# THE CELL AND PROTOPLASM

## Symposium at Stanford University

By Prof. C. V. Taylor,
Stanford University, California

THE Symposium on the "Cell and Protoplasm", which was convened at Stanford University, California, during June 30-July 5, began on the last evening of sessions of the Pacific Division of the American Association for the Advancement of Science and closed on the day of registration for the Sixteenth National Colloid Symposium. This time relation would partly account for the large attendance at the Cell Symposium, which increased with each session to an audience of about seven hundred at the closing lecture on "Structural Units" by Prof. J. D. Bernal, of the University of London.

The attendance and growing interest that marked this commemorative Symposium can be accredited primarily to the excellence of the papers presented —all by investigators of merited eminence. Their number included several distinguished biologists whose names have been familiar in the front ranks of biological science for more than a quarter of a century. Among the other participants were those whose more recent physical and chemical analyses of the cell and its protoplasmic constituents have greatly extended the confines of biological knowledge and have doubtless pointed the way for further productive researches of fundamental importance.

In all, thirteen American men of science participated, representing eleven universities and research institutes, and three visitors from Europe: Prof. A. Szent-Györgyi of Hungary, Prof. Hugo Theorell of Sweden and Prof. J. D. Bernal of Great Britain.

Of the papers presented, one each morning, afternoon and evening of the five-day session, the first three were concerned with early and modern concepts of the cell and protoplasm.

In the initial address on "An Historical Account of Cell and Protoplasm Concepts", Prof. E. G. Conklin of Princeton University re-emphasized the social nature of scientific discovery in which the end result of successive achievements of many minds is often given undue recognition. Thus credit for the formulation of the cell theory as commonly accorded to Schleiden and Schwann was believed unwarranted because their accounts had been antedated many years by the published results of various workers, beginning notably with Robert Hooke (1667).

The second paper, by Prof. Robert Chambers of New York University, on "The Micromanipulation of Living Cells", essentially resolved our modern concepts of the living cell as a protoplasmic unit to the unified nature and interrelations of its molecular constituents. As illustrated in a motion picture, the protein constituents of the living protoplasm tend to retain their integrated state in the presence of an engulfed droplet of oil, although upon cytolysis, induced through mechanical disruption of the nucleus or otherwise, a protein 'skin' forms and becomes wrinkled around the oil droplet. This film formation likewise ensues when such a droplet is added to a protein solution on a microscope slide. This integrated protein framework of living protoplasm, which may be demonstrated also by the air-driven ultracentrifuge, strongly indicates a multimolecular organization for the living cell.

Another modern aspect of protoplasmic constitution, gained chiefly through studies on protoplasmic elaborations, was presented in the third paper, by Prof. I. W. Bailey of Harvard University, on "The Cell Wall and Protoplasm". Clearly the structural nature of the cellulose wall laid down by the plant protoplasm should offer invaluable clues to the nature and spatial relations of the protoplasmic constituents. Hence, the regularity of a highly specific and characteristic structural pattern—whether concentric, radial, ramifying or radio-centric—of a given cell wall evidently reflects a potential pattern in the arrangement and orientation of the protoplasmic constituents from which the wall is derived. All evidence thus far indicates that the cellulose matrix of the cell walls of higher plants is a continuous rather than a discontinuous system of anastomosed chain-molecules the long axis of which is oriented parallel to the long axis of the cellulose fibril. They exhibit positive anisotropy and sharply defined extinction angles in monochromatic polarized light. At present, there is no reliable evidence that the structural framework of the cell wall is ever composed of any randomly oriented chain-molecules.

Complementing these three papers on the cell and protoplasm, there followed two on the cell and chromosomes. The first of these was given by Prof. H. S. Jennings of the Johns Hopkins

University on "Chromosomes and Cytoplasm in the Protozoa", and the second by Prof. Richard Goldschmidt of the University of California on "Genes and Chromosomes".

The well-known nuclear cycle that recurs with each cell division during the ontogeny of a multicellular organism was cited by Prof. Jennings as crucial evidence of material interchanges between the nucleus and cytoplasm. Accordingly, each condensed chromosome enlarges during the later phases of mitosis to become vesicular from material taken up from the cytoplasm. Within the resulting contiguous chromosome vesicles which constitute the reformed nucleus, the newly acquired cytoplasmic material is altered and, as such, is returned again to the surrounding cytoplasm upon the subsequent breakdown of the vesicular wall of each chromosome, the residue of which again condenses for the following mitosis. These cyclic interchanges and transformations apparently provide the essential mechanism of cellular differentiations both in the ontogeny of multicellular organisms and in the racial variations of these and of unicellular organisms. As illustrative of the latter, De Garis's recent results from crossing large and small races of Paramecium were discussed. These results showed that the ex-conjugants, having unlike cytoplasms but like nuclei, retained their size differences for about twenty-two generations, whereupon these differences gradually disappeared. Evidently, therefore, the two different cytoplasms were finally transformed by the like nuclei such that the two races of unequal size came to have the same size.

By what mechanism of the nucleus the cyclic modifications, and so racial differences, may be effected was discussed in the succeeding paper by Prof. Goldschmidt. His thesis tended to discount the commonly accepted gene theory of Mendelian heredity, and proposed instead a chromosome theory in which the occurrence of genes as discrete entities, arranged bead-like in a definite order, need not be assumed. Taking into account the similarities in chromosome form and structure in the cells of all organisms, wherein a visible fibril-like core may represent a single protein unit of definite stoichiometric properties along its axis, it would be more in accord with recent X-ray, chemical and polarimetric analyses to identify the chromosome as a chemical unit. Such a unit might show any amount of differential chemical complexity in different chain-molecules of similar length. This concept would ascribe to the chromosome a linear pattern and would account for mutational changes (the bar-effect, mosaicism, position-effect, etc.) as due to changes in the chromosomal pattern (inversions, translocations, duplication of parts, etc.) rather than to changes within discrete particles, or genes, of molecular order.

The demonstrable interrelations between cytoplasm and nucleus which would account for differentiation in both the individual and the race obviously represent but one of the two major components in the fundamental phenomena of living things. The other essential component is, of course, the environment. The role of environmental factors was well exemplified in the three papers that followed on developmental aspects of the cell and its relation to the organism. These included: "Cellular Differentiation and External Environment" by Prof. C. M. Child of the University of Chicago and Stanford University, "Cellular Differentiation and Internal Environment" by Prof. Ross G. Harrison of Yale University, and "Cell and Organism" by Prof. C. A. Kofoid of the University of California.

The external environment of the primordial cell, according to Prof. Child, is a determining factor in its differentiation at the very onset of development. The cell's primordial pattern is essentially a surface-interior pattern which reflects an intimate relationship with its environment. Also, by action of a suitable differential in its external environment, its polar or axiate differentiation is duly determined. Any one or more of various environmental differentials may induce this superimposed axiate pattern, as demonstrated, for example, in Whitaker's experimental studies on the ova of Fucus, as well as in numerous previous experiments by Prof. Child. Accordingly, the axiate pattern arises as a gradient which is initially quantitative in nature and which, once established, constitutes a gradient in rate of metabolic activity. Moreover, this environmentally induced axial or metabolic gradient then conditions the production and transfer of active constituents (chemical substances) in differentiating cells and so predetermines the course of later development.

Factors of differentiation in later stages of early development were discussed by Prof. Harrison as factors of the internal environment. These were illustrated especially from his extensive transplantation experiments on amphibian larvæ. Here the developmental pattern, which has progressed well beyond the initial axiate stage of Prof. Child's account, has demonstrably a primary cellular locus ('organizer') in the region of the dorsal lip of the blastopore and later various secondary loci, which determine organ differentiation throughout ensuing development. Depending upon the age of donor and of host as well as on the piece removed and its disposition where transplanted, the fate of the transplant and its effect upon the organogeny of the host were strikingly illustrated. From this it was evident that the fate and effect of a developing part are a function of its relation to other parts. This fundamental relationship obviously marks the internal environment of cellular differentiation.

In the succeeding paper, presented by Prof. Kofoid, it was emphasized, however, that even were the roles of both genetic and environmental factors of ontogenetic development well understood, that knowledge, essential as it must be, could constitute only part of any adequate understanding of the cell and organism. For the organism, beginning its individuality as a primordial cell, represents in its complete life-history not only the ontogeny that follows its unicellular stage but also the phylogeny preceding that stage. All organisms exhibit in this fundamental respect comparable life histories which may include, even for numerous so-called unicellular forms, a multicellular as well as a unicellular phase. Accordingly, it is only in terms of their total life-history, as an expression of their evolutionary and developmental history, that the cellular organization of living things can have basic significance and so the results of fundamental investigations a satisfactory basis of interpretation.

The two lectures that followed, one on "Chemical Aspects of Microorganisms" by Prof. C. B. van Niel of Stanford University, and the other on "The Structure of Viruses" by Dr. W. M. Stanley of the Rockefeller Institute, marked a transition from considerations of the cell and protoplasm of the more conspicuously cellular organisms to a discussion of the subcellular bacteria and of those ultramicroscopic, reproducing entities, the viruses, whose systematic status, whether animate or inanimate, apparently remains a problem of great moment.

Recognizing Schwann's important contribution not only to the formulation of the cell theory but also to the concept of yeast cells as vital agents of alcoholic fermentation, Prof. van Niel recounted the later developments of that concept, beginning especially with Pasteur, which have now led to a distinctly basic and far-reaching generalization. This generalization affirms that all chemical activities of living organisms are fundamentally hydrogen transfer reactions. Postulated first by Wieland for respiration as essentially a dehydrogenation of the respiratory substrate with oxygen or some other agent as the final hydrogen acceptor, this concept has become expanded by Kluyver and others to its present broadest generalization. Thus enzyme activity in metabolic processes serves primarily to facilitate hydrogen transfer reactions. It now appears that in the catabolic process this leads to the formation of products from which are directly synthesized the building stones of cell growth and differentiation by means of thermodynamically spontaneous reactions. This comprehensive generalization re-emphasizes the processes common to living things as the fundamental processes, the elucidation of which provides our point of departure for an adequate understanding of the more complex vital phenomena.

In this respect, the succeeding discussion on the viruses by Dr. Stanley was distinctly of fundamental significance. It now appears that these entities represent a margin of animate Nature beyond the limits of cellular organization as commonly understood, yet they exhibit properties of organic synthesis and reproduction characteristic of the living cell. Evidently their size relations alone are not definitive, since they are larger than some well-known microbes but several times smaller than some protein molecules. Their apparently complete dependence on a living cell for their reproduction would place them among obligate parasites the nutrient requirements of which are highly specific and, at present, beyond experimental duplication. Their essential nature, however, may have a counterpart in the chromosomal genes of the cell nucleus or in other known protoplasmic constituents such as the enzymes—a relationship which would obviously carry fundamental implications.

Some of the major advances in modern researches on the cell have had to do with its active protoplasmic constituents. Résumés of some recent results were presented on "Enzymes" by Prof. Hugo Theorell of the University of Stockholm, on "Plant Hormones", by Prof. F. W. Went, California Institute of Technology, and on "Vitamins" by Prof. A. Szent-Györgyi of the University of Szeged.

The common theme of these discussions demonstrated the essential relations between these several active constituents. The common role of enzymes in the formation or release of linkages within the carbon chain is referable initially to the prosthetic groups; and for a number of well-known enzymes, the vitamin nature of their active groups is now established. Thus, Prof. Theorell isolated the prosthetic group of the 'yellow enzymes' from the protein component by means of electrophoresis and identified this active group with vitamin $B_2$. It is now known also that vitamin $B_1$, including its pyrophosphate derivative, is identical with the prosthetic group of the enzyme carboxylase, and that the anti-pellagra vitamin is identical with the nicotinic acid amide, which is the essential part of the prosthetic group of various dehydrogenases.

