Meetings

Bioenergetics

Living organisms utilize a multiplicity of energy-yielding and energy-trapping mechanisms. Some facts and speculations about these mechanisms for energy transfer were presented at a symposium on bioenergetics held 15–16 October 1964 in London, Ontario, under the auspices of the Biochemistry Division of the Chemical Institute of Canada.

One of the highlights of the meeting was the talk by R. Y. Stanier (University of California) on comparative aspects of the cytological structures involved in energy transformations. Most aerobic bacteria possess no recognizable intracellular membrane components comparable to mitochondria. While it is possible to isolate fragments (presumably derived from outer cytoplasmic membranes) which contain oxidative enzymes, it is probably correct to view the whole bacterial cell as the smallest respiratory unit. The forms of photosynthetic structures present great variety in procaryotic cells. The chromatophores of the purple bacteria resemble the internal lamellae seen in chloroplasts of higher forms. The lamellae may appear as intrusions of the cytoplasmic membrane, as vesicular elements in the cytoplasm, or as transitions between the two forms. These primitive structural analogues of the chloroplast may be interpreted as early experiments on the path to evolution of the discrete, membrane-contained organelles of present-day higher organisms.

Mechanisms and intermediates of the phosphorylation reactions associated with photosynthesis were explored by D. I. Arnon (University of California) and A. T. Jagendorf (Johns Hopkins University). Central to these and subsequent discussions on photosynthesis was the role of ferredoxin, a protein of low molecular weight which contains stoichiometric proportions of nonheme iron and sulfide, and has a redox potential at physiological *p*H in the neighborhood of that for the hydrogen electrode. One of the more interesting functions of this strongly reducing electron carrier is its ability to promote net synthesis of pyruvic acid from acetyl coenzyme A and CO_2, a reaction of special significance to *Chromatium*, in which the primary products of photosynthesis are amino acids. The photoreduction of ferredoxin by activated chlorophyll appears to be a primary reaction in photosynthesis, leading to generation of high-energy phosphate compounds, the production of reduced pyridine nucleotides for CO_2 fixation, and the evolution of molecular oxygen. By alternating light and dark periods of exposure of isolated chloroplasts it is possible to demonstrate formation in the light of a high-energy compound with a short half-life which is capable of subsequently transferring its energy equivalents to adenosine triphosphate in the dark. The nature of the intermediate is not established as yet, but its formation is associated with structural changes in the chloroplast and the uptake of hydrogen ions. Jagendorf provided some illuminating speculations concerning the relation of these events to the known contractile changes of mitochondria and to Mitchell's theory of the anisotropic membrane-bound adenosine triphosphatase.

The primary event of photosynthesis, the quantum-trapping, charge-separating mechanism which leads ultimately to the splitting of water into molecular oxygen and reducing equivalents of hydrogen, was described by N. E. Good (Michigan State University) and B. Kok (Research Institute for Advanced Studies, Baltimore). The process appears to involve an aggregate of chlorophyll molecules which acts as a light-gathering structure analogous to a lens, transferring and focusing the energy from a single photon to a photochemically active electron sink. Kinetic studies based on combinations of selective inhibitors of oxygen evolution with the photochemically active site have shown that the aggregate unit possesses approximately 2500 chlorophyll molecules for each active oxygen-producing site. Losses in the energy transfers are compensated by an extension of half-life of the activated pigment molecules; an additional factor extending the stability of the excited pigments involves reaction with an associated electron carrier (probably cytochrome *f*) in a charge-transfer complex. The entire process of photosynthesis may be viewed as an extension of the lifetime of energy storage forms from that of the activated singlet state of chlorophyll ($T_{\frac{1}{2}} \sim 10^{-8}$ sec) to that of reduced pyridine nucleotide ($T_{\frac{1}{2}} \sim 1$ sec).

The problem of energy production in certain autotrophic bacteria was broached by W. W. Umbreit (Rutgers University) and H. Lees (University of Manitoba). The inability of obligate autotrophs such as *Thiobacillus thiooxidans* to grow with carbon sources other than CO_2 is unexplained, since the organism possesses a full complement of enzymes for glucose metabolism and converts C^{14}-glucose into cellular protein. *Thiobacillus* can be adapted to survive in the absence of CO_2 in a glucose medium under certain conditions but does not remain viable. The reduction of pyridine nucleotide for CO_2 fixation by *Nitrobacter* appears not to proceed by a direct pathway, since the redox potential for the oxidation of nitrite to nitrate is too high to permit a direct coupling of the two reactions. The inital electron transfer is to cytochrome *c*; reduction of pyridine nucleotide occurs by an energy-requiring reversed electron flow from cytochrome *c*, a reaction demonstrated previously in mammalian mitochondria and also reported for *Escherichia coli*.

