either 0.5 ml. 0.05 M magnesium sulphate or 0.5 ml. of an autolysed cell-free culture of *Cl. welchii* in Evans's peptone broth.

The relation of magnesium deficiency to the smooth-rough transformation

In some aspects the filamentous cells of cultures of *Cl. welchii* in magnesium-deficient media resemble the cells of rough variants of bacilli, for the smooth-rough transformation in some species is associated with recognizable changes in morphology (Wilson, 1930) and with an alteration in the method of cell division (Nutt, 1927). It has been reported (Henry & Stacey, 1943) that continued subculture of streptococci and certain other organisms in magnesium-deficient media gave rise to consistently rough colonies. However, in this case the smooth-rough transformation could not be induced in cultures of several strains of staphylococci and streptococci by continued subculture in 'ammonia precipitated' Evans's peptone media.

During the normal routine of subculturing the laboratory organisms, a rough variant of *Bact. lactis aerogenes* was isolated from a culture of the organism on Evans's peptone agar. It differed from the smooth variant in the following respects; in liquid media a fibrous pellicle was formed which showed a marked tendency to creep up the sides of the tube, the culture fluid remaining clear.

In contrast to the smooth form which grew throughout the liquid and gave rise to a considerable increase in viscosity, no polysaccharide was produced. On agar plates the variant grew rapidly, covering the surface with a spreading growth composed of irregular waxy colonies. The latter were completely removed from the agar on touching with the loop. In distilled water the growth proved difficult to emulsify, and in saline agglutination occurred. Stained smears and impression preparations showed the bacteria to be in chains.

Table 5. *Analysis of the rough and smooth forms of Bacterium lactis aerogenes*

Cells	Yield dry cells (g. from 25 Roux bottles)	Ash (%)	Magnesium (%)	
			In ash	In cells
Smooth organism	3.1	5.14-5.18	0.20	0.01
Rough organism	2.9	7.18-7.84	2.89	0.17

Twenty-five Roux bottles were inoculated with each variant and incubated at 37°. After 24 hr. the cells were removed in distilled water, washed twice at the centrifuge with distilled water and dried with ethanol and ether. Analysis of the two forms gave the results of Table 5 which show that the rough variant of *Bact. lactis aerogenes* contained a considerably greater percentage of magnesium than did the smooth form. It is, therefore, tentatively concluded that the inhibition of cell division which occurs in magnesium-deficient media bears no relation to the smooth-rough variation.

The similarity in morphology observed between the cells of cultures of rough bacilli and of cultures in magnesium-deficient media would suggest that the conclusion that an alkaline medium usually favours the *S* → *R* transformation (Hadley, 1927) should be accepted with reserve unless such observations are substantiated by immunological studies, since in alkaline media, magnesium may be precipitated as phosphate.

DISCUSSION

The production of filamentous forms of bacteria is well known and is observed when organisms are grown in the presence of sub-bacteriostatic amounts of certain antibacterial agents; as, for example, when *Bact. typhosum* is grown in the presence of methyl violet (Ainley-Walker & Murray, 1904), *Streptococcus viridans* in the presence of sulphonamide (Tunncliffe, 1939), *Cl. welchii* and other organisms in the presence of penicillin (Gardner, 1940) and *Bact. lactis aerogenes* in the presence of *m*-cresol (Spray & Lodge, 1943). It has also been claimed (Henrici, 1928) that filaments of *Escherichia coli* are produced when the surface tension of the medium is diminished by sodium ricinoleate to 35-40 dynes/cm. A similar effect has been observed in cultures of *Lactobacillus hevelticus* in which the surface tension of the medium has been lowered by the addition of sub-bacterio-static amounts of sodium glycocholate (Stacey & Webb, unpublished). In these latter cases it remains to be established whether the production of the long forms is a direct response to the lowering of surface

tension or is due to the chemical nature of the surface active substances. Filaments have also been observed in bacterial cultures which have been subjected to the action of β - or γ -rays (Porter, 1946).

