★ *Fifteenth Symposium of the Society for General Microbiology* ★

FUNCTION AND STRUCTURE IN MICRO-ORGANISMS

M. R. POLLOCK & M. H. RICHMOND

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CONTRIBUTORS

BRITTEN, R. J., Carnegie Institution of Washington, Department of Terrestrial Magnetism, Washington, U.S.A.

BURGE, R. E. AND HOLWILL, M. E. J., Department of Physics, Queen Elizabeth College, University of London.

FITZ-JAMES, P. C., University of Western Ontario, Faculty of Medicine, London, Ontario, Canada.

HALVORSON, H. O., Department of Bacteriology, University of Wisconsin, Madison, Wisconsin, U.S.A.

HAYES, W., M.R.C. Microbial Genetics Research Unit, Hammersmith Hospital, London.

HOLTER, H., Carlsberg Laboratorium, Physiological Department, Copenhagen, Valby, Denmark.

KORNBERG, H. L., Department of Biochemistry, University of Leicester.

LAMPEN, J. O., Institute of Microbiology, Rutgers University, New Brunswick, New Jersey, U.S.A.

LASCELLES, J., Microbiology Unit, Department of Biochemistry, University of Oxford.

MAZIA, D., Department of Zoology, University of California, Berkeley, California, U.S.A.

MCQUILLEN, K., Department of Biochemistry, University of Cambridge.

NEWTON, B. A. AND KERRIDGE, D., Sub-Department of Chemical Microbiology, Department of Biochemistry, University of Cambridge.

ROGERS, H. J., National Institute for Medical Research, Mill Hill, London.

SAGER, RUTH, Department of Zoology, Columbia University, New York, U.S.A.

STACEY, K. A., M.R.C. Microbial Genetics Research Unit, Hammersmith Hospital, London.

WOLPERT, L., Department of Zoology, King's College, Strand, London.

WOODS, D. D., Microbiology Unit, Department of Biochemistry, South Parks Road, Oxford.

EDITORS' PREFACE

There has been great progress during the last 10 years in understanding the mechanisms of biosynthesis of proteins, nucleic acids, carbohydrates and lipids in the living cell; but relatively little is yet known of the orientation in space and time of these molecules and of the systems responsible for their formation. Nevertheless, advances in molecular biology, electron microscopy and the biochemical and physical dissection of cell components have encouraged biologists to speculate on the spatial interrelationships of macro-molecules and on the manner in which the chemical reactions they support and facilitate are co-ordinated. Indeed, it can no longer be considered premature to attempt to relate concepts of form and function, based on observations on the biochemistry and biophysics of the living cell, directly with those arising from studies in morphology and dynamics.

Advances in magnification techniques now begin to enable us to relate the shapes of what can be seen in thin-section electron micrographs to the 3-dimensional configuration of macromolecules as inferred from studies on their chemical and physico-chemical properties.

We even have a fair idea of how large protein molecules, and even larger molecules of nucleic acid, are built up by linear extension. Probably this takes place in most cases from a single growing point and can be regarded, chemically, as a relatively simple process. But what of the 2- and 3-dimensional structures such as cytoplasmic membranes, cell walls, mitotic spindles, mitochondria and all the other numerous organs and organelles upon which the metabolism and reproduction of the cell depend? How do they grow? How, in other words, are they built up in a coherent and fully-functional fashion, and to what extent is the repetitive pattern on which their structures are based rigidly and uniformly adhered to?

Can this type of synthesis be viewed as an extension, in one or two additional planes, of the linear, unidirectional growth undergone by certain proteins and nucleic acids? And if so, how many 'growing points' are there, and what is the chemical, physico-chemical and enzymic basis of the numerous branching points that must be involved?

Or do the component parts come together as previously synthesized macromolecules? And if so, are covalent linkages then forged, and are pre-existent covalent bonds in the macromolecules broken? What guides the specific associations of these components to form the char-

acteristic patterns that emerge? Do the molecules 'find each other' by random collision and adhere to each other simply through complementary surface groupings? Or are there specific guiding and orientating 'foundation' macromolecules whose sole function is to gather the relevant component pieces together in a coherent 3-dimensional structure by means of a pre-formed pattern of complementary specific groupings on their surfaces? What contribution does the form and mutual orientation of such macromolecular structures make to the co-ordination and efficiency of the metabolic processes that take place within or upon them?

