CONTENTS

I

THE ORGANIZATION OF CELLS AND OTHER ORGANISMS

1. The cell concept: old *causes célèbres*

THE changing nature of the cell concept and of concepts immediately related to it*—from the container (the 'cells' of Robert Hooke's cork (1667)) to the contained (the *Energide* of Sachs, 1892); from *Gallerte* (Treviranus, 1816) or *Schleim* (Schleiden, 1838), with or without *Körperchen* (Purkinje, 1836) or *Körnchen* (Valentin, 1835), to *Cytoplasma* (Kölliker, 1867) and *Kernplasma* (Strasburger, 1879); from 'sarcode' (Dujardin, 1835) to universal *Protoplasma* (Cohn, 1850); from the 'substance glutineuse, simple et homogène' of Dujardin to the immensely complex heterogeneous system which we know today—should serve as a permanent warning against a belief in the fixity of concepts, or in their value at any moment in time, save as a means of communication, of rapid reference to the present state of knowledge.

The cell theory of Schwann (1839), though it undoubtedly served to consolidate the view that living things are composed of cells, was not, as defined by Schwann, co-terminous with that view. It was not merely a generalization relating to static structure, but rather—as Baker (1948a) emphasized—a theory of development; a theory that organic tissues result from a common principle of development (*Entwicklungsprinzip*), namely, the formation of cells (*Zellenbildung*). For Schwann, the observational basis of this generalization was not, as one might suppose, the fact that tissues are compartmentalized into units each consisting of *Kern* and *Cytoblastem*, but the process of cell formation believed to have been observed by Schleiden (1838). Nuclei were held to form by 'crystallization' in the structureless, fluid *Cytoblastem*; and a new cell was delimited by the subsequent development of a cell membrane.

Once this erroneous theory of cell formation was abandoned, as it was definitively by Virchow (1858), there was—in Schwann's sense—no cell theory left. Yet such was the force of the complex of new ideas associated with Schwann's theory that, at the end of the century, Oskar Hertwig (1893a), though well aware of profound changes in the content of the theory, could write: 'the doctrine that animals and plants conformly consist of such very small particles [= 'microscopically detectable *elementary units*'] . . . is called the *cell theory*'. As Sedgwick (1896) recognized, to say that

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* Aschoff, L., Küster, E. and Schmidt, W. J., *Hundert Jahre Zellforschung*, *Protoplasma-Monographien* 17, Borntraeger, Berlin (1938); Cameron, G. R., *Pathology of the Cell*, Oliver and Boyd, Edinburgh and London, 1952.

organisms are 'cellular' (provided the meaning of the word 'cell' is agreed on) was no 'theory' but, by the nineties, a statement of fact. The generalization had undergone an important extension, however, with Haeckel's formulation of the origin of the Metazoa in the *Generelle Morphologie* (1866) and in the *Schöpfungsgeschichte* (1872); and in its developed form the theory asserted 'that organisms of Metazoa are aggregations or colonies of individuals called cells, and derive from a single primitive individual—the ovum—by successive cell-divisions' (Sedgwick, op. cit., p. 214). This was a dogmatic statement, and at two points it opened the door to future difficulties: in the qualification of the single individual—the ovum—as 'primitive', and in the identification of the Metazoa with 'aggregations or colonies'.

The recent restatement of the 'cell theory' by Baker (1948*a*, 1949, 1952), couched in seven propositions, though it served as a peg on which to hang a much-needed review in English of the history of basic concepts in cytology, demonstrated afresh that there is no single theory to which it is now desirable to give the name 'cell theory'. For the several propositions are either (for a majority of biologists) matters of fact, or assertions, the truth of which depends on the precise meaning attached to the terms brought into relation, or untestable hypotheses.

Most biologists have now forgotten the heated discussions at the turn of the century which sprang, partly from observation, partly from a tidying up of the concept: 'the cell', and partly from attempts to reconcile abstractions with observed reality; as Sedgwick (1895) wrote: 'the cell' is 'a kind of phantom which takes different forms in different men's eyes'. He does not seem to have realized that its phantasmal character is due to its being a concept. Bourne (1896) took Sedgwick to task for inquiring 'What, after all, is a cell?', but he succeeded only in showing that there were many possible answers to the question, and frankly concluded that 'an argument about definition would soon land one in the regions of scholasticism'. From a distance, it is instructive to observe how, as with unanswered letters, the passage of time so shifts the basis of discussion that questions once of passionate interest come to require no reply and are dropped, unanswered, from the common field of interest.

In relation to the field of ideas about cells there were two main types of controversy. The first centred on the question: how far are cells discrete units, and how far is the reality a protoplasmic reticulum with nuclei at the nodes—a continuum? The question was prompted by Sedgwick's supposed observation of incomplete separation of the blastomeres in the segmenting ovum of *Peripatus*. As Manton (1949) showed, the peculiarities of the cytoplasm observed by Sedgwick and figured in his paper of 1887 were due to the condition of his material; but the question was perhaps prompted, in part and subconsciously, by the desire to preserve some material basis—intercellular connexions or cytoplasmic continuity—

for the behaviour of living things as unitary organisms. The fact that Bourne (1896), while emphasizing the distinction and complete isolation of the cells formed in the segmentation of the egg, for example, could yet in the same paper condemn the view that the metazoan organism is composed of independent and isolated units, indicates that there was an unresolved conflict between the view of the metazoan as an aggregate of isolated individuals called cells and its behaviour as an integrated unit.

To some extent the conflict remains unresolved; but from our present position we can see that the discussion hinged in part on what is to be understood by 'isolated' in this context. We are now so aware of the constant exchanges between cell and cell, and cell and environment, that the idea of a cell as a unit *isolated* by its membranes is quite foreign. The exchanges of substances between one region and another in a syncytial cytoplasmic continuum, and in a mass of cells partitioned by cell membranes, might admittedly be expected to occur in somewhat different ways —by simple or trapped diffusion in the first instance, by simple or facilitated diffusion and active transport in the second. But these differences are not absolute, and even the largest biologically active molecules, such as antibodies, can move across cell membranes in certain material and at certain times: the hypothetical completely isolated units do not exist.

It must not be forgotten that if the Metazoa are not in Sedgwick's sense syncytia, the Metaphyta, to a considerable extent, are. Protoplasmic connexions between cells in the bodies of plants are frequent, and the extent to which, in the tissues of the higher plants, movement of cytoplasm and nuclei occurs through pores in the cell wall, and along plasmodesmata, has recently been demonstrated (Lou *et al.*, 1957).

The second type of discussion turned on the status of those organisms commonly referred to as 'unicellular'. To Dobell's reasons for rejecting this term in favour of 'noncellular' (Dobell, *1911*), Baker (1948*b*) did not perhaps do justice; for though the discussion may appear to have revolved about a verbal quibble, Dobell's protest, like that of Sedgwick in favour of the syncytium, was directed against an over-simplification and over-generalization. At the time of Dobell's polemic, the cytoplasmic organization of the Protista (as he preferred to call them) tended to be minimized; for all but protozoologists they had become the simplest organisms of the evolutionist. In effect, Dobell's protest was a plea for accepting them as organisms in their own right, organized in a different way from that of the Metazoa: a subkingdom, not a phylum. The way of escape from Dobell's logical difficulty was shown by Woodger (1929), when he pointed out that 'the cell' is a highly abstract concept of minimal content, a type of organization in which a mass of cytoplasm is associated with one or more nuclei. 'A cell' seen under the microscope as a part of a metazoan, on the other hand, is a concept of a lower grade of abstraction, a perceptual object. Without prejudice to their status, we may safely say that many Protozoa retain the type or mode of organization known as 'the cell' throughout life. But this controversy also has lost its emotional content. Now that the electron microscopists, following on the heels of the French school of protozoologists, have begun to turn their attention to ciliates and flagellates, there is no need to doubt any longer the structural and functional complexity of these organisms.

