

TABLE I

INCREASE IN DRY WEIGHT OF PROTOPLASTS AND
INTACT CELLS

| Incubation time hours | Suspension density | |
|-----------------------------|-----------------------|----------------------|
| | Intact cells μg/ml | Protoplasts μg/ml |
| 0 | 104 | 82 |
| 2 | 234 | 172 |
| 4 | 524 | 248 |

Suspensions of intact cells or protoplasts were incubated with gentle aeration at 28°C in a medium containing glucose and peptone with sucrose as stabiliser.

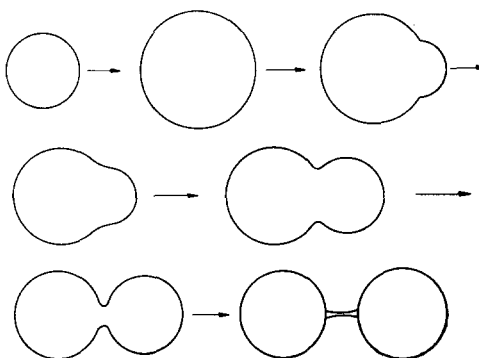


Fig. 3. Probable sequence of forms during the growth and division of protoplasts of *Bacillus megaterium*.

it was noticed that after about 6 hours' incubation there appeared non-spherical forms among the greatly enlarged protoplasts. As time went on these developed into dumb-bell shaped bodies such as appear in Figs. 1 and 2. The probable sequence of forms is represented diagrammatically in Fig. 3. Identical findings have been made on several occasions and there is every reason to believe that these sub-cellular preparations can grow and divide.

A fuller account of this work will appear later.

The author is grateful to Mr. R. W. HORNE for making the electron micrographs.

KENNETH MCQUILLEN

Department of Biochemistry, University of Cambridge (England)

¹ K. MCQUILLEN, *Biochim. Biophys. Acta*, 17 (1955) 382.

² K. MCQUILLEN, *J. Gen. Microbiol.*, 13 (1955) iv.

³ M. R. J. SALTON AND K. MCQUILLEN, *Biochim. Biophys. Acta*, 17 (1955) 465.

⁴ S. BRENNER AND G. S. STENT, *Biochim. Biophys. Acta*, 17 (1955) 473.

⁵ M. R. J. SALTON, *J. Gen. Microbiol.*, 13 (1955) iv.

⁶ C. WEIBULL, *J. Bacteriol.*, 66 (1953) 688.

⁷ C. WEIBULL, Characterization of Bacterial Protoplasts, in *Bacterial Anatomy*, ed. E. T. C. SPOONER, Cambridge University Press, Cambridge, England (in press).

⁸ K. MCQUILLEN, Capabilities of Bacterial Protoplasts, in *Bacterial Anatomy*, ed. E. T. C. SPOONER, Cambridge University Press, Cambridge, England (in press).

⁹ K. MCQUILLEN AND M. R. J. SALTON, *Biochim. Biophys. Acta*, 16 (1955) 596.

Received August 9th, 1955

The binding of sulfate and phosphate ions by salmine

In an earlier report¹ on the molecular weight of the protamine, salmine, it was noted that the sedimentation constant of the phosphate salt is appreciably higher than that of the chloride salt. As reported, this indication of phosphate ion binding was confirmed by conductivity and electrophoresis measurements, and was found to exist in the case of the sulfate salt also. Recently GOLDWASSER AND MATHEWS² reported the binding of sulfate ion by mucoproteins and bovine serum albumin. In view of the paucity of sulfate binding data noted by these authors, and the general interest in all types of protein-ion binding, we felt it desirable to report in more detail our preliminary studies of salmine. While the binding of multivalent anions by proteins may be a phenomenon of some importance, the binding by salmine of phosphate, in particular, may have special significance in the problem of the linkage between protamine and nucleic acid in the nucleoprotamine. The physical constants submitted here are not to be accepted as final values, since we have evidence that commercially prepared salmine, at least, is not homogeneous. The problem of the heterogeneity of salmine is currently being investigated in this laboratory.

A commercial sample of salmine sulfate, obtained from Krishell Laboratories, Inc., was dissolved in water and put through an anion exchange column to convert it to the free base. Samples of the base were then titrated to pH 8.5 with 1 N HCl, H₂SO₄ and H₃PO₄ to convert them to the corresponding salts. Conductivities of these solutions and the free base were measured

in a conductivity cell with sealed platinum electrodes at 25° C. Mobility measurements were made in an Aminco-Stern electrophoresis apparatus at 1.2° C on solutions which had been dialyzed against buffers which were 0.2 μ in an appropriate salt, *i.e.*, 0.2 M KCl, 0.2 μ K_2HPO_4 and 0.2 μ Na_2SO_4 for the salmine chloride, phosphate and sulfate, respectively. All three buffers for electrophoresis contained 0.005 μ veronal and had a pH of 8.5. The samples used for conductivity and mobility determinations were all about 2% in salmine concentration. Viscosity measurements were made in an Ubbelohde type viscometer on both salt-free and salt-containing solutions. The concentrations used in the viscosity experiment were determined by Kjeldahl analysis. We have determined the per cent of nitrogen in salmine chloride to be 26.8 and in salmine phosphate to be 26.2.

Table I contains the descending mobilities and the conductivities of the salmine compounds investigated. The mobility data show qualitatively that sulfate and phosphate ions are bound much more than chloride ions. A quantitative measure of binding cannot be made by conventional methods since the very high isoelectric point of salmine ($2/3$ of the amino acid residues are arginine) makes it difficult or impossible to make measurements around the isoelectric pH. Also we have found that the compound undergoes a degradation when left at a high pH.

TABLE I

| Compound | Mobility $\times 10^5$ ($cm^2 volt^{-1} sec^{-1}$) | Conductivity $\times 10^3$ (ohms $^{-1} cm^{-1}$) | Calculated anion conductivity $\times 10^3$ (ohms $^{-1} cm^{-1}$) |
|-------------------|---|---|---|
| Free base | | 8.1 | |
| Salmine chloride | + 8.0 | 4.8 | 4.0 |
| Salmine sulfate | + 2.5 | 2.0 | 3.6 |
| Salmine phosphate | + 2.9 | 1.6 | |

The conductivity data are more difficult to interpret, since the contribution of the anion to the total conductivity varies from one anion to the other. In order to lend more significance to the experimental conductivity data, we have calculated the contributions of the anions to the total conductivity for the chloride and sulfate salts. These were calculated according to the equation

$$K = \frac{c \lambda}{1000}$$

where K , the conductivity, is in ohms $^{-1} cm^{-1}$, c , the concentration, is in g equiv/l and λ , the equivalent anion conductance, is in ohm $^{-1} cm^2/g$ equiv per ml. The concentration of anions, obtained from the titration data, was $6.0 \cdot 10^{-2}$. The equivalent anion conductances for this concentration were calculated from published data on the equivalent conductances and transference numbers of various concentrations of NaCl and $1/2$ Na_2SO_4 .³ In this way an equivalent conductance of 67 was obtained for the chloride ion and 60 for the $1/2$ sulfate ion. Using these values the anion conductivities in column three of Table I were calculated. It is seen that while the measured conductivity of the salmine chloride is larger than that of the anion conductivity alone, in the case of the sulfate the experimental conductivity is appreciably less than that of the calculated value. This lowering is due to two factors, first, there are fewer free anions to conduct and second, the salmine ion is conducting less current since part of its charge has been neutralized by bound anions.

If the chloride ion is much less bound, as the conductivity and mobility data indicate, then certain predictions can be made about the viscosity behavior of salmine chloride. Dissolved in water, it should display polyion characteristics, that is, the reduced viscosity should increase with dilution but decrease on addition of free salt. Table II, which compares the viscosity of the chloride and phosphate salts, shows that salmine chloride indeed behaves like a polyelectrolyte in this respect. The relative insensitivity of the phosphate salt to dilution and salt addition is further proof that the positive charges of salmine have been greatly neutralized by the bound phosphate ions.

TABLE II

| Salmine salt | Solvent | Concentration (g. dl. $^{-1}$) | η_{sp}/c (dl. g. $^{-1}$) |
|--------------|---------------------|------------------------------------|------------------------------------|
| Chloride | Water | 1.91 | 0.062 |
| Chloride | Water | 1.09 | 0.067 |
| Chloride | Water | 0.761 | 0.070 |
| Chloride | 0.2 M KCl | 1.30 | 0.047 |
| Chloride | 0.2 M KCl | 0.759 | 0.048 |
| Phosphate | Water | 1.70 | 0.055 |
| Phosphate | Water | 1.05 | 0.056 |
| Phosphate | 0.2 μ phosphate | 0.802 | 0.054 |

Laboratory of Physical Biology, National Institutes of Health,
Bethesda, Md. (U.S.A.)