More important still, perhaps, is the problem of the genetic continuity of these multi-dimensional structures. To what extent is their form and orientation within the cell rigidly determined by the DNA of the nuclear chromosomes? How often does some extra-nuclear DNA (or RNA) contribute to their genetic control? Is it possible that the form of the growing structure is influenced to some extent by the existing design and properties of the structure itself? In other words, could the pattern in which are associated a number of protein, carbohydrate or lipid molecules (having, individually, structures that are exclusively determined—directly or indirectly—by a nucleic acid code) be influenced, *to some extent*, by the existing molecular pattern upon, or around, or in, which they often appear to conglomerate? And, again, if so, to what degree might the internal or external environment of the cell be able, by its action on such a structure, to exercise a heritable influence—albeit within narrow limits—upon cell function, independently of the control exercised by its DNA?

As with many others of the Society's April Symposia, attempts to deal with problems of this size and complexity may well be considered far too ambitious a project. From time to time there were temptations to restrict its scope and consider only the Bacteria. The absence both of a nuclear membrane and the highly organized mechanism for chromosome partition (mitosis) present in larger organisms, and the apparently direct participation of the DNA in the minute-to-minute metabolic life of the cell differentiates them rather sharply from other types of micro-organism. [After all, from the point of view of function and structure at the cellular level, yeast may have more in common with mammalian liver than with any of the eubacteria: the degree of similarity extends even to the fine chemical structure of their enzymes.] Such a restriction would have eased our own problems and have been logically and conceptually a simpler task for the contributors to handle. But the

Society has always felt the need to fight 'separatist' tendencies amongst its members—whether they be in the form of bacteriological arrogance or movements for virological independence. Microbiology as a discipline is indeed in a rather precarious state. As André Lwoff himself has recently pointed out, 'Quant au mot microbe, il connaîtra encore des temps difficiles; il convient donc de l'entourer de beaucoup de soins et de sollicitude'.* So while it is still recognized as being in existence and in need of support, we felt those temptations should be resisted, and we believe that both this book and the Symposium will have a wider appeal as a result.

In any case, the intention has never been to try and organize a complete review of so vast a subject. The aim was to offer a well-distributed assortment of authoritative articles written in a critically informative but speculative vein with the object of stimulating discussion and provoking new ideas and fresh approaches to work in the field. Fortunately it will be impossible to prove that this, also, was too ambitious. Substantial progress is assured, during the next 10 years, in the understanding of the molecular basis of cell ultra-structure and its physiological and genetical control, in relation to its mechanical, physico-chemical and biochemical functions. It will always be possible to boast that this Symposium has played a vital part in such progress. And there may even be a few who will believe the claim to be justified.

The editors would like to thank contributors most sincerely for their generous co-operation in entering into the speculative spirit of this Symposium, by touching—if only lightly—upon many of the questions referred to above, without seriously having attempted to answer any of them. To do more than this would have been premature—and anyway quite impossible!

Our thanks are also due to Dr Joel Mandelstam for his supplementary editorial aid at a critical juncture, precipitated by a nearly disastrous coincidence of chickenpox, postal strike, and an unusually high proportion of 'intrinsically' late MSS. We are also deeply grateful to Miss Joan Fleming and Miss Sheila Petrie for painstaking and invaluable proof checking and correcting of bibliographical references and format conventions, at all stages of publication.

M. R. POLLOCK
M. H. RICHMOND

National Institute for Medical Research,
Mill Hill, London, N.W.7

* Lwoff, A. (1963). Protistes, Microbes et virus. Remarques sur l'évolution des mots. *Revista de Biologie*, **4**, 55.

D. D. WOODS

It is only as we go to press that news has come of the tragic death of Donald Woods.

Quite apart from the disastrous loss to microbiology as a whole and in particular to our Society, for which he did so much, the impact of his absence will be felt most directly and acutely at this very Symposium. He had advised and encouraged the organizers during the early stages of its planning, and was himself to act as Chairman for the first two sections. Moreover he has provided a charming and beautifully written introductory essay, full of characteristic dry wit and wisdom, which may well be the last article he ever produced. The editors and all other participants are deeply in his debt.