2. *The* unit of life confronted with bacterium and virus

There remains, however, another aspect of the expanded cell theory which must be examined afresh; and that is the extension of the original concept to the view that the cell is *the* unit of life; the generalization that the behaviour which we recognize as 'living' is only shown by systems having the organization (nucleus, or nuclei, and cytoplasm) of one or more cells. The validity of this generalization has been brought in question by relatively recent developments in the fields of virology and of transformation and transduction in bacteria: what is the status of these various 'infective agents'? To some extent the answer to the question of validity depends on how far it is practicable or desirable to extend current definitions of 'nucleus' and 'cytoplasm'. These are convenient labels for inherently complex perceptual objects. For example, a bacterial 'nucleus', as revealed by the electron microscope, appears to have no bounding membrane, unlike the nuclei of typical cells. Furthermore, though the occurrence in bacteria of a process resembling mitosis has been claimed, this is hotly disputed. In the light of these statements, is it legitimate to extend the term 'nucleus' to the Feulgen-positive particle in a bacterium? Is it legitimate to refer to a bacterial 'cell'? And moreover, in view of the absence of many components discriminated in bona fide cells, are we prepared to recognize the Feulgen-negative material in a bacterium as 'cytoplasm'?

The question of status is still more acute in the case of the viruses, as will be seen later (p. 24). At this stage, it may be stated that many viruses show no such dual organization as will allow even the most attenuated form of the cell concept to be retained. They are not cells. Furthermore, in isolation, in the form in which their structure can be examined by X-ray analysis, and their chemical composition by direct methods, they neither do anything, nor are they subject to change. They are highly 'organized', in the sense in which a protein or nucleic acid crystal is organized, but not in the sense in which a respiring, assimilating, growing metazoan cell is organized. They 'live' only in producing more of their kind, when they enter a specific type of living cell, where they seem to seize upon and direct to their own ends the synthetic processes of the host cell; in this they are to some extent comparable with self-perpetuating cell organelles. If they are not cells, how are we to think of them?—for the cytologist cannot afford to ignore them. Are they 'organisms'?

Lwoff (1957) wittily defended the view that viruses are neither organisms nor molecules but viruses: 'that viruses should be considered as viruses because viruses are viruses'. The statements are as unexceptionable as any expression of identities must be, and as suitable for inducing prehypnotic meditational states as similarly turned statements about a rose by Stein (1922). But they do not absolve us from the necessity of relating viruses to other aspects of experience. It is conceded by Lwoff that viruses and organisms have a few characters in common, characters also shared with cellular organelles; but the presence of common characters does not suffice to justify our referring all three entities to one class of *organisms*. (It would suffice, however, to unite these three types of entity in a single class, whatever name we give to it.) The conclusion reached: that viruses are not organisms, follows from the particular definition of 'organism' framed by Lwoff: 'an independent unit of integrated and interdependent structures and functions'. In later restatements the definition is curtailed, however: 'an organism is the result of the integration of its dependent and interdependent parts'; and again: 'the essential character of an organism, independence, with all its implications, transcends the characters of its parts, dependence.' The 'independence' of living organisms, however, is always a qualified independence; it is never absolute, and in most living organisms it is susceptible of great fluctuations of degree—between states of 'suspended animation' and full activity, for example. 'Function', that is, the dynamic aspect of structure—structure changing in time—may also range between zero (the state of the chromatin of a mature sperm head (p. 109), for example) and full activity (the state of the chromatin in a developing oocyte nucleus (p. 130), say). If, as happens in Lwoff's restatements of his definition, 'function' is omitted, his definitions also apply to any molecule. They apply to still smaller entities; and indeed the term 'organism' was extended by Whitehead (1926) to all concrete enduring entities in nature whatsoever. This meaning of the word organism: 'a whole consisting of dependent and interdependent parts, compared to a living being' (*Shorter Oxford English Dictionary*) has been English usage since the eighteenth century; and perhaps a way out from Lwoff's omphaloscopic 'virus is virus is virus . . .' lies in accepting that the organisms with an energy flux (p. 21) in which the biologist has hitherto for the most part been interested are not the only types of wholes that consist of dependent and interdependent parts.