Studies of oxidative phosphorylation in *Mycobacterium phlei* were described by A. F. Brodie (University of Southern California). The oxidation of malic acid by cytochrome-containing particles from this organism proceeds via two different enzyme reactions. The first is the typical pyridine nucleotide–linked dehydrogenase; the second path proceeds by a flavin-mediated reduction of a naphthoquinone. Depletion of particulate naphthoquinone by irradiation with long-wave ultraviolet light causes inactivation of oxidation and phosphorylation with a variety of substrates. Restoration of activity on addition of synthetic and natural quinones revealed two sites of in-

teraction with the electron-transport system for pyridine nucleotide–linked substrates, and an additional site for succinate. The session on bacterial energetics was fittingly concluded by R. M. Hochster (Canada Department of Agriculture, Ottawa) with a discussion of some unsolved problems in this area. Major difficulties in assessing the efficiency of oxidative phosphorylation in microbial systems arise from high phosphatase activity and the existence of bypasses in the oxidative reactions.

The dynamics of carbohydrate metabolism in mammalian cells was discussed by G. R. Williams (University of Toronto) and E. Shrago (University of Wisconsin). By continuous monitoring of $C^{14}O_2$ released from carboxyl-labeled substrates by respiring liver mitochondria in vitro it was possible to calculate the rate constants for utilization of intermediates of the tricarboxylic acid cycle. The rate constant for fumaric acid was considerably greater than that for succinic acid, which suggests that succinic dehydrogenase is a limiting component of the cycle. Studies in vivo of enzymes in animals made diabetic by alloxan treatment revealed marked alterations in pathways of carbohydrate metabolism. The most dramatic change was the marked increase in activity of the soluble enzyme phosphoenolpyruvic acid carboxykinase in liver; since the increase was reversed by injection of insulin it would appear that this enzyme plays a key role in the endocrine regulation of gluconeogenesis.

Dissection of the mitochondrial oxidative phosphorylation process into its partial reactions was presented by C. L. Wadkins (Johns Hopkins University). A protein of low molecular weight which catalyses an exchange reaction between adenosine diphosphate and adenosine triphosphate may be extracted from liver mitochondria; simultaneously, the phosphorylation coupled to cytochrome *c* oxidation is abolished and may be restored by addition of the purified exchange enzyme to the depleted mitochondria. The exchange enzyme appears to correspond in its action and properties to the coupling factor III described by Green and co-workers. Kinetic studies indicate that ferro-cytochrome *c* combines at two sites on the exchange enzyme; interaction at one site is blocked by dinitrophenol, while azide or oligomycin A prevent combinations at both sites.

W. Chefurka (Agricultural Research Institute, London) presented comparative data on the lability of phosphorylation reactions in mammalian mitochondria and insect sarcosomes. The rapid aging of insect mitochondria is related to the rapid release of free fatty acids from mitochondrial lipids. The most sensitive indicator of the aging process is the adenosine triphosphate cleavage which occurs in the presence of dinitrophenol. The disappearance of the latter enzyme on aging may be reproduced in vitro by addition of fatty acid.

The symposium was thoroughly and efficiently organized by a local committee under the chairmanship of K. P. Strickland. Fortunately, the organization was not so rigid as to preclude free discussion of the stimulating data and ideas presented in the formal papers. Moreover, the speakers and participants widened their field of interest to take part in several interesting functions concerned with nutrition, digestion, and the products of fermentation. Plans are under way to publish the papers in the *Canadian Journal of Biochemistry*.

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Nerve as a Tissue

The biochemical and biophysical bases of brain functions are being increasingly studied and understood. In view of the rapid development of this field, the choice of "Nerve as a Tissue" as the subject of the fourth Lankenau conference on tissue, held 12–13 November 1964 in Philadelphia, was especially appropriate.

At the session on morphology, S. L. Palay (Harvard Medical School) discussed the way in which nerve cells and their processes are interconnected and, especially, the way in which the surrounding cells are related to the nerve cell. He showed that the glial fibers and glial processes are distributed throughout the neuropil of the system in a specific pattern. Palay suggested that the terminals within a single compartment of the glia arise from the same cell or from cells that have similar significance with respect to the postsynaptic element; the compartments of glia isolate the postsynaptic membrane from neighboring synapses which have a different significance with respect to the postsynaptic element. This Palay interpreted to mean that terminals lying within adjacent or neighboring compartments may be presumed to originate from different perikarya.

J. D. Robertson (Harvard Medical School and McLean Hospital) talked about intimate details of membrane structure, the relations between cells and membranes at their contact points, and the study of these relations by the use of x-ray diffraction techniques in conjunction with electron microscopy. G. D. Pappas (Columbia University) discussed ultