Proteus vulgaris yields *N*-acetylchondrosin. On the bases of these findings, together with analytical results, the product is considered to be *N*-acetylchondrosin sulphate.

3. When chondroitinase action is not allowed to proceed to completion a series of oligosaccharides is obtained, the principal member being identical with the single product obtained by exhaustive degradation. This series of oligosaccharides is identical with that arising from the prolonged degradation (48 hr.) of chondroitin sulphate with testicular hyaluronidase.

4. Evidence suggests that this oligosaccharide series consists of di-, tetra-, hexa- and octa-saccharide homologues, the smallest member being *N*-acetylchondrosin sulphate.

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REFERENCES

- Belcher, R., Nutten, A. J. & Sambrook, C. M. (1954). *Analyst*, **79**, 201.
- Cifonelli, J. A., Ludoweig, J. & Dorfman, A. (1957). *Fed. Proc.* **16**, 709.
- Davidson, E. A. & Meyer, K. (1954). *J. Amer. chem. Soc.* **76**, 5686.
- Dische, Z. (1947). *J. biol. Chem.* **167**, 189.
- Dodgson, K. S. & Lloyd, A. G. (1957a). *Biochem. J.* **65**, 4P.
- Dodgson, K. S. & Lloyd, A. G. (1957b). *Biochem. J.* **66**, 532.
- Dodgson, K. S., Lloyd, A. G. & Spencer, B. (1957). *Biochem. J.* **65**, 131.
- Dodgson, K. S. & Spencer, B. (1953). *Biochem. J.* **55**, 436.
- Dorfman, A. (1955). *Methods in Enzymology*, vol. 1, p. 166. New York: Academic Press Inc.
- Feingold, D. S., Avigad, G. & Hestrin, S. (1956). *Biochem. J.* **64**, 351.
- French, D. (1951). *Science*, **113**, 352.
- French, D. & Knapp, D. W. (1950). *J. biol. Chem.* **187**, 463.
- French, D. & Wild, G. M. (1952). *Abstr. Pap. Amer. Chem. Soc.* 121st meeting, 4P.
- French, D. & Wild, G. M. (1953). *J. Amer. chem. Soc.* **75**, 2612.
- Hoffman, P., Meyer, K. & Linker, A. (1956). *J. biol. Chem.* **219**, 653.
- Linker, A., Meyer, K. & Hoffman, P. (1956). *J. biol. Chem.* **219**, 13.
- Lloyd, A. G. & Dodgson, K. S. (1957). *Biochem. J.* **66**, 45P.
- Markham, R. (1942). *Biochem. J.* **32**, 790.
- Meyer, K., Davidson, E. A., Linker, A. & Hoffman, P. (1956). *Biochim. biophys. Acta*, **21**, 506.
- Meyer, K. & Rapport, M. M. (1951). *Science*, **113**, 596.
- Moore, S. & Stein, W. H. (1948). *J. biol. Chem.* **176**, 367.
- Orr, S. F. D. (1954). *Biochim. biophys. Acta*, **14**, 173.
- Partridge, S. M. (1948). *Biochem. J.* **42**, 238.
- Partridge, S. M. (1949). *Nature, Lond.*, **164**, 479.
- Payne, W. J. & Kieber, R. J. (1954). *Arch. Biochem. Biophys.* **52**, 1.
- Schütte, E. & Greiling, H. (1955). *Hoppe-Seyl. Z.* **302**, 55.
- Weissmann, B. & Meyer, K. (1954). *J. Amer. chem. Soc.* **76**, 1753.
- Weissmann, B., Meyer, K., Sampson, P. & Linker, A. (1954). *J. biol. Chem.* **208**, 417.
- White, L. M. & Secor, G. E. (1953). *Arch. Biochem. Biophys.* **43**, 60.

Electrophoretic Studies on Bacteria

2. THE EFFECT OF ENZYMES ON RESTING SPORES OF *BACILLUS MEGATERIUM*, *B. SUBTILIS* AND *B. CEREUS**

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In a previous paper (Douglas, 1955) the electrophoretic behaviour of resting spores of *Bacillus subtilis* and *Bacillus megaterium* was reported, as a function of pH and ionic strength, together with the effect on the behaviour of addition of surface-active agents. It was concluded that the outermost, electrokinetically effective, surface layers of the *B. subtilis* and *B. megaterium* spores were essentially non-ionogenic, acquiring their charge by

the adsorption of ions, and that they were different with respect to either or both of their chemical and physical structures. Comparison with the corresponding behaviour of bacterial cells and of protein, lipid and other surfaces, as recorded in the literature, suggested that the effective surface of *B. subtilis* spores was essentially polysaccharide; for the *B. megaterium* spores the evidence suggested surface lipid, with possibly a protein component in a complex so as to render the normal ionogenic groups inoperative.

* Part 1: Douglas (1955).

Enzymes have been used with considerable success to indicate the presence of particular types of biological polymer in the surface layers of bacterial cells. The enzymes used include lysozyme (Epstein & Chain, 1940; Wiebull, 1953; Salton, 1955), trypsin and lipase (Dyar, 1948), ribonuclease (Harris, 1953). In some of the studies (Dyar, 1948; Harris, 1953) electrophoresis was used as the means of observing changes in surface composition.

In seeking further information concerning the surface composition of resting spores of *B. subtilis*, *B. megaterium* and *Bacillus cereus* therefore, their electrophoretic behaviour before and after treatment with active preparations of a series of enzymes has been determined, essential control experiments being performed. The enzymes used were lysozyme, hyaluronidase, trypsin, ribonuclease, lipase, lecithinase and a glutamylpeptidase.

The present paper reports the results obtained and draws modified conclusions concerning the nature of the spore surfaces, on the basis of these enzyme effects together with mobility-pH results obtained for model particles having adsorbed surface layers of isolated bacterial peptides (Douglas & Shaw, 1957).

EXPERIMENTAL

Spores. The spores used in the present work were from the same strains of *B. subtilis*, *B. megaterium* and *B. cereus* as those employed by Powell and co-workers in a series of biochemical studies (Powell & Strange, 1953; Strange & Powell, 1954; Strange & Dark, 1956). They were supplied as suspensions in sterile water containing about 2×10^{10} spores/ml., and stored as such.

Enzymes. Samples of crystallized lysozyme (egg white), trypsin (bovine pancreas) and ribonuclease (bovine pancreas) were obtained from Armour Chemicals Ltd., Hampden Park, Eastbourne, Sussex. A second sample of lysozyme from L. Light and Co., Colnbrook, Bucks, behaved identically with the sample from Armour Ltd.

An active preparation of hyaluronidase (Hyalase, 1000 units/mg.; Bengers Ltd.) was kindly supplied by Dr E. London, Bengers Ltd.

Lipase in the form of an active, acetone-dried extract from pig pancreas was kindly supplied by Mr R. Strange, Microbiological Research Establishment, Porton, Wilts.

The lecithinase preparation was a filtrate of a glycerol-treated culture of *Clostridium welchii* type A (Macfarlane & Knight, 1941) obtained from M.R.E., Porton.

The glutamylpeptidase (Thorne, 1956) was generously supplied by Dr C. B. Thorne (then at M.R.E., Porton) as an active preparation in aqueous solution.

Enzyme treatment of spores. A brief review of the literature concerning the susceptibility of bacteria to enzymes showed that the concentrations of enzyme and temperature used have varied considerably with the object of the experiments. Thus lysozyme was used at a final concentration of $4 \mu\text{g./ml.}$ and 34° to study the changes in appearance produced by it in cells of *B. megaterium* (Welshimer, 1953),

whereas to isolate proptoplasts of the same organism 0.5 mg./ml. was used at room temperature (Wiebull, 1953).

In the present work 37° has been used as the temperature for the treatments of active enzyme, which have been carried out in phosphate media, $I=0.1$, of appropriate pH. Periods of treatment of 3, 6 and up to 24 hr. have been used with each of a number of enzyme concentrations to determine the change in electrophoretic behaviour produced by each of the enzymes. When a change was found the enzyme concentration was increased until no further mobility shift resulted. The upper concentration limits quoted below exceed those at which limiting shifts were observed with some of the enzymes.

