The Composition of the Spore Coats of *Bacillus megatherium*, *B. subtilis* and *B. cereus*

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A peptide containing glutamic acid, alanine, $\alpha\epsilon$-diaminopimelic acid and hexosamine was found to be present in exudates from germinating spores and in extracts from disintegrated resting spores (Powell & Strange, 1953; Strange & Powell, 1954). It was suggested that the peptide might be derived from the spore coat when this underwent a change in permeability during germination or was damaged mechanically. In the present study of the chemical composition of spore coats, these were obtained by mechanical disintegration, a method which has been used by a number of workers to obtain bacterial cell walls (Salton & Horne, 1951; Salton, 1953; Cummins, 1954). There seemed little hope of demonstrating the presence of the peptide in the coat of the resting spore by this method, as the peptide appeared to be so readily freed during disintegration. It was found, however, that spore-coat preparations of *Bacillus megatherium* still contained considerable amounts, and *B. subtilis* smaller amounts, of bound hexosamine which was slowly released in the form of the peptide previously isolated. This process and the effect of various enzymes on it are described. A similarity was found between the effect of lysozyme on spore peptide and lysozyme substrate, which is a mucopolysaccharide considered to exist within certain bacterial cell walls as an insoluble complex (Meyer, Palmer, Thompson & Khorazo, 1936; Epstein & Chain, 1940; Meyer & Hahnel, 1946; Salton, 1952), and this is also described.

MATERIALS AND METHODS

Spore suspensions of *B. megatherium*, *B. subtilis* and *B. cereus* were prepared as previously described (Powell & Strange, 1953; Strange & Powell, 1954).

Germinated spores of *B. megatherium* were obtained by heating resting-spore suspensions for 2 hr. at 60° and allowing them to germinate spontaneously in distilled water (Powell, 1951). They were freed from exudate by centrifuging and washing with distilled water.

Crude alkaline phosphatase. This was prepared from calf-intestine mucosa as described by Schmidt & Thannhauser (1943), but was not treated with activated alumina. The substrate was suspended in 0·02 M-veronal/sodium veronal buffer, pH 9·3, containing 50–100 units of enzyme/ml., final concentration.

Crystalline lysozyme. This was kindly supplied by Dr D. Herbert, who had prepared it according to the method of Alderton & Fevold (1946). The substrate was usually suspended in 1:10 McIlvaine's buffer at pH 5·3 containing 40 μg. of enzyme/ml., and 0·4% (w/v) NaCl, final concentration.

Crude lipase. This was obtained from pig pancreas as an acetone-dried powder (Willstätter & Waldschmidt-Leitz, 1923). The substrate was suspended in 0·02 M-NH_3/NH_4Cl solution, pH 8·9, containing 0·25–1 mg. of crude enzyme/ml., final concentration.

Crude papain. This was obtained from Baird and Tatlock (London) Ltd. The substrate was suspended in 0·02 M phosphate buffer, pH 7·6, containing the equivalent of 1 mg. of water-soluble extract of crude enzyme/ml., final concentration.

Crude ficin. This was obtained from L. Light and Co. Colnbrook, Bucks. The substrate was suspended in 0·02 M acetate buffer, pH 4·5, containing 1 mg. of enzyme/ml., final concentration.

Pepsin. Twice-crystallized pepsin was obtained from L. Light and Co. The substrate was suspended in 0·01 N-HCl containing 250 μg. of enzyme/ml., final concentration.

Trypsin. 'Tryptar' brand from Armour Laboratories, Chicago, U.S.A., was used. The substrate was suspended in 0·02 M-$NaHCO_3$, pH 9·2, containing 20 μg. of enzyme/ml., final concentration.

Incubation conditions are given in the Results section.