Enzyme specificity, however, is evidently not due to the prosthetic group but to its associated protein molecule—thus denoting a relationship between activating and reacting components of the cell which may come to account fundamentally for all biological specificity. According to Prof. Went, therefore, the more generalized activity of the growth hormones can be attributed to their identity with prosthetic groups. This was well illustrated by the multiple effects of auxin in cell elongation, bud inhibition, root formation and probably other functions inside the plant. The initiation of these growth processes, or their inhibition, is traceable to the effect of diffusing or free-moving auxin on the translocation of other essential growth factors. But the specificity of this effect is inherent in the co-growth factors of the reacting tissues. The production of these essential active groups by some cells, such as those of the growing tip of a coleoptile, and the transport of these groups to other cells of the plant, which through cellular differentiation have lost this producing capacity, afford an excellent illustration of the interdependence of cells and the functional integration of the various differentiated organs. These relationships obviously underlie a unity in the plant organism that is entirely comparable with that in the animal.

These considerations of enzymes and growth hormones clearly indicate the essential nature of the vitamins. As re-emphasized by Prof. Szent-Györgyi, vitamins may be identified with the prosthetic group of certain enzymes, and differ from a hormone chiefly through the accident of nomenclature, according to the source of production. Thus for rats or plants, ascorbic acid is not a vitamin since they themselves are able to synthesize it. In the same sense, thiamin is a vitamin for animals, a hormone in some plants and a vitamin for other plants, depending only on their powers of synthesis. Obviously these relationships give further evidence of the fundamental unity in the plant and animal kingdoms, and, in terms of the enzyme concept noted above, the vitamins constitute an important key to a better understanding of the essential nature of protoplasm and the cell.

The three concluding papers, as originally planned, effectively linked this Symposium with the National Colloid Symposium which directly followed. These included: "The Molecular Structure of Protoplasm" by Prof. O. L. Sponsler of the University of California at Los Angeles, "Protoplasm and Colloids" by Prof. L. V. Heilbrunn of the University of Pennsylvania, and "Structural Units" by Prof. J. D. Bernal of the University of London.

The general concept of the living cell as an organized protoplasmic unit, which was stressed in foregoing discussions, evidently presupposes for its protoplasm a fundamental architecture, that is, an integrated spatial arrangement of the protoplasmic constituents.

An analysis of this architecture was presented by Prof. Sponsler, as based on the known molecular constitution of protoplasm and computed from now fairly well-established dimensions of its protein chains and their linkages through hydrogen bonds. Assuming a degree of protoplasmic homogeneity, it was concluded that the protoplasm comprises parallel protein chains, of dimensions about 1000 A. by 10 A. by 4·5 A., which are laterally united by water hydration centres and which, in turn, compose a sponge-like framework intercalated with water containing the various solutes. From this elementary protoplasmic architecture is derived the fundamental pattern of the primordial cell which, through developmental differentiation, gives rise to the tissue cells and organs of the adult organism, as recounted in the earlier discussions.

The colloidal properties of protoplasm and its cellular differentiation were variously exemplified in the paper presented by Prof. Heilbrunn. His recent investigations have demonstrated especially a localization in the cortex of the cell of calcium which, upon appropriate stimulus, is released within and so effects a gelation of the protoplasm involving contraction. Further evidences of this gelating effect were found upon exposing cut surfaces of cells to various concentrations of calcium salts. Thereupon a reversible limiting membrane was formed on the cut surface, or a bulb-like contraction was locally induced, due to the penetrated calcium. Thus the age-old problem of contractility, a common property of protoplasm, may find its solution normally in the localization and release of calcium in the cell's cortex.

Recalling the emphasis given throughout the Symposium to the structural aspects of protoplasm, Prof. Bernal, in presenting the final paper, urged that consideration of the energy relations was equally important, since not only do the energies involved determine the sort of structure possible but also their nature must be known in order to account for that structure. These energies relate primarily to the protein constituents of the protoplasm, a model for which may be found in the tobacco mosaic virus when contained in known salt solutions. Here the virus entities, which are long protein molecules, become orientated in striking spindle-like patterns, or tactoids, and their regular distances apart vary directly with the concentration of the salt solution. Evidently long-range forces between the virus entities are operative through the ionic atmosphere of the surrounding medium with which the former are in equilibrium. The magnitude and direction of forces inside the tactoid pattern are different from those on the outside. These differences, in fact, account for the pattern formation. Apparently analogous forces may similarly account for the formation of the mitotic figure and the ensuing phenomena during the mitosis of the living cell.

These papers are soon to be published in book form by the American Association for the Advancement of Science.