Lysozyme was used in phosphate buffer, pH 6.8, and at final concentrations between 2 and $100 \mu\text{g./ml.}$ Trypsin and ribonuclease were used at concentrations within the same range and at pH values 8.0 and 6.8 respectively. The hyaluronidase preparation was used at pH 5.9 and at concentrations $50 \mu\text{g./ml.}$ to 1 mg./ml. The solid lipase preparation as received was assumed 5% active and an amount sufficient to give a final 'active' concentration of $20 \mu\text{g./ml.}$ extracted by suspension in phosphate buffer, pH 6.8; the insoluble residue was centrifuged off and the supernatant liquid used in the spore treatments. The original active solutions of lecithinase and of glutamylpeptidase were used at final volume dilutions of 10 to 100 times, in phosphate buffers of pH values 6.8 and 8.0 respectively.

The general experimental procedure for the enzyme treatments was as follows. A small volume of stock spore suspension in water was spun down in an angle centrifuge, the supernatant liquid decanted and the spores were resuspended in a sufficient volume of phosphate buffer, $I=0.1$, of pH appropriate to the enzyme being used, to give a number concentration of about 2×10^{10} spores/ml. A volume (1 ml.) of this was diluted to 10 ml. with the same buffer containing the enzyme at the desired concentration, in a 15 ml. centrifuge tube. This was transferred to a water thermostat at 37° for the incubation period, and stirred from time to time with a sterilized glass rod to maintain the spores in suspension. The tube was then removed, the spores were spun down and the supernatant buffer was discarded. The spores were washed three times by resuspending in 0.05 N-NaCl and spinning down. Finally the washed spores were resuspended in 100 ml. of Michaelis buffer, $I=0.05$, to give a spore concentration of about 2×10^8 spores/ml., which was convenient for the electrophoretic observations.

Control experiments. To allow for the possibilities that (i) heating to 37° alone, (ii) the inactivated enzyme preparation, (iii) adsorption of (a) the enzyme or (b) other components in the enzyme preparations, might be the cause of any shift in electrophoretic behaviour observed after the above treatments, the following control experiments were performed. The possibility that germination might be the cause could be ruled out since phase-contrast microscopy was used to confirm that the spores, on which electrophoretic measurements were made, retained their appearance as resting spores.

The action of (i), incubation alone, was determined by performing experiments under the conditions described in the previous section, but without the addition of the enzyme preparation. Possibility (ii) was taken account of by repeating the experiments as for active enzyme but with inactivated materials. Possibility (iii) was checked by

allowing parallel systems of spores plus enzyme to stand for up to 3 hr. at room temperature. This was to permit adsorption but to minimize any enzyme attack. As in the experiments at 37°, the spores were then centrifuged, washed three times with 0.05N-NaCl and finally resuspended in Michaelis buffers of pH 3 and 7, $I=0.05$. Their mean electrophoretic mobility was then determined.

Buffer systems. KH_2PO_4 - Na_2HPO_4 buffers, $I=0.1$, were used for all the enzyme and control experiments. The electrophoretic measurements, on the other hand, were carried out in Michaelis buffers, $I=0.05$, as in the previous work on resting spores (Douglas, 1955). The buffer NaCl and HCl used were all of A.R. or equivalent quality. Sterile solutions and distilled water were used throughout the work.

Electrophoresis. The determinations of electrophoretic mobility were made with the same type of rectangular-section micro-cell (10 mm. wide $\times$ 0.2 mm. deep) and technique as previously described (Douglas, 1955). All the electrophoretic measurements relate to 25°. All the mobility values reported are mean values (averaged for at least 24 observations in any one determination) except in Fig. 2, where the histograms record the frequency of individual spore velocities.

In making the twenty-four or more observations, one 'stationary level' in the cell (Douglas, 1947) was focused and two spores were timed each way; the other 'stationary level' was then focused and four more timings were made; the first level was refocused, four timings were made—and so on. This gave a minimum of three sets of four observations for each level, located three times; A slight drift velocity, up to 5% of the spore velocity, was sometimes observed in the absence of an applied field; this was allowed for by averaging the reciprocals of the times in each set of four, as standard procedure. The agreement between the sets of observations at the two 'stationary levels' gave a check on cell performance.

RESULTS

Mobility-pH relations for untreated spores.

Mobility-pH relations, in Michaelis buffers pH 2-9 and $I=0.05$, for resting spores of the strains of

Fig. 1. Mobility-pH curves for resting spores and bacterial peptides in Michaelis buffers, $I=0.05$ at 25°. □, Resting spores of *B. cereus*; △, resting spores of *B. subtilis*; ○, ○, different samples of resting spores of *B. megaterium*; ---, exudate hexosamine peptide adsorbed on Nujol droplets; ····, DL-polyglutamic acid (*B. subtilis*) adsorbed on Nujol droplets.

B. megaterium and *B. subtilis* used have already been reported (Douglas, 1955). Fig. 1 records the behaviour of further samples of these spores, as determined in the present work, together with the curve for resting spores of *B. cereus* used by Powell and co-workers. The mean mobility values recorded were reproducible to within 2% experimentally.

The mobility against pH behaviour was remarkably consistent for samples of *B. subtilis* spores reaped from different cultures; the smaller number of *B. cereus* samples studied showed similar consistency. For *B. megaterium* the different samples studied gave mobility against pH curves of the same form, but varying in quantitative values between the two extreme curves given, which relate to spores reaped from cultures grown at different times. Nevertheless, the several *B. megaterium* samples showed the same response (within 2%) to enzyme treatments, etc., in terms of the relative changes in electrophoretic mobility thus produced.

Fig. 1 also includes the corresponding curves for a hexosamine peptide associated with the spore coat (Strange & Dark, 1956) and liberated into the medium on germination (Powell & Strange, 1953), and a DL-polyglutamic acid, produced extracellularly by vegetative *B. subtilis* (Thorne, 1956). These curves were obtained for the compounds adsorbed from solution in the Michaelis buffers on to liquid paraffin (Nujol) droplets, under conditions giving a complete surface layer (Douglas & Shaw, 1957).

Electrophoretic homogeneity of spore samples. In order to assess the significance of any shift in spore mobilities, both individual and mean, information concerning the electrophoretic composition of the spore suspensions is required, for resting spores and for spores variously treated. In particular the coefficient of variance (V) of individual velocities and the standard error of their mean are required, if possible.

Fig. 2 presents summations of the individual observations from several separate experiments made in Michaelis buffer, pH 7, $I=0.05$, 25°, both for resting spores and treated spores. These histograms are typical representatives of many more relating to other pH values and other spore treatments. The dotted curves are the Gaussian or normal distribution curves fitted to the observations in the usual way.

Electrophoretic mobilities of treated spores. Fig. 3 records the limiting mobility values, in Michaelis buffer, pH 7, at 25°, for ungerminated spores after the different enzyme and control treatments. They are expressed relative to the mobility of untreated resting spores as unity; as will be seen later, this enables the significance of the mobility shifts to

be assessed on sight, from knowledge of the standard error limits for these mean mobility values. For the three organisms, the effects of incubation at 37° with inactivated enzymes were the same as with incubation alone (*I* in Fig. 3). The adsorption experiments at room temperature gave the same final mobilities as the untreated control (*C* in Fig. 3) in all cases. Mobility values at pH 3 were also obtained, to allow for the possibility of coincident behaviour for treated and untreated spores at pH 7 or near, even when some significant change in surface had been produced (Harris, 1953). It was found that when a significant shift in the mobility occurred at pH 7, one was observed at pH 3 also; when none occurred at pH 7 there was none at the lower pH. Accordingly, Fig. 3 records only the findings at pH 7.

DISCUSSION

Before considering the other results presented in this paper it is pertinent to discuss the electrophoretic homogeneity of the initial resting spores, and any changes produced by subsequent treatment. The histograms were always unimodal, as shown in Fig. 2, and corresponded closely to

normal distribution curves with a coefficient of variation (*V*) of about 6%. For resting spores of *B. cereus*, as illustrated, the larger variance found (*V* about 9%) may be the result of combining observations on spores of different ages. Newly reaped spores and 1-year-old spores gave mobilities of -1.2 and -0.9 μ /sec./v/cm. respectively at pH 7. This mobility variation may in turn be associated with the falling off with age of a lytic activity exhibited by these *B. cereus* spores (Strange & Dark, 1957). The other histograms related to spores of similar ages; *B. subtilis* spores showed no variation with age.

As already mentioned, the overall variance (V^2) includes (i) the effect of the Brownian movement, (ii) experimental errors in timing and in locating the 'stationary levels', (iii) any electrophoretic heterogeneity of the spore samples, and possibly (iv) a small error due to imperfect elimination of drift. Even if (i) and (ii) were negligible the homogeneity of the spore samples with respect to electrophoresis, and in turn to their surface composition,

Fig. 2. Histograms of number of spores against electrophoretic mobility for normal and treated spores, and the corresponding Gaussian distributions. Mobilities were determined in Michaelis buffer, pH 7, $I=0.05$ at 25°. *B. cereus*: (a) untreated, resting spores; S.D. 0.09; (b) trypsin-treated, ungerminated spores; S.D. 0.06. *B. megaterium*: (a) untreated, resting spores; S.D. 0.16; (b) incubation control, ungerminated; S.D. 0.18. *B. subtilis*: (a) untreated, resting spores; S.D. 0.13; (b) lysozyme-treated ungerminated spores; S.D. 0.10; (c) ribonuclease-treated, ungerminated spores; S.D. 0.13.

Fig. 3. Limiting electrophoretic changes, measured in Michaelis buffer, pH 7, $I=0.05$ at 25°, for spores treated with enzymes in 0.1M-phosphate buffer, at 37°. Values are relative to those for untreated resting spores. *C*, Untreated, resting spores; *I*, incubation alone; *L*, incubation with lysozyme; *T*, incubation with trypsin; *R*, incubation with ribonuclease; *Gp*, incubation with polyglutamylpeptidase preparation; *H*, incubation with hyaluronidase preparation; *Lp*, incubation with lipase preparation; *Lc*, incubation with lecithinase preparation; *L+T*, incubation with lysozyme, followed by incubation with trypsin; *L+H*, incubation with lysozyme, followed by incubation with hyaluronidase; *Gp+L*, incubation with glutamylpeptidase, followed by lysozyme.

could be considered satisfactory; when allowance is made for (i) and (ii) the degree of uniformity appears very great. In the electrophoretic measurements, the potential gradient was adjusted so that the spores took approximately 8–10 sec. to cover $60\ \mu$ at the stationary level. Thus assuming the spores to approximate to spheres of diameter μ and that their diffusion coefficient $D = kT/6\pi\eta r$, the Brownian displacement $\Delta = \sqrt{2Dt}$ is about $3\ \mu$, i.e. 5% of $60\ \mu$. Hence error (i) contributes about 0.0025 to V^2 ; this is probably an overestimate as the spores are ellipsoidal and charged. The excursion time can be judged to 0.1 sec. and the stationary level located to better than $1\ \mu$ in $40\ \mu$ (cell depth 200–250 μ); thus error (ii) may contribute about 0.0005 to V^2 . The observed V^2 is nearly always about 0.0036 ; deducting contributions from errors (i) and (ii), and remembering that there may still be a small residual drift error (iv), we conclude that the true coefficient of variation of the mobility is not more than 2.5%. In fact, since Sheppard's correction for grouping has not been applied in the above general argument, it is possible that the spores are strictly homogeneous with respect to electrophoretic mobility. It is of interest to note that hydrocarbon droplets also show a coefficient of variation of about 6% in Michaelis buffer, pH 7 and $I = 0.05$, the same apparatus and technique being used. Further, the remarkable homogeneity suggested for the resting spores appears to be maintained after the various treatments to which the spores have been subjected in the present work.