Preparation of spore coats. Spore suspensions containing about 10^{10} spores/ml. were disintegrated in a Mickle (1948) tissue disintegrator with Ballotini beads, size 12. 'Capryl alcohol' (Hopkin and Williams Ltd.) (three drops) was added to each vessel and disintegration time was controlled by the examination of stained films at suitable intervals. The process was stopped when practically no intact spores remained, but before the coats had been broken up into unidentifiable fragments. This time, 15–30 min., varied for different spores. The resulting suspension was centrifuged in an angle centrifuge at 1400 *g* for 15 min. The cloudy supernatant liquid was removed and the deposit washed by centrifuging at room temperature with M-$NaHCO_3$ (3 ×), H_2O (2 ×), N-HCl (3 ×) and H_2O (3 ×). The washing procedure was designed to extract material adhering to the coats, and for this purpose it was carried out over as wide a pH range as possible without damaging their appearance. It was found that strong alkali caused them to become gelatinous, so sodium bicarbonate solution was used on the alkaline side. The washed material was dried over H_2SO_4 in a vacuum desiccator. Yields of dried spore-coat material, expressed as percentage of dried weight of spores, were about 35 for *B. subtilis*, 22 for *B. megatherium* and 26 for *B. cereus*.

Preparation of lysozyme substrate from Micrococcus lysodeikticus. Acetone-dried cells of *M. lysodeikticus* were extracted with 0·5N-NaOH at room temperature, as described by Meyer & Hahnel (1946). The crude mucopolysaccharide fraction obtained contained 19% of hexosamine (as glucosamine) and gave a positive Molisch reaction.

Preparation of B. megatherium *spore-exudate peptide.* This was prepared as described by Strange & Powell (1954).

Total nitrogen was estimated by the micro-Kjeldahl method, with the catalyst of Chibnall, Rees & Williams (1943); *α-amino nitrogen* by the ninhydrin–CO_2 technique of Van Slyke, Dillon, MacFadyen & Hamilton (1941); *hexosamine* by the method of Elson & Morgan (1933) with the modification of Immers & Vasseur (1950); *acetylhexosamine* by the method of Morgan & Elson (1934); *total phosphorus* by the method of King (1932); *total carbohydrate* (excluding hexosamine) by the method of Sørenson & Haugaard (1933); reducing power by the method of Hagedorn & Jensen (1923), and *deoxyribose* by the method of Morse & Carter (1949). *Amino acids* and *amino sugars* were detected by paper chromatography (Consden, Gordon & Martin, 1944).

Lipid content. Spore coats (40–50 mg.) were hydrolysed with 6·5N-HCl in sealed tubes for 2 hr. and the hydrolysates extracted with ether as described for bacterial cell walls by Salton (1953).

Electron microscopy. Spore-coat preparations were examined and photographed by Mr W. F. Harris of the Chemical Defence Experimental Establishment, Porton, Siemens apparatus with an accelerating voltage of 50 kv being used.

RESULTS

To demonstrate that the preparations used consisted essentially of integuments, electron micrographs of the spore coats of *B. subtilis*, *B. megatherium* and *B. cereus* are shown in Figs. 1–4. Differences in texture and mode of rupture are apparent. The coats of resting *B. megatherium* spores (Fig. 3) appear much more opaque and rough than those of the germinated forms (Fig. 4). In Fig. 4 it can also be seen that the spore coat of *B. megatherium* has two layers, as shown by Robinow (1953).

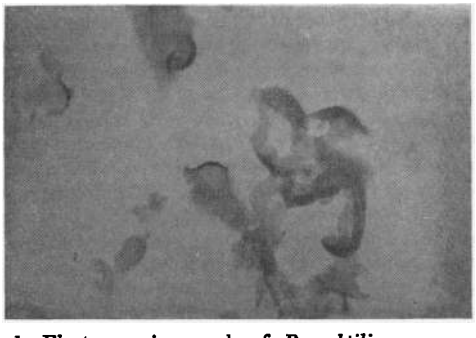

Fig. 1. Electron micrograph of *B. subtilis* spore coats. Magnification, ×8000.

Analyses of the spore coats of B. megatherium, B. subtilis *and* B. cereus

Spore coats of the three organisms gave strong positive biuret and positive Molisch reactions. Table 1 shows the results of analyses of spore coats. α-Amino acid nitrogen and hexosamine were

Fig. 2. Electron micrograph of *B. cereus* spore coats shadowed with gold–palladium alloy. Magnification, ×8000.