There is no objection to maintaining distinctions between categories of organisms of which the 'lower' are not merely included in, but encapsulated by, the 'higher'. Cellular components and viruses may be placed in the general class of organisms, without prejudice to the organismal status of the cell in which they both find themselves. A cell is also an organism, but of a higher level of structural complexity, though of a lesser degree of independence and structural complexity than the metazoan or metaphytan of which it is a member. Many would restrict the term 'organism' to this last category.

When Burnet (1957) spoke of a virus as 'a stream of biological patterns', however, he was looking away from the inert virus particle (which Lwoff cannot accept as an organism) to the phenomenon of virus-in-action. From this standpoint, the virus is more readily identified with the biologist's conception of an organism as an entity (a pattern) replicating in time, even though its degree of independence is lower than that of a cell.

It is not necessary that virus particles should be 'living' for them to be classed as organisms; for the antithesis of 'living' and 'dead', as Pirie (1937) so ably argued, is a linguistic convenience of everyday life, but not a scientifically valuable or valid distinction. The biologist, like the physicist, has perpetually to reconsider old names and invent new; for never, in the history of thought, has a new term, or a new meaning-extension of an old term, been used at first with that degree of definable precision that it later acquires. One of the more striking powers of human beings is that they constantly and usefully talk about things which, according to philosophers, they have no business to be talking about at all. The nature of thought and communication we shall perhaps begin to understand when philosopher and scientist have both been psychoanalysed; and when the activities of philosopher, scientist, and psychiatrist have been scrutinized by the ethologist and cyberneticist.

3. The antithesis: structure and function

There is a further piece of gymnastics to which the reader is invited to subject himself before coming to more solid fare. It is that of standing on its head—or examining while he himself stands on his head, as did St. Francis to gain a new vision of the world—the pair: structure and function. We tend to think of them as static, quasi-permanent structure, in, through, and about which function is manifested as a relatively evanescent perturbation. But in fact, it is known from tracer experiments, and in other ways, that these solid-seeming structures are all, in varying degrees, in a state of flux.

It is in order to avoid the traditional dichotomy between 'structure' and 'function' and to emphasize the need to abandon too static a view of structure, that the topics to be dealt with here have been brought together under the title of 'organization'. For a term so frequently used in relation to aspects of human society, and implying not a static structure but an integration of activities in time, seems a more suitable designation for what it is desired to convey than the dichotomous 'structure and function'. At the molecular and immediately supramolecular level of texture with which we shall be largely concerned, there is indeed no dichotomy. There are still too many lacunae in our knowledge for it to be possible to talk consistently in terms of 'organization' in this sense, as Young (1951) has already attempted to do at a higher anatomical and functional level; but it is time to urge acceptance of the fact that at the molecular level function is changing structure, and the seemingly 'static' organism is revealed as a process. To add a time dimension to our molecular pictures of cell organelles is still only possible to a limited extent; but a beginning has to be made.

How microscopic structures arise, in the sense of the sort and shapes of molecules of which they are formed, and how these are arranged, in supramolecular textures, is to some extent, and in general terms, known. If they are evanescent structures, it is possible to imagine a reversal of the process by which they come into being, leading to their disappearance; if they are more permanent, they can be envisaged as relatively slowly changing stock-piles of the end-product of a synthetic process. Now one of the most striking properties of the sorts of molecules which give rise to microscopic structures is that they tend to aggregate spontaneously, and will form structures *in vitro* in a suitable ionic environment. This suggests that it is possible to carry one stage farther the distinction between what is primary and what secondary in living systems. It would appear that what is primary is not, unfortunately, the structures we can so readily discriminate optically, isolate mechanically, weigh, measure, and analyse both chemically and structurally, but the activity in time that gives rise to substances which in turn, in a suitable environment, spontaneously generate structure.

To write of 'activity' may well seem one degree worse than writing about 'organization'. But it is salutary to make the effort to think of all parts of a cell as slices in time of solid graphs; the differentials of processes of synthesis and destructive catabolism; waxing and waning with the time-course of a steadily and cyclically varying *activity*; assembled or dispersed according to the sense of concentration gradients that arise in ordered sequence, in the directed march of historically-determined chemical events.