Although no impressively large changes in mobility result from enzyme treatment (Fig. 3) the shifts of 15% or more observed for some enzymes are significant. The standard error of the mean mobilities ($\sim V/\sqrt{n}$, where n is the number of individual observations in a mobility determination) reported here is about 1.5% at a maximum. Electrophoretic shifts were observed only with spores of *B. megaterium* and of *B. subtilis*. For *B. subtilis* spores lysozyme, hyaluronidase and the glutamylpeptidase separately produced significant shifts; the combinations of enzyme treatments were used in an attempt to enhance these shifts, but without any very marked effect. *B. megaterium* spores showed a pronounced shift with the glutamylpeptidase, a lesser shift with the lipase preparation, but none with lysozyme, etc. *B. cereus* was unco-operative throughout.

Since the electrophoretic behaviour of a particle surface reflects its chemical nature, it is reasonable to assume that the electrophoretic shifts reflect changes in the spore surfaces produced by enzyme attack. It is known that both lysozyme and hyaluronidase attack polysaccharide substrates rich in *N*-acetylhexosamine, whereas the glutamylpeptidase is reasonably specific for polypeptides

containing glutamic acid (Thorne, 1956). It is therefore suggested that the surface of *B. subtilis* spores includes both *N*-acetylhexosamine-containing and glutamylpeptide-containing material and that the *B. megaterium* spore surface contains a glutamylpeptide component and possibly some lipid.

This view is supported by the mobility-pH curves for model particles having adsorbed surface layers of a hexosamine peptide or DL-polyglutamic acid, which closely resemble the curves for *B. subtilis* and *B. megaterium* spores respectively (Fig. 1). From the known surface and interfacial-film behaviour of polysaccharides and polypeptides (Davies, 1953), it seems probable that when adsorbed on oil droplets the hexosamine peptide will be orientated with both components in the surface and with any charged or polar side-groups preferentially in the aqueous medium; polyglutamic acid is likely to behave similarly, especially when the carboxyl groups can ionise.

It is thus possible that the outermost surface layers of the resting spores of both *B. subtilis* and *B. megaterium* consist essentially of this hexosamine peptide, which is largely liberated by these organisms on germination, orientated differently so that both its hexosamine and peptide parts appear in the surface of *B. subtilis*, but only the peptide part appears in that of *B. megaterium*.

These suggestions are consistent also with the action of enzymes on the isolated spore coats of these organisms and on the exudate peptide. Thus Strange & Dark (1956) have shown that lysozyme accelerates the liberation of the hexosamine peptide from the spore coats and also leads to degradation of the hexosamine peptide itself, as indicated by diffusion through a cellophan sac. Further, the glutamylpeptidase used in the present work was subsequently found to degrade the hexosamine peptide with the production of smaller peptides (Strange & Thorne, 1957).

Despite the negative response to enzymes, the mobility against pH for *B. cereus* spores is similar in form to that for the other spores and suggests that they have the same surface-charge groups, $-\text{CO}_2\text{H}$, at lower density. Essentially the same hexosamine peptide is associated with the spore wall and liberated on germination (Strange & Dark, 1956). The surface of *B. cereus* spores might also contain hexosamine peptide, orientated in a way different from those suggested for *B. subtilis* and *B. megaterium*, so that the enzyme-sensitive links in the hexosamine peptide are shielded from external attack. Alternatively, the exosporium may be the cause of the lack of response with *B. cereus*. This is rather speculative, but a recent study by the authors (Douglas & Parker, 1957) of the cation-charge-reversal spectra of the resting

spores of the three organisms, and of the hexosamine peptide adsorbed on oil droplets, again suggests closely similar chemical compositions for their surfaces, with $-CO_2H$ predominating as the effective charge group (Kruyt, 1949).

Finally, the relative resistance of the spores to the variety of enzymes used stands in marked contrast to the sensitivity of the vegetative cells to at least some of these enzymes. Thus *B. megaterium* spores show no electrophoretic shift on treatment with lysozyme; further, they appear unchanged by phase-contrast microscopy and remained ungerminated and viable. On the other hand, vegetative cells of *B. megaterium* are well known for their sensitivity to lysozyme (Welshimer, 1953; Wiebull, 1953; Salton, 1955). This has been confirmed for vegetative cells grown from the present spores (Douglas & Parker, 1958). The insensitivity of the resting spores to enzymes is paralleled by their relative resistance to a variety of bactericidal agents, and is perhaps to be expected if the function of spore formation is regarded as protective (Williams, 1952). The problem remains of explaining this insensitivity in terms of the chemical and physical composition of the spore coat, which appears to be somewhat similar to that of the vegetative cell wall (Salton, 1955).