The conclusion reached from a survey of such observations is that the production of filaments in rapidly growing cultures is due to inhibition of cell division while growth, in the sense of synthesis of cellular substances, is unaffected. The diverse nature of the agencies which are able to produce such marked changes in morphology suggests that the enzymes of the cell-dividing mechanism are much less stable than are the enzymes concerned in synthetic processes. Lodge & Hinshelwood (1948) suggested that there are two independent factors, referred to as (*L*) and (*D*), which respectively control elongation and division during bacterial growth. It is assumed that (*D*) must be present in more than a critical concentration before division can occur. The enzyme systems responsible for the (*L*) and (*D*) factors are easily thrown out of balance and filaments are formed when elongation proceeds normally, but the concentration of (*D*) is below the critical. The rate of synthesis of the latter factor was thought to be decreased by the presence of antibacterial substances such as *m*-cresol (Spray & Lodge, 1943).

The present study has shown that cell division in cultures of *Cl. welchii* in complex peptone media is dependent on the presence of magnesium ions. In magnesium-deficient media cell division is inhibited and filamentous cells are produced. The observation that increased polysaccharide synthesis occurs in cultures of *Cl. welchii* in media from which the magnesium has been removed by precipitation with ammonium hydroxide may be explained by an alteration of enzyme balance, occurring when cell division is inhibited and giving rise to others.

Although magnesium appears essential for the growth of most bacterial species, and is almost invariably included in defined media, relatively little attention has been paid to its role in the growth and reproduction of bacterial cultures. That the element stimulates growth is evidenced by the work of Hotchkiss (1928), in which an increase in crop yield of *Esch. coli* in 1 % peptone was secured by the addition of magnesium chloride in low concentrations. Lodge & Hinshelwood (1939) found that a standard inoculum of *Bact. lactis aerogenes* failed to grow in a synthetic medium which contained less than two parts per million of magnesium. The conclusion of Olitzki & Bromberg (1931) that magnesium was completely unimportant for the growth of *Brucella* in complex media cannot be accepted, since peptones themselves contain relatively high percentages of magnesium (cf. Table 3). More pertinent to the present investigations are the observations of Palgen (quoted by Buchanan & Fulmer, 1930) that the average length of the cells in cultures of either *B. mesentericus* or *B. subtilis* is decreased by the addition of magnesium sulphate.

The nature of the enzyme or enzymes which constitute the cell-dividing mechanism is unknown. The process can apparently only occur in conjunction with the other stages of the growth and reproduction of bacterial cells, for the addition of magnesium or of an autolysed normal culture to existing filamentous cells does not result in division. Of great interest in this con-

nexion is the discovery that deoxyribonuclease exists in pneumococcal autolysates (McCarty & Avery, 1946).

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Figs. 1-10

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EXPLANATION OF PLATE

Magnification, $\times 1200$. Cells of *Cl. welchii* from cultures in:

- Fig. 1. 2% Difco Bacto-peptone broth.
- Fig. 2. 2% Evans's bacteriological peptone broth.
- Fig. 3. Difco peptone-agar.
- Fig. 4. Evans's peptone-agar.
- Fig. 5. 2% Evans's peptone from which Mg^{++} had been removed by precipitation with ammonium hydroxide ('Ammonia precipitated Evans's peptone').
- Fig. 6. 'Ammonia precipitated Evans's peptone' (30 ml.) + 1.5 mg. $FeCl_3$.
- Fig. 7. 2% Difco peptone broth + 0.1% magnesium sulphate.
- Fig. 8. 2% Difco Bacto peptone previously extracted with ether at pH 8.5 ('ether extracted Difco peptone').
- Fig. 9. 2% 'Ether extracted Difco peptone' + 0.16% magnesium sulphate.
- Fig. 10. 4% 'Ether extracted Difco peptone'.

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