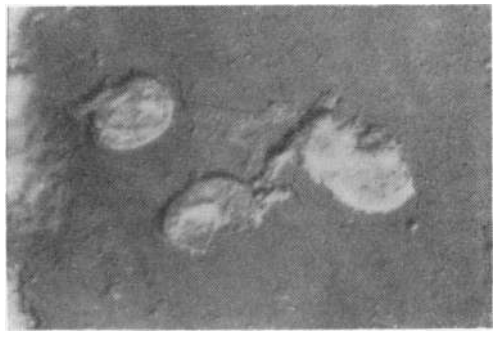

Fig. 3. Electron micrograph of *B. megatherium* spore coats shadowed with gold–palladium alloy. Magnification, ×8000.

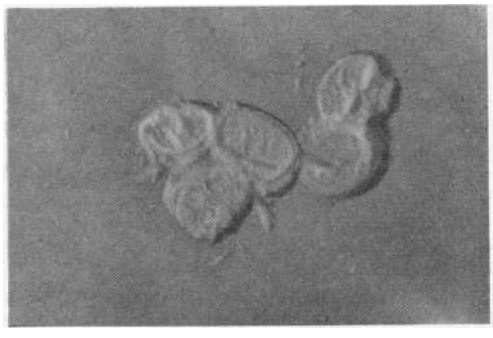

Fig. 4. Electron micrograph of coats of *B. megatherium* pre-vegetative forms shadowed with gold–palladium alloy. Magnification, ×8000.

estimated after hydrolysis with 6·5N-HCl for 18 hr. at 106° in sealed tubes. The results for hexosamine are reported in terms of glucosamine and were obtained by measurement of the Elson & Morgan colour at 530 mμ., which is the optimum for the known amino sugars. Measurements were also made at 520 mμ., which is the optimum for the unidentified amino sugar present in the spore peptides (Strange & Powell, 1954). The value for $-\log T_{530\ m\mu.}/-\log T_{520\ m\mu.}$, where T represents the

The amount of lipid found in acid-hydrolysed spore coats was small. With *B. subtilis*, whole spores were investigated with regard to the possibility that an outer layer of lipid was present which was lost on disintegration. Dried, viable, resting spores were extracted successively for several hours with ethanol and ether in a Soxhlet apparatus. On drying the combined extracts under reduced pressure, an insignificant residue remained. The spores remained viable.

Table 1. *Composition of spore coats of* B. megatherium, B. subtilis *and* B. cereus (*g.*/100 *g.*)

	Source of coats			
	B. megatherium resting spores	*B. megatherium* pre-vegetative forms	*B. subtilis* resting spores	*B. cereus* resting spores
Total nitrogen	12·8	13·4	13·1	13·2
α-Amino-N (ninhydrin–CO_2)	5·6	6·3	8·2	9·5
Hexosamine	10–15 (0·98)*	8–10 (0·97)	1–2 (0·96)	2–3 (1·07)
Total P	0·3	0·4	1·6	1·2
Total carbohydrate as glucose	1·0	0·8	1–4	4
Sulphated ash	1·4	1·0	2·8	2·8
Lipid (after acid hydrolysis)	—	0·95	1·1	0·9

* Figures in parentheses give the value $-\log T_{530\ m\mu.}/-\log T_{520\ m\mu.}$ of the Elson & Morgan colour.

percentage transmission, is given in Table 1 and is to be compared with that of glucosamine, which is 1·08 and 0·9 for the purest sample of the unidentified amino sugar so far obtained. It appeared that *B. megatherium* and *B. subtilis* spore coats contained both glucosamine and the unidentified amino sugar, whereas *B. cereus* spore coats contained glucosamine only. Although the total nitrogen content was similar in all the spore-coat preparations examined, α-amino acid nitrogen was lower and hexosamine nitrogen higher in those from *B. megatherium* than in the other two organisms. In no case was all the nitrogen of spore coats accounted for as α-amino and hexosamine. Spore coats were examined for the presence of nucleic acids as follows: a sample (20–100 mg.) was treated with 5 ml. of 5 % trichloracetic acid for 15 min. at 90° (Schneider, 1945) and the suspension centrifuged. The supernatant fluid was removed and the deposit treated with a further 5 ml. of 5 % trichloroacetic acid as before. The combined supernatant fluids were filtered through sintered glass (5/3) and examined for ultraviolet light absorption and deoxyribose content. Compared with a standard of sperm deoxyribose nucleic acid (DNA) the amount of nucleic acid present in extracts of *B. megatherium* and *B. subtilis* coats was equivalent to less than 0·5 %, and, for *B. cereus*, less than 1 % of the weight of material used. After acid hydrolysis, followed by paper chromatography, most of the common amino acids were found in all the preparations. In addition, *B. megatherium* coats contained considerable amounts of glucosamine, the unidentified amino sugar and αε-diaminopimelic acid.