SUMMARY

1. Change in electrophoretic behaviour has been used to assess the susceptibility of the surfaces of resting spores of *Bacillus subtilis*, *Bacillus megaterium* and *Bacillus cereus* to attack by a variety of enzymes including lysozyme, trypsin and lipase.

2. *B. subtilis* spores were affected by lysozyme, hyaluronidase and a polyglutamylpeptidase, *B. megaterium* by the glutamylpeptidase and lipase, *B. cereus* by none of the enzymes.

3. The results, taken in conjunction with the mobility-pH curves for the resting spores and for bacterial peptides, are consistent with the surfaces of the resting spores being composed essentially of differently orientated layers of the same hexosamine peptide, which is partially liberated from the sporecoats on germination.

4. Analysis of histograms of mobilities against numbers of spores shows the initial spore samples to be remarkably homogeneous electrophoretically, and that this homogeneity is maintained after the various enzyme treatments.

REFERENCES

- Davies, J. T. (1953). *Trans. Faraday Soc.* **49**, 949.
 Douglas, H. W. (1947). *J. sci. Instrum.* **24**, 103.
 Douglas, H. W. (1955). *Trans. Faraday Soc.* **51**, 146.
 Douglas, H. W. & Parker, F. (1957). *Trans. Faraday Soc.* **53**, 1494.
 Douglas, H. W. & Parker, F. (1958). *Biochem. J.* **68**, 99.
 Douglas, H. W. & Shaw, D. J. (1957). *Trans. Faraday Soc.* **53**, 512.
 Dyar, M. T. (1948). *J. Bact.* **56**, 821.
 Epstein, L. A. & Chain, E. (1940). *J. Exp. Path.* **21**, 339.
 Harris, J. O. (1953). *J. Bact.* **65**, 518.
 Kruyt, H. R. (1949). In *Colloid Science*, vol. 2, ch. ix, p. 276. London: Elsevier.
 Macfarlane, M. G. & Knight, B. C. J. G. (1941). *Biochem. J.* **35**, 884.
 Powell, J. F. & Strange, R. E. (1953). *Biochem. J.* **54**, 205.
 Salton, M. R. J. (1955). *3rd Int. Congr. Biochem., Brussels. Conférences et Rapports*, p. 404.
 Strange, R. E. & Dark, F. A. (1956). *Biochem. J.* **62**, 459.
 Strange, R. E. & Dark, F. A. (1957). *J. gen. Microbiol.* **16**, 236.
 Strange, R. E. & Powell, J. F. (1954). *Biochem. J.* **58**, 80.
 Strange, R. E. & Thorne, C. B. (1957). *Biochim. biophys. Acta*, **24**, 199.
 Thorne, C. B. (1956). *6th Symp. Soc. gen. Microbiol.* p. 68.
 Welshimer, J. (1953). *J. Bact.* **66**, 112.
 Wiebull, C. (1953). *J. Bact.* **66**, 688.
 Williams, O. B. (1952). *Bact. Rev.* **16**, 89.

Electrophoretic Studies on Bacteria

3. THE GROWTH CYCLE OF *BACILLUS MEGATERIUM*, THE BEHAVIOUR OF CELLS AND THE CHANGES PRODUCED BY LYSOZYME*

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In previous studies some attempt was made to characterize and determine the composition of the outermost surface layers of resting spores of *Bacillus megaterium*, *Bacillus subtilis* and *Bacillus cereus* electrophoretically, by measuring their

mobility-pH behaviour in buffered media (Douglas, 1955) and the changes produced by treatment with a variety of enzymes (Douglas & Parker, 1958). In independent biochemical studies, a hexosamine-containing peptide of characteristic composition was shown to be a common constituent of spore coats of these species (Strange & Dark, 1956). It

* Part 2: Douglas & Parker (1958).