There is nothing 'vitalistic' about this 'activity'. Its essence is already known, even though it is impossible, as yet, to begin to envisage the complexity of what is going on from moment to moment with respect to all simultaneous aspects of activity. Before coming to consider in what this activity consists, it may be well to interject that between the activity and the structures generated there is undoubtedly a reciprocal relationship, so that the notion of activity proceeding in isolation from structure is an unjustifiable abstraction; but there is, none the less, a simple sense in which activity precedes macromolecular structure.

In the most general terms, this activity is chemical interaction based on conformity or congruity of patterns of molecular structure. It manifests itself: (1) in enzyme catalysis, by which metabolic energy is made available; (2) in the generation of microscopic from molecular structures by extended condensation and by polymerization in one-, two-, and three-dimensional aggregates; (3) in the coupling of energy on molecular and supramolecular structures leading to function—that is, to textural or dimensional changes correlated with microscopic or macroscopic mechanical, electrical, or other phenomena. In all such reactions, a specific pattern of chemical groupings is the unique cause of specificity of interaction; and this same chemical specificity is the basis of the existence, in no mystical sense, of biological *personae*, of individuals.

The manifestations of chemical interaction, based on congruity of molecular pattern, mentioned under the second and third headings of the last paragraph, will be examined in detail in subsequent chapters. Here it is proposed only to consider briefly the essential features of enzyme catalysis in relation to metabolic processes and the energetic coupling of synthetic and other reactions.

4. Catalysts and the general principles of their structure and mode of action

The existence of specific substances to which such processes as digestion are due was postulated more than half a century before their isolation; so that while Schwann inferred the existence of 'pepsin' in 1836, Buchner did not observe fermentation with a cell-free yeast extract until 1897. It is striking to note that, even earlier than Schwann's bold inference, came the recognition by Berzelius (1835) that the activities of such substances, and of those accelerating inorganic and organic chemical reactions without themselves being changed, could be regarded as special instances of a general phenomenon of catalysis; such 'catalysts' being substances which influence the rate of reactions but do not—in low concentration at least—change the equilibrium state. Although catalysts are unchanged and do not appear in the reaction products in simple proportions, they always enter into combination with one (or both) components of the reaction; and in the most general terms, it is the possibility and consequence of this combination which lead to acceleration of the rate of reaction.

In the following pages an attempt has been made to summarize present views on the mode of action of enzymes in terms of molecular mechanisms.* Be it understood, the intention is not to give an explanation intelligible without any previous knowledge of chemistry; familiarity, at least in outline, with the classical picture of chemical energetics as applied to biochemical reactions, is assumed. To a biologist it would seem to be of evolutionary significance that the molecular mechanisms on which the phenomenon of enzyme catalysis rest are now seen to belong, in principle, to the field of general chemistry. 'Since it is independent of the assumptions of atomic theory, classical energetics can give no information on mechanisms

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* My debt to the *Symposium on the mechanism of enzyme action, sponsored by the McCollum-Pratt Institute of the Johns Hopkins University*, edited by W. D. McElroy and B. Glass (1954), is evident.

at the molecular level' (Klotz, 1957); and for the biologist and morphologist it is exhilarating, after a century of thermodynamics, to have at least a glimpse, however indistinct, of mechanism.

The splitting of hydrogen peroxide proceeds slowly in the absence of a catalyst, because random thermal collisions rarely excite the peroxide molecule to such a level as will enable one or two electrons to transcend a particular potential energy barrier and fall to a lower energy level, thereby breaking a chemical bond. The effect of combination with a catalyst is to weaken the bond in question, so that a comparatively small increase in kinetic energy from thermal collision enables the change in electronic structure to take place. This weakening of the strength of primary bonds occurs, as Eyring *et al.* (1954) re-emphasized, in systems which are not normally thought of as comparable with enzyme-substrate complexes.

In general, whenever a molecule makes strong electrostatic hydrogen bonds with other molecules, it is to be expected that the primary bonds holding the molecule together will be weakened. Thus the strength of the H-Cl bond is lowered in water as compared with the gaseous state, because as a result of the electrostatic bonds formed between chlorine and water, the pair of bonding electrons uniting hydrogen ion and chlorion are pulled over completely on to the anion, and both hydrogen and chlorine are fully ionized. Such modification of the strength of a primary bond can occur internally, and may be a phenomenon of great importance for the understanding of the properties of large and complex molecules. The halogen-substituted acetic acids—for example chloracetic and in greater degree trichloracetic acid—are stronger than the unsubstituted acid, because the Cl atoms tend to withdraw charge from the adjacent O-H bond; that is, the electrons of the hydrogen atom are caused to draw away from the proton, which is therefore readily set free when electrostatic bonds with water are established. The same authors have summarized the ways in which primary bonds may be weakened: (*a*) by electrostatic interaction; (*b*) by 'electron-hungry' groups pulling (or electron groups pushing) electrons out of a bond; (*c*) by geometrical distortion of bonds—for example, the straining of a bond as a result of repulsion between two halves of a complex molecule united by a primary bond. Any process which extends a primary bond by as much as 10 per cent. will unsaturate it and turn it into a reactive fractional bond. It is to be supposed that the function of an enzyme is to do just this.

5. The structure of enzymes and the mechanism of enzyme catalysis

Up to the present, no protein-free enzyme has been isolated; and it may well be that all enzymes are catalytically active proteins. In many enzymes, groups themselves possessing slight catalytic activity—the prosthetic groups—are united with a globulin-like component, the apo-enzyme;

in others, no such detachable active groups have been detected. The latter class includes the hydrolysing enzymes; while those concerned with oxidation and reduction generally include prosthetic groups. It is probable, however, that a prosthetic group of some kind, though not detachable, is present in the hydrolases as well, since in some members of the class activity depends on the presence of metallic ions which participate in complexes.

The role of metallic ions in prosthetic groups was reviewed in an account of chelation and catalytic properties by Calvin (1954). Just as in the most general sense, the essential mechanism of enzyme catalysis, namely the weakening of primary bonds by electrostatic or geometrical strain, is already widespread in small-molecule, inorganic systems, so also is the more specifically characteristic type of complex found in metallo-enzymes adumbrated in inorganic systems. As first systematically explored by Werner at the beginning of the century, many metallic ions are able to form 'co-ordination' complexes with a number of atoms or groups of atoms ('ligands'). The latter are united to the ion by bonds that are more homopolar than electrostatic in character; and the normal electrovalency of the ion is left unchanged. If the ligands themselves are united, the complex is said to be 'chelated', because of the pincer-like grip exerted by the groups on the metal ion. Now certain types of chelate rings can combine with organic compounds, and in doing so they weaken the internal bonds of the compound. To this extent the chelate ring may be compared with an enzyme, and the organic compound with which it forms a complex, to a substrate. For example, the Cu or Ni chelate of salicylaldehyde forms a complex with an α-amino acid; and in this complex, reactions occur which are virtually unobtainable except in the complex: racemization, oxidative deamination, and ester exchange (Martell and Calvin, 1952).

The variety of possible metallo-compounds of this type is enormous, and as yet only a beginning has been made with their systematic examination. Even at this stage, however, it is clear that other types of association than that of chelation will have to be considered, if the role of certain metals in enzymes is to be explained. For example, while Cu, Fe, and Ni are active both in chelation and in the prosthetic groups of enzymes, Mg and Mn are among the most weakly chelating ions, and yet play a very important role in enzymic activity. For this reason, Klotz (1954) suggested that these metal ions are not present as chelated compounds, and that they exert a stabilizing action on the activated complex—holding it in the strained position—precisely because their weak co-ordinating powers are unsatisfied.

One of the most interesting and suggestive examples, where the role of the rest of the molecule in relation to the prosthetic group is clear, is catalase, discussed by K. H. Meyer (1942) and by K. G. Stern (1942). Here the increasing catalytic activity of the iron atom as a result of its entering