Release of hexosamine-containing peptide from spore coats of B. megatherium

It was observed that when coats of resting or germinated spores of *B. megatherium* were left suspended in distilled water, soluble hexosamine-containing material was released. The hexosamine was liberated from this material by hydrolysis with 6N-HCl. From these suspensions a non-dialysable peptide was isolated in the same way as from spore extracts (Strange & Powell, 1954), and after acid hydrolysis it was found to contain glucosamine, the unidentified amino sugar, alanine, glutamic acid, αε-diaminopimelic acid and acetic acid. A similar further release of peptide occurred when intact germinated spores of *B. megatherium* were suspended in water. The rate of release of peptide from spore coats depended on the pH of the suspending medium. Thus, weighed amounts (10 mg.) of dried *B. megatherium* germinated spore coats were suspended in 1 ml. volumes of 0·01N-HCl and various 0·02M buffers up to pH 10·9. The suspensions were incubated in stoppered tubes at 37° for 40 hr. and centrifuged. The supernatant fluid was removed and the deposit washed twice with small volumes of water. The supernatant fluids and washings were dried at 80° and hydrolysed in sealed tubes with 6·5N-HCl at 106° for 18 hr. Hexosamine was determined in the hydrolysates after neutralization and the results are given in Table 2. Maximum liberation of peptide occurred at pH 5·0–8·0.

Liberation of peptide at pH 7·0 was unaffected by the presence of chloroform, mM-NaCN or

24 mM-NaF. When the spore-coat preparations were heated at 100° for 30 min. in distilled water and then suspended in buffer at pH 7, liberation of hexosamine-containing material still occurred, but in reduced amount.

Spontaneous release of peptide did not appear to be due to slow leaching out of material from inside the spore coats. When these were further disintegrated into small, unidentifiable fragments, then washed and suspended in buffer, peptide was released in the same way.

and to a small extent with trypsin, pepsin and lipase (Table 3). As the amount of peptide associated with *B. megatherium* spore coats is considerably greater than with *B. subtilis*, values for liberated hexosamine for *B. megatherium* are higher throughout the series. The results with *B. cereus* coats showed that only very small amounts of peptide were liberated but that some enzymes increased the rate of liberation slightly. As spore coats gave a positive biuret reaction the effect of trypsin in higher concentration and at lower pH

Table 2. *Spontaneous liberation of combined hexosamine from spore coats of* B. megatherium *suspended in solutions at various* pH *values and incubated for* 40 *hr. at* 37°. *Results expressed as* μg. *of glucosamine/* 10 *mg. dry wt. of spore coats*

pH*	2·2	3·05	4·0	5·0	6·95	7·95	8·8	10·0	11·0
Hexosamine in supernatant (μg.)	23	30	39	53	69	61	40	15	17

* 0·02 M buffers used except at pH 2·2 (0·01 N-HCl).

Table 3. *Liberation of combined hexosamine from spore coats on incubation with enzymes for* 48 *hr. at* 37°

Results expressed as μg. of hexosamine/10 mg. dry wt. of spore coats.

pH*	7·6			4·5			5·3			9·3		
Enzyme	*Papaya* extract			*Ficus* extract			Lysozyme			Alkaline phosphatase		
Source of coats	Test	Control	Diff.	Test	Control	Diff.	Test	Control	Diff.	Test	Control	Diff.
B. megatherium pre-vegetative forms	342	108	234	124	39	85	236	47	189	164	51	113
B. subtilis resting spores	66	10	56	37	6	31	46	21	25	43	8	35
B. cereus resting spores	10	4	6	18	4	14	—	—	—	34	12	22

pH*	9·2			2·1			8·9		
Enzyme	Trypsin			Pepsin			Lipase		
Source of coats	Test	Control	Diff.	Test	Control	Diff.	Test	Control	Diff.
B. megatherium pre-vegetative forms	100	82	18	87	88	0	97	58	39
B. subtilis resting spores	25	9	16	18	5	13	7	5	2
B. cereus resting spores	8	5	3	22	5	17	—	—	—

* 0·02 M buffers used except with pepsin (0·01 N-HCl).

Effect of enzymes on the liberation of peptide from spore coats

For these experiments, duplicate weighed amounts of dried spore coats were suspended in 1 ml. of buffers of appropriate pH values (Table 3) and enzyme was added to one of them. After incubation at 37° for 48 hr. the suspensions were centrifuged, the supernatant fluids and washings dried at 80° and analysed for hexosamine content. No significant amount of hexosamine was found in acid hydrolysates of any of the enzymes themselves at the concentrations used. With *B. megatherium* and *B. subtilis* spore coats there was increased liberation of hexosamine-containing material during treatment with lysozyme, *Papaya* extract, *Ficus* extract and alkaline phosphatase was studied. Spore coats of *B. megatherium* (10 mg.) were suspended in 1 ml. of 1:4 McIlvaine's buffer (pH 8·0) containing 50 μg. of trypsin for 24 hr. at 37°. The suspension was centrifuged and the deposit treated with 1 ml. of buffer + enzyme as before. The process was repeated, giving a total treatment period of 72 hr. The difference between the hexosamine content of the combined supernatants and that found in control without enzyme was 26 μg. A control solution of buffer and enzyme retained activity for 72 hr., as shown by its rapid effect on a gelatin film. After thorough washing with distilled water, the treated coats gave a strong positive biuret reaction and their microscopic appearance was found to be little changed.

The progressive effect of lysozyme on the liberation of combined hexosamine from *B. megatherium*

resting-spore walls was shown by the following experiment. Duplicate amounts (30 mg.) of coats were suspended in 1 ml. of 1:10 McIlvaine's buffer (pH 5·3), and 60 μg. of lysozyme was added to one. Both suspensions were incubated at 37° for 24 hr. and centrifuged. The supernatant fluid was removed and replaced with fresh buffer and enzyme as before. This process was repeated over a period of 10 days and the supernatants were dried at 80°. Analyses of these (for hexosamine) after acid hydrolysis showed that the liberation of hexosamine-containing material was speeded up by lysozyme (Fig. 5), but the total amount of hexosamine liberated was very similar in each case (with

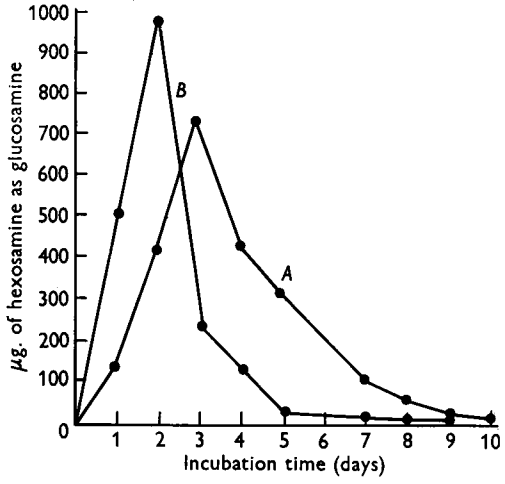

Fig. 5. Rate of liberation of combined hexosamine from 30 mg. of *B. megatherium* spore coats: *A*, in 0·02M phosphate/citric acid buffer, pH 5·3; *B*, in buffer + lysozyme (60 μg.).

lysozyme, 1·86 mg.; control, 1·96 mg.). Thus about two-thirds of the hexosamine initially present in the coats was liberated as a soluble complex in each case.

Effect of lysozyme on spore peptide

Because lysozyme increased the rate of liberation of combined hexosamine from spore walls, its effect on spore peptide was studied. When *B. megatherium* spore-exudate peptide was used, the following results were obtained:

Reducing power. A 0·25% (w/v) solution of peptide in 0·9% saline was incubated at 37° for 2 hr., together with a similar solution containing lysozyme (0·1 mg./ml.). Reducing power expressed as glucose of the lysozyme treated solution increased by 2·7%.

Acetylhexosamine. For estimation of combined acetylhexosamine, the optimum time of heating the solution of the material with Na_2CO_3 has to be found (Aminoff, Morgan & Watkins, 1952). For solutions of spore peptide this was 15 min. On the solutions used for reducing-power estimation, acetylhexosamine was nil before and 0·9% after treatment with lysozyme (as acetylglucosamine).

Diffusion through cellophan. B. megatherium spore-exudate peptide has an average molecular weight of 15300 (Record & Grinstead, 1954), and when solutions of it are dialysed in cellophan sacs little diffuses through. A 4% (w/v) solution in 1:10 McIlvaine's buffer containing 0·4% (w/v) NaCl, of pH 5·3, was treated with lysozyme (1·0 mg./ml.) for 24 hr. at 37°. Fresh lysozyme was added (0·5 mg./ml.) and the solution incubated for a further 24 hr. A similar solution of peptide without lysozyme was incubated for the same period. The two solutions were transferred to cellophan sacs and dialysed against several changes of distilled water for 3 days at 2°. After freeze-drying it was found that 90% of the control material had remained in the sac, whereas only 54% of that treated with lysozyme was recovered. Sedimentation coefficients of these materials in a Spinco analytical ultracentrifuge were 1·18 and 0·77 Svedberg units respectively.

Viscosity measurements. A 1% (w/v) solution of peptide was incubated for 2 hr. at 37° with lysozyme (0·1 mg./ml.) with a similar control solution without enzyme. Flow times in a micro-viscosimeter (Ostwald) of the control and test in a water bath at 25° were 176 and 157 sec. respectively. That of 0·9% saline containing lysozyme (0·1 mg./ml.) was 137 sec.

Similarity between the aminopolysaccharide present in M. lysodeikticus *and spore peptide*

The crude lysozyme substrate prepared from *M. lysodeikticus* by us contained less hexosamine than that prepared by Meyer & Hahnel (1946) and was relatively insoluble in slightly acid solutions. On acid hydrolysis, followed by paper chromatography, it was found to contain relatively large amounts of glutamic acid, alanine, lysine, glucosamine and an unidentified amino sugar. The last substance behaved in a manner identical with that present in spore peptide, i.e. it occupied a similar position on phenol/collidine chromatograms, on which it reacted with ninhydrin, ammoniacal $AgNO_3$ and Partridge's (1948) modification of the Elson & Morgan reagent and on ascending paper chromatography, *tert.*-butanol:6N-HCl:water (60:1:29 by vol.) being used as solvent. No $\alpha\epsilon$-diaminopimelic acid was detected.

The increases in reducing power and in acetylhexosamine after treatment of this material with lysozyme under the conditions described for spore peptide were 6·7 and 2·4% respectively.

DISCUSSION

The preparations studied in this investigation appeared to consist essentially of spore integuments as far as could be judged by electron-microscope examination. It appeared at least possible with *B. subtilis* and *B. megatherium* that the hexosamine-containing peptide was associated with the spore coat. With *B. cereus*, it seemed that the peptide was readily and completely released during disintegration. The breakdown of an insoluble peptide complex either by depolymerization or detachment from some other constituent of the spore coat may well be one of the first steps of the germination process, preceded perhaps by activation of an enzyme system with an action similar to that of lysozyme (see below).

It is interesting to compare the composition of the spore peptide with that of the cell-wall constituent of *Corynebacterium diphtheriae* isolated by Cummins & Harris (1954). This material also contained glutamic acid, alanine, $\alpha\epsilon$-diaminopimelic acid, glucosamine and an unidentified sugar amine with similar properties to those of the unidentified constituent of the spore peptide.

The microscopic appearance of the spore-coat preparations was unaffected by the action of the enzymes tested, but lysozyme, *Papaya* extract, *Ficus* extract and alkaline phosphatase increased the rate of liberation of hexosamine-containing material. The crude preparations of ficin and papain almost certainly contained some lysozyme (Meyer, Hahnel & Steinberg, 1946), and it is possible that some was present in the alkaline-phosphatase preparation (Goldsworthy & Florey, 1930). It is most likely, therefore, that the release of hexosamine–amino acid complex was due not to the splitting of peptide bonds by proteinases in the crude extracts but to the action of lysozyme. This is supported by the finding that trypsin, which may or may not be a pure enzyme, and pepsin had little or no effect compared with lysozyme. It was interesting to find that lysozyme did not attack resting spores, suggesting that the peptide substrate was either not present at the surface or that it was present in some resistant form which became susceptible to attack during germination or disintegration.

The action of lysozyme on spore-coat preparations led us to consider the possibility of a similarity between lysozyme substrate isolated from *Micrococcus lysodeikticus* (Epstein & Chain, 1940; Meyer & Hahnel, 1946) and the spore peptide. Salton (1952) concluded that the cell walls of *M. lysodeikticus* consisted of a highly polymerized mucopolysaccharide which was the lysozyme substrate, but we used instead the method of Meyer & Hahnel for extracting substrate from whole organisms in an attempt to obtain a water-soluble material more analogous to the spore peptide. The authors mentioned, and Salton (1954), have shown that the action of lysozyme on the substrate was to increase reducing power, decrease viscosity and liberate groups reacting as acetylhexosamine, and similar results were obtained with spore peptide. Although the substrate from *M. lysodeikticus* was impure, its composition as revealed by paper chromatography showed some striking similarities to that of the spore peptide. Thus it contained relatively large amounts of the same constituents with the exception of $\alpha\epsilon$-diaminopimelic acid which would appear to be replaced by lysine. Epstein & Chain (1940) reported that 46 % of their material was dialysable after treatment with lysozyme, and it is interesting to note that a similar result was obtained with the spore peptide. Since the latter consisted of one component according to available physical criteria, it must be assumed that the action of lysozyme ceases after the molecules of peptide are reduced to a certain size. This is in agreement with the findings of Salton (1954), who showed that the action of lysozyme on the isolated cell walls of certain lysozyme-sensitive bacteria was to liberate material of which the major component had a molecular weight of between 10 000 and 20 000.

SUMMARY

1. Coats isolated from spores of *Bacillus megatherium* and *Bacillus subtilis* after mechanical disintegration contain an insoluble hexosamine complex of characteristic composition. Traces of this complex are found in *Bacillus cereus* spore coats.

2. This complex is liberated in a soluble form when spore coats are suspended in distilled water or buffer solutions, maximum liberation occurring between pH 5·0 and 8·0.

3. Lysozyme accelerates the rate of liberation of the complex but then it is in a modified form.

4. The composition of the complex is similar to that of the mucopolysaccharide present in cells of *Micrococcus lysodeikticus*.

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The Effect of Magnesium and Calcium Ions on Adenosine Triphosphatase in the Nervous and Vascular Tissues of the Brain

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During the course of histochemical work on the distribution of adenosine triphosphatase in brain tissue, it has been found that the enzyme is present in both the vascular and the nervous elements (Naidoo & Pratt, 1951). It is therefore considered valuable, in the study of properties of brain adenosine triphosphatase, to take account of the existence of the enzyme in tissues of widely different embryological origins within the same organ. Since the brain is well supplied with blood vessels, the proportion of mesodermal tissue is substantial.

A detailed study of brain-adenosine triphosphatase activity was made by Gore (1951). Enzyme activity showed two maxima with variation of pH—one at pH 7·4 and the other at pH 8·2. The enzyme was activated by magnesium but inhibited by calcium ions. There is some variation in the properties ascribed to the enzyme by different workers. In previous histochemical work optimum activity has been found at a lower pH, 6·5 (Naidoo & Pratt, 1951). Some workers report activation of brain adenosine triphosphatase by magnesium ions, with inhibition or no activation by calcium ions (Epelbaum, Sheves & Kobylin, 1949; Gordon, 1950, 1953; Lowry, Roberts, Wu, Hixon & Crawford, 1954; Cseh, Hermann & Zombori, 1954). On the other hand, the enzyme has been reported as activated by calcium ions (DuBois & Potter, 1943; Feldberg & Mann, 1945). The work of Gore (1951) suggests that there are adenosine triphosphatase fractions in brain which differ in solubility in various aqueous media. According to Lowry *et al.* (1954) brain adenosine triphosphatase releases only the terminal phosphate group and is inhibited by adenosine diphosphate.

The purpose of the present work is to investigate the extent to which the properties of adenosine triphosphatase are likely to depend on the cytological origin of the enzyme, and whether there is any evidence to suggest that some of the inconsistencies in the reported properties of the enzyme are in fact due to its origin from both the vascular and the nervous tissue. This paper reports a study, by histological and by quantitative methods, of the comparative effect of magnesium and calcium ions on the enzyme of the vessels, on the one hand, and on that of the nerve and glial cells, on the other.