ABBREVIATIONS AND SYMBOLS

Unless otherwise indicated in the text of individual articles, the system recommended by *The Biochemical Journal* in *Suggestions and Instructions to Authors*, revised 1964, also adopted by the *Journal of General Microbiology*, has been applied so far as possible.

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DIFFERENTIATION IN DEVELOPMENT, HEREDITY AND SURVIVAL

THE ARCHITECTURE OF THE MICROBIAL CELL

D. D. WOODS

Microbiology Unit, Department of Biochemistry, University of Oxford

The introduction to a symposium must almost of necessity be a collection of random and rather superficial thoughts; if the organizers have done their work well there remains but little that the opener can consider in depth. He certainly must not steal the thunder of later contributors or debate their views before they have had an opportunity to put them forward. Perhaps one service he can try to perform is to give some indication to the non-specialist of the nature and interrelationships of the specific topics to be discussed.

A designed relationship between structure and function is inherent to all good architecture. This was one reason for the choice of the title of this introductory contribution; the other is a rather remarkable coincidence. October 1632 saw the birth of two great Europeans, one Dutch, one British; they were born within 4 days of one another, each survived to his ninety-first year and they died (in 1723) within 6 months of one another. One was Antony van Leeuwenhoek, universally regarded as the founder of microbiology through his observations of his animalcules. The other was Christopher Wren, perhaps the most famous of British architects. But their lives crossed more directly than this. Christopher Wren, then a professor of astronomy, was one of the group of scientists who founded the Royal Society of London in 1660; it was to this society that van Leeuwenhoek communicated from 1673 onwards a large part of his historic observations; he was elected a Fellow of the Society in 1680, the year that Wren became President. Wren was one of the observers when Robert Hooke in 1677 gave a demonstration of some of van Leeuwenhoek's observations. Van Leeuwenhoek, however, never attended a meeting of the Royal Society in person and there is no record that he ever met Wren.

It is not clear to me why a biochemical microbiologist should have been asked to open the present Symposium. I can only suppose with John Ruskin,* the first Slade Professor of Fine Art in my University:

* It is recorded in the *Dictionary of National Biography* that John Ruskin revoked his bequest to the University of Oxford of paintings by Turner when the University would not provide funds to extend the School of Drawing he had established, but at the same moment voted money for a laboratory in which vivisection took place.

'You know there are a great many odd styles of architecture about you; you don't want to do anything ridiculous; you hear of me, among others, as a respectable architectural man-milliner, and you send for me, that I may tell you the leading fashion.'*

A biochemical microbiologist, at his convenience, wears many hats, sometimes biochemical, sometimes microbiological, sometimes genetical, sometimes even pathological; this is the first time I have worn an architectural one and I do not feel comfortable in it. Furthermore, in the context of John Ruskin, I am disturbed by Christopher Wren's dictum: 'Architecture aims at Eternity; and therefore is the only thing incapable of modes and fashions in its principles.'†

In buildings the correlation of structure and function is a matter of deliberate design. In a micro-organism the design has emerged through the trial and error of mutation and selection. How nearly perfection has been attained is perhaps one of the purposes of this symposium to try to assess. Some of our contributors will in turn be describing the structure and function of the genetic apparatus itself, in which such mutational changes occur. In this field the analysis is reaching the stage of molecular events—the ultimate level of the molecular architecture of the microbial cell. It is at this level too that the process of self-replication is initiated. Although template mechanisms perhaps have their parallel in some modern techniques of prefabrication in building practice, complete self-replication has not been achieved in this field, although a visitor from some other planet who landed, peradventure, in one of our garden cities or housing estates might be forgiven for deducing that it had.