M. J. CALLANAN
W. R. CARROLL
E. R. MITCHELL

¹ W. R. CARROLL, M. J. CALLANAN AND E. R. MITCHELL, Abstracts of Papers, *Am. Chem. Soc.* 126th meeting, Sept., 1954, p. 87C.

² E. GOLDWASSER AND M. B. MATHEWS, *J. Am. Chem. Soc.*, 77 (1955) 3135.

³ See D. A. MACINNES, *The Principles of Electrochemistry*, Reinhold Publishing Corporation, 1939, pp. 339, 85.

Received August 9th, 1955

BOOK REVIEWS

Outlines of Enzyme Chemistry. Von J. B. NEILANDS UND PAUL K. STUMPF, mit einem Kapitel über die Synthese der Enzyme von ROGER Y. STANIER, John Wiley and Sons, Inc., New York, 1953, x + 315 Seiten, \$ 6.50.

Das ständig wachsende Interesse für die Lehre von den Enzymen hatte in den letzten Jahren das Erscheinen zahlreicher Darstellungen des Gebietes zur Folge, unter denen wir Handbücher, umfangreichere Lehrbücher und kürzere Einführungen finden. Das vorliegende Werk von NEILANDS UND STUMPF gehört zur letztgenannten Kategorie; wie im Vorwort ausgeführt wird, will es den fortgeschrittenen Studenten in die allgemeine Enzymologie einführen und auch dem Forscher auf benachbarten Gebieten das für ihn notwendige enzymologische Rüstzeug vermitteln.

Die noch verhältnismässig jungen Autoren sind den Fachkollegen bereits durch eine Anzahl interessanter Experimentalarbeiten wohlbekannt. Durch das vorliegende Buch haben sie auch einen beachtlichen Beweis ihres didaktischen Geschicks gegeben. Wir finden eine ausgezeichnete Gliederung, eine meist glückliche Auswahl des Materials und auch, dass in vielen Fällen neueste Erkenntnisse berücksichtigt werden. Der europäische Leser wird vielleicht durch die besonders starke Betonung physikalisch-chemischer Gesichtspunkte überrascht werden; diese spiegelt aber eine zur Zeit in den angelsächsischen Ländern vorherrschende Tendenz wieder, die ja auch in den kürzlich abgehaltenen Symposien über den Mechanismus der Enzymwirkung (Johns Hopkins University) und über die physikalische Chemie der Enzyme (Faraday Society) ihren Ausdruck fand. Immerhin haben die Autoren auch biologische Gesichtspunkte der Enzymologie nicht ganz vernachlässigt; wir finden in den späteren Kapiteln des Buches klare Darstellungen des Zusammenwirkens der Enzyme in wichtigen Prozessen des Intermediärstoffwechsels. Der Referent vermisst hier allerdings jeden Hinweis auf den Vorgang der Blutgerinnung, der doch ohne Zweifel ein wichtiger enzymkatalysierter Prozess ist, ebenso werden die Vorgänge der Verdauung ziemlich vernachlässigt. Auch ein etwas ausführlicher Hinweis auf die Beziehungen zwischen Genen und Enzymen wäre erwünscht.

Zusammenfassend darf jedoch gesagt werden, dass das Werk, welches sich auch durch guten Druck und gefällige Aufmachung auszeichnet, für den eingangs genannten Leserkreis sehr geeignet ist. Es kann aber auch dem engeren Fachkollegen, der sich mit den modernen Auffassungen in der Enzymologie auseinandersetzen will, empfohlen werden.

O. HOFFMANN-OSTENHOF (Wien)

The Life of Bacteria. Their Growth, Metabolism and Relationships, by KENNETH V. THIMANN, Professor of Biology, Harvard University. The Mac Millan Company, New York, 1955, 775 pages, numerous illustrations, \$ 13.50.

In recent years several textbooks on bacteriology have appeared. The book by the well-known professor of biology at Harvard University, KENNETH V. THIMANN differs from these in many respects. It is obvious that the writer has entered the bacteriological field with the spirit of the biologist who wishes to acquaint himself with another interesting group of living organisms. This implies that in composing the book a serious attempt has been made to write a complete life-history of the bacteria. Accordingly morphology, cytology and physiology have enjoyed an equal share of the author's attention, and phenomena of general biological interest such as growth, adaptation, heredity, mutation and evolution have also been duly treated. When we read in the Preface that the book has been in preparation for more than fifteen years it is at once clear that we should welcome it as the result of a labour of love. It is this attitude of the author towards his subject which is a characteristic feature of the book, and which greatly adds to its attractiveness. In view of the author's devotion to his subject it is not surprising that, in several places,