Cell Function in Relation to Structure

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Introduction

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TRADITIONALLY the concept of regulation of biological structures and activities has implied the maintenance of constancy under variable conditions. The problem of regulation has been referred to the external environment of the cell. When, however, we examine the events inside the cell, we are compelled to deal with the obverse aspect of regulation. Given a relatively constant environment and endowed with a relatively constant *total* composition, how can the cell bring about the rapid changes in the *level* of various biochemical activities associated with physiological changes? How, with a stable environment and presumably constant genetic endowment, can patterns of differentiation be laid down? What are the patterns of interaction of the recognized "organs" of the cell: nucleus, cytoplasm, and its differentiated structures, and the cell surface? Although the several papers of this symposium deal with a broad spectrum of cell activities, the central problem is dynamic regulation, or regulation viewed as directed change.

Molecular Aspects of Growth and Differentiation

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IN AN effort to reformulate the problems of morphogenesis, particularly of growth and differentiation, in terms which would lend themselves to attack and verification by modern physicochemical methods, the static concepts of cellular morphology and the anorganismic concepts of an oversimplified protoplasm physiology have been reconciled, or rather bridged, by a concept of "molecular and particulate ecology." This concept takes account of the organizing function of the structural order in which the chemical reactions of the cell take place. The role of stereochemical configuration of macromolecules in protoplasmic reproduction, cell growth, cell differentiation, cell association, tissue formation, and wound healing has been suggested and supported by various examples of experimental work from the author's laboratory.

Regulations in the Heart Muscle Fiber

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THE "staircase" in muscular reactions, described by Bowdich, should be an expression of the function of the actomyosin system. The staircase can be studied quantitatively in a steady state by varying the frequency of impulse. It can be studied better in isometric than isotonic contraction.

It was found that the staircase could be abolished by reducing the potassium concentration of the perfusion fluid and by decreasing the temperature. If both frequency and temperature are varied we can state the limits of frequency or temperature at which a given substance abolishes the staircase.

Fresh serum, if used as perfusion fluid, abolishes the staircase. Analysis showed that the substance responsible for this action belongs to the steroids, that adrenal extracts act likewise, and that desoxycorticosterone in concentrations of a few micrograms per ml also has the same effect, which was specific as far as studied. Its action was duplicated by substances of the digitalis group. The effect is developed gradually, suggesting that these substances act on the membrane and modify its selective activity in such a way as to make it establish a new intracellular ionic atmosphere, in the presence of which the staircase is abolished.

The Function of Enzymes on the Surface of the Cell

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THE cell surface not only regulates cell activity by its properties as a semipermeable membrane, but it actively participates in enzyme reactions. For example, the surface of the cell contains saccharases and phosphatases. The function of the phosphatases is digestive. Nonutilizable substances in the medium are split into products that can be utilized. In addition, the cell surface contains loci that are intimately involved in hexose metabolism. These loci can be inhibited by the uranyl ion. The characteristics of this inhibition, its kinetics, and its temperature coefficient are consistent with the hypothesis that enzyme reactions are involved. It is suggested that hexoses are actively transported into the yeast cell by a mechanism involving phosphorylation by enzymes located on the surface of the cell.

The Nature of Intermediates in Protein Synthesis

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THE problem of protein biosynthesis may be divided into two broad categories: first, the nature of the activating and coupling mechanisms at the amino acid level; and second, the processes involved in the final assembly of the protein molecule from its immediate precursors.

Activation at the amino acid level appears to depend, at least for its primary energy source, on ATP. Recent studies on the incorporation of C^{14}-labeled glutamic acid into tissue proteins suggest that a precursor, not in equilibrium with the amino acid itself, may be involved. Thus, the incorporation of carboxyl-labeled glutamic acid can, to a large degree, be accounted for on the basis of the fixation of $C^{14}O_2$ released by oxidation and not by direct incorporation.

The final product of protein synthesis may be formed either by an all-or-none template assembly of activated free amino acids or through the intermediary production of peptide precursors. To test these hypotheses, pure radioactive proteins were prepared *in vitro*, containing C^{14}-labeled alanine or aspartic acid. By specific proteolytic degradation, followed by chromatographic separation, fragments of the protein structures could be isolated, and the specific radioactivity of the labeled amino acids, derived in this way from different points in

the protein molecules, could be compared. The observed "asymmetry" in radioactivity supports the hypothesis that peptide intermediates are involved as precursors in protein synthesis.

Biological Activity of Compressed Monolayers of Proteins

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SINCE many studies in the literature point to the orientation of cellular proteins at protoplasmic interfaces, the possibility was considered that the proteins of cell structures consisted of surface-spread molecules. To approach the problem, studies were made of several kinds of protein molecules spread at air-water interfaces. The films thus formed were compressed to insoluble fibers to render them amenable to measurement.

To date, three protein systems have been studied. The pepsin-albumin system demonstrated that surface-spread pepsin retained its hydrolyzing properties. The mode of its action was shown to be the breakdown of the surface-spread pepsin-albumin fiber, which behaved as a stabilized enzyme-substrate complex. The second protein, catalase, was studied by J. G. Kaplan. It was found that the surface-spread catalase and the same enzyme *in situ* (erythrocytes) showed identical properties of resistance to heat inactivation and actual activation by heat at temperatures of 50°, 56°, and 60°, whereas the same enzyme in solution and eluted from the cells showed no such properties. The third case studied was the intracellular proteinactomyosin. Here, the surface-spread protein had not only a strong ATP-ase activity but also, when compressed to a fiber, contractile properties in the presence of ATP. The contraction was such that the actomyosin fibers could perform mechanical work, and was reversible with changes in salt concentration. Thus repeated contractions with ATP in 0.05 *M* KCl were obtained, relaxation occurring in 0.3 *M* KCl without ATP. The contracted state could be maintained in 0.05 *M* KCl without ATP. It was concluded that surface-spreading plus the subsequent compression brought about an alignment and intermolecular bonding of the molecules in such a way as to convert chemical energy into mechanical energy.

Post-Fertilization Respiration of the Sea-Urchin Egg and the Oxygen Uptake of Reactivated Oxidase Systems

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THE increase in oxygen uptake of the sea-urchin egg at fertilization can be understood as an adaptation to preserve the life of the unfertilized cell, in order to increase its chance of fertilization. The oxidases operating before and after fertilization are identical. The oxidase is an iron porphyrin and resembles the mammalian cytochrome oxidase very closely. The absorption bands of cytochromes *a*, *b*, and *e* have been observed in the egg, whereas the cytochrome *c* band was found only in the sperm. In the egg, the cytochrome *c* concentration seems to be lower than 5×10^{-4} μg/mg dry matter. It is likely, however, that there is no fundamental difference in composition between the mammalian and the echinoderm cytochrome systems, but merely that the relative concentrations of the components differ.

In view of the fact that a proper colloidal structure is of paramount importance for the optimal functioning of the cytochrome system, the difference in respiratory rate of the sea-urchin egg before and after fertilization may be related to and caused by the colloidal rearrangements following fertilization.

Studies on Developing Muscle Tissue

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THE material reported here is part of a systematic study of the mechanism of the embryonic development of muscle tissue. The earliest phase investigated so far is the development of the somite from the 10- to the 50-somite stage (30–92 hr of incubation). The observations on somite tissue included measurements of linear dimensions, of volume, and of quantities of protein nitrogen (PN), ribose nucleic acid (RNA), and desoxyribose nucleic acid (DNA). The volume of an average somite increases about 26 times. This equals approximately the increase in wet weight. On the other hand, the amounts per somite of PN, RNA, and DNA, representing the dry weight, accumulate to about 15 times, 15 times, and 10 times their respective initial levels. Thus the increase in wet weight is twice that of the dry weight. The rate of accumulation of PN, RNA, and DNA changes considerably and reaches two marked peaks for all three substances at about the 25-somite stage and the 45-somite stage.

Analyses were carried out on older muscle tissue (eighth day of incubation to 5 weeks after hatching) for RNA, DNA actomysin, phosphocreatine (PC), and adenosinetriphosphate (ATP). Although a few fibers can be observed on the seventh day, significant amounts of actomyosin were found no sooner than the eleventh day of incubation. Subsequently, a rapid increase of the actomyosin content was observed and continued until the fifth week after hatching, but, no parallel increase in nucleic acids was noticed. The formation of larger quantities of phosphate esters (PC, ATP) took place even later (fourteenth and sixteenth day).

In comparing the early and later phases of development of muscle tissue we find, so far, two differences: In the first phase, growth and differentiation are accompanied by an increase in water content and an accumulation, at comparable rates, of proteins and nucleic acids. In the later phase, proteins are formed rapidly while the content of nucleic acids remains at about the same level and the content of water decreases. This suggests that different mechanisms are involved in the early and late development of muscle tissue.

Nucleus-Cytoplasm Interactions

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THE use of a technique for isolating of mammalian tissue nuclei in quantity with the aid of citric acid disclosed that the rate of incorporation of radioactive phosphorus into the nucleus of the "resting" liver cell was very rapid and that its removal was also very rapid. The nuclear phosphorus was found to be primarily in the nucleoprotein, and later it was shown that it was in the pentosenucleic acid (PNA) and not in the desoxyribonucleic acid. In rapidly proliferating tissue the radioactive phosphorus was incorporated into the desoxypentosenucleic acid (DNA) but was removed from it very slowly, if at all.

Some Enzymes of Cell Nuclei

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THE composition of nuclei isolated from avian erythrocytes and from the liver, kidney, thymus, pancreas, heart, and intestinal mucosa of the calf or horse was studied with a view to ascertaining (*a*) whether nuclei, like their corresponding cytoplasms, are chemically differentiated, and (*b*) whether certain enzymes might be universally distributed among all nuclei and hence significant to some general aspect of nuclear metabolism. Commonly, such studies as have been made on the properties of isolated nuclei have been based on analyses of nuclei isolated in aqueous media. This procedure is unreliable, since the transport of proteins into or out of the nuclei in the course of their isolation cannot be avoided. By the use of a modified Behrens procedure in which nuclei are isolated in nonaqueous media, this major source of error is largely, if not entirely, eliminated. A comparison of the compositions of nuclei isolated in this way shows that nuclei are as diverse in their properties as their tissues of origin.

This investigation also indicates that enzymes related to nucleoside metabolism occur in high concentration in the nucleus, and that the enzymatic activities of nuclei are appreciably affected by the physiological state of the cell.

Colloidal Regulation of Protoplasmic Activity

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THE blood of higher animals is in a constant state of balance between the factors which tend to promote clotting and those which tend to prevent it; in protoplasm, also, there is a similar balance between clotting and anticlotting factors. The thromboplastin of ground-up or injured cells not only hastens blood clotting but also causes the clotting of the protoplasm of normal cells. This is clearly demonstrated in experiments in which extracts of injured tissues cause a gelation in the protoplasm of sea urchin eggs. Heparin itself can prevent protoplasmic gelation and cell division; so too can the bacterial polysaccharide of Shear. This polysaccharide has some of the properties of a heparin. But the most potent anticlotting and antimitotic substance has been obtained in extracts of the starfish ovary. The clotting and anticlotting substances that can be obtained from living tissues not only affect the mitotic gelation and the division of cells, they also act on heart muscle, the cells of which are apparently permeable to these substances. There is also much additional evidence indicating that the impetus which makes a cell active is a liquefaction of the cortex and a gelation in the interior. On the basis of this hypothesis, it is possible also to interpret the electric changes that accompany response and the changes in rate of respiration.

Calcium as an Indicator and Regulator of Cell Activities

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THE postulate is offered that the aging process involves a decrease in efficiency of the mechanisms regulating reconstitution or self-synthesis. In general, it appears true that actively growing cells do not age. There is increasing evidence from our laboratory that a ribonucleoprotein complex containing calcium is associated with the surface of cells. Variations with age in the composition of this cortical layer may limit synthetic processes by conditioning the transport of materials across the cell surface. Several studies indicate that calcium does increase with age in the cell cortex.

The Nature of Virus-Host Specificity

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THE first step in the invasion of bacterial host cells by bacteriophages of the T- system consists in an attachment reaction that is completely reversible. In an optimal concentration of the necessary salts, the rate of attachment of any virus approaches 100% collision efficiency on a molecular basis. The reaction velocity is independent of temperature between 1° and 37° C. It is proposed, therefore, that the initial virus-host attachment consists in the establishment of electrostatic bonds between ionic groups on the surfaces of the two particles. The second step of virus invasion is an irreversible, strongly temperature-dependent reaction that can be inactivated by agents like heat, ultraviolet radiation, or Zn^{++}, which do not interfere with the original attachment. Hence this step appears to be an enzymatic transformation, affecting some vital cell constituent.

The ability of a bacterial cell to resist invasion by a specific virus is a genetic characteristic and can be due to failure of either the first or second reaction to occur. Both types of mutants have been isolated. Cell mutants that resist the initial attachment of specific viruses still unite with related viruses in a reaction whose velocity approaches 100% collision efficiency. It is postulated that such a mutant cell differs from the wild-type in the distribution pattern of the ionic groupings on its surface so that a noncomplementary electrostatic configuration is presented to the particular virus to which it is immune.

Regulation of the Submicroscopic Organization of the Mitotic Spindle

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THE mitotic spindle disappears reversibly when a cell in metaphase or anaphase is treated with colchicine, hypotonic medium, low or high temperature, or high hydrostatic pressure. As the structure of the spindle in most living cells is invisible with the ordinary light microscope, the effects of these agents on the organization of the spindle have been studied in the past, mostly in fixed and stained preparations. By developing an improved polarization microscope, however, the author could see the finer structures of the spindle in living and functioning cells, specifically of *Chaetopterus pergamentaceous*, as well as the pollen mother cell of *Lilium longiflorum*. Neither the ordinary tactoid hypothesis nor the line of flow hypothesis can account for the observed distribution of birefringence within the living spindle. It appears very likely that the spindle is a special kind of gel structure composed of elongated and oriented micelles, linked together with fairly labile bonds.