The trial and error of mutation and selection has undoubtedly led to the intracellular structures whose functional activities are beginning to be defined. Yet the successive stages in their evolution are not yet clear. Some of the matters reviewed by Dr Lascelles (p. 32) suggest the cell membrane as a possible ultimate precursor of specialized structures such as chromatophores and mitochondria. Again, we have to trace the evolution from a thread of DNA of separate chromosomes and of a discrete nucleus. By and large we are still at the stage of clearly defining structures within, and bounding, the microbial cell and ascribing to them special functions in the economy of the organism. The advance in knowledge of this kind that has followed the invention of the electron microscope and associated techniques has been dramatic. This will be clear enough both from other contributors and the demonstra-

* *The Crown of Wild Olive*, §53, lect. ii, Traffic.
† *Parentalia.*

tions. Yet there are necessarily limitations, and from some points of view the electron micrograph is as like the living object as is a petrified forest to a living forest with its complexity of form and movement. We still need to add a dimension of time in our techniques.

In investigations of structure we still need gentler methods for taking the organism apart. Although our methods of demolition have become less drastic and more controlled, we are still very far, at the cellular level, from the desirable aim of the true dissection that is essential if possible artifacts of an original harsh treatment are to be excluded.

Consideration of the possible evolution of structures with specialized function within a single microbial cell, or intracellular differentiation, leads naturally to the problem of cellular differentiation into tissues of specialized function. In micro-organisms there is to be found, on the one hand, what is an almost halfway situation between intracellular differentiation and tissue differentiation, that is spore formation and the reverse process of germination. On the other hand, there are very simple types of tissue differentiation into two or three forms of tissue only, as in the slime moulds. Microbiologists therefore have some promising systems to study and progress made from various aspects is summarized by Dr Halvorson (p. 343) and Dr Fitz-James (p. 369).

Some types of microbial locomotion are dependent on the development of specialized organelles, including flagella and cilia (pp. 220, 250). There has been a considerable increase in recent years of knowledge of the fine structure, chemical composition and biochemical activity of such organelles and this, together with physical aspects of locomotion, naturally forms part of our Symposium, as does also the type of locomotion that depends, not on special structures, but on changes in shape of the organism and movement of the fluid contents. I do not know whether we shall learn which organism holds the blue riband of the Petri dish or of the tryptic meat broth pond. Some of the problems here are related to naval or perhaps submarinal architecture. One matter that does not seem to have been discussed much so far is the biological significance of power of movement in organisms of the size of bacteria which are anyway subject to Brownian movement.

Scientists who have been concerned with the design of new laboratories have often felt, I think, that their architectural colleagues have been concerned with a pleasing external appearance even at some sacrifice of the functional requirements of the laboratory as a whole. In modern building practice the external walls frequently serve only a minor structural function, but the wall remains the main item of structural engineering of the micro-organism. Many micro-organisms

are remarkably resistant to mechanical forces, including osmotic pressure; indeed, this is one of the difficulties in taking them to pieces by gentle methods. The nature of the outer layers of bacteria is thus of peculiar interest and Dr Rogers (p. 186) will review the recent great progress in this field. I do not know whether knowledge is yet sufficiently advanced to explain in physico-chemical terms the high tensile strength of the macromolecular complex which forms the bacterial wall; in diatoms there is what might be called a reinforcement of silica. The macromolecular nature of the wall might suggest the possibility of a template type of mechanism in its synthesis. This is not the case, and in any event would be improbable because of the variety of types of molecule involved; in our analogous field of building technology one could scarcely hope to precast as a whole a structure containing, for example, glass, plastic, wood and concrete.

The structural function is, of course, only one of the functions of the outer skins of either a building or a micro-organism. There must also be means of communication between the outside and inside, including provision for the physical passage of commodities. The microbial edifice must surely be the most burglar-proof of any. There are facilities only for the entry of desired materials; specificity of selection is high. Such selection is traditionally assumed to be a function of a cytoplasmic membrane underlying the cell wall, although of course the incoming material will have first to permeate the wall. One of our contributors, Dr Britten (p. 57) will be dealing with the uptake and retention of small molecules. An even more difficult problem is set by the ability of certain large molecules to permeate micro-organisms. While this is achieved (see Dr Holter's contribution (p. 89)) in certain micro-organisms, for example, the amoebae, by a process of invagination (pinocytosis, phagocytosis) both for particles and large molecules, this is not the case in bacteria. Many large molecules are degraded to smaller molecules outside the organism by exocellular enzymes, but a notable exception is deoxyribonucleic acid (DNA) which certainly gains access intact to bacteria which are subject to transformation (modification of the genotype) by DNA preparations from other strains. Recent work suggests that during the limited period of the growth cycle that the organism is transformable there is a temporary interruption to cell-wall synthesis in the case of *Diplococcus pneumoniae* (Ephrussi-Taylor & Freed, 1964) and a temporary breakdown of cell wall in the case of *Bacillus subtilis* (Young, 1964).

Once the small molecules have entered the organism they are used, directly or indirectly, either as sources of energy or as building stones

for the elaboration of larger molecules necessary for the life of the organism. Energy metabolism may be compared with the power equipment of a building—lighting, heating and so on, variously derived from external supplies of electricity or generated internally by the burning of gas, coke or fuel oil. Dr Lascelles (p. 32) traces the structures associated in the cell with energy generation; again it appears that the cytoplasmic membrane is of great significance. Professor Kornberg (p. 8) reviews the interweaving and control of the metabolic pathways generating and utilizing energy. This is a study of the microbial edifice at a dynamic molecular level and a situation which could only have an ordinary architectural analogy in terms of a built-in, do-it-yourself maintenance system coupled with facilities for the prefabrication of units for the construction of an identical building next door!

The metabolic changes essential for the production of energy and of structural units are dependent on the controlled concurrent production of the enzymes which catalyse these changes. This is essentially a matter of protein synthesis that is in turn dependent on nucleic acid synthesis. The fundamental advances in this field both at the molecular and structural level are dealt with by Dr McQuillen (p. 134) and Dr Stacey (p. 159).

With the consideration of the replication of DNA the wheel has turned full cycle and we are back again, this time at the molecular level, with the genome, and to the trial and error of mutation and selection which must have been responsible for the evolution of the highly successful structure/function relationship that is found in present-day micro-organisms. The possible importance of membranes as precursors of other structural entities has already been emphasized and Dr Ruth Sager (p. 324) will discuss, in her contribution on non-chromosomal heredity, the possibility that DNA carried in membrane structures may control the synthesis of functional organelles from the various macromolecules. Dr Hayes (p. 294) and Dr Mazia (p. 379) will cover down to the molecular level the relation of structure to function in the genome.

Successful architecture is more than just a proper relationship between structure and function. If I may return again to my first Slade Professor in Oxford: 'We require from buildings, as from men, two kinds of goodness: first, the doing their practical duty well: then that they be graceful and pleasing in doing it; which last is itself another form of duty.'*

Do we ask also of our microbes such an element of beauty? Even if we do not, we certainly get it. Yet science has become so austere that

* John Ruskin, *Stones of Venice*, vol. II, chapter 6, section 73.

we no longer mention it in our publications. Could we not occasionally let ourselves go? There is something refreshing, and informative even scientifically, about the following passage (Dobell, 1958) from one of Antony van Leeuwenhoek's letters to the Royal Society: '. . . and the whole water seemed to be alive with these multifarious animalcules. This was for me, among all the marvels I have discovered in nature, the most marvellous of all; and I must say, for my part, that no more pleasant sight has ever yet come before my eye than these many thousands of living creatures; seen all alive in a little drop of water, moving among one another, each several creature having its own proper motion.'

I wonder how many present-day microbiologists derive aesthetic enjoyment from observing their organisms with the phase-contrast microscope? I suspect that many do; perhaps we might inquire during this meeting. How many chemical microbiologists who use 'Celite' in their work have ever put a little under the microscope and been entranced by the myriad beautiful forms of the silicious skeletons of the diatoms of which it is composed? It is fascinating to speculate what other masterpieces we might have had if a modern microscope had been available to Leonardo da Vinci.

The subject under review in this symposium is at a particularly lively and fluid state of development. This means also that we must avoid preconceived notions and in particular too great a desire to seek uniformity and analogy with the so-called higher organisms. We do not wish to fall into the position that:

> The stone which the builders refused
> Is become the head stone of the corner.*

Morphologists, geneticists, biochemists and biophysicists are uniting to solve the problems at all levels. Not so very long ago a microbe might have proclaimed:

> I am a little world made cunningly
> Of elements and an angelic sprite.†

I pass you on now to my more sophisticated colleagues who are expert at the identification of sprites.

* Psalm cxviii. 22.

† John Donne, *Holy Sonnets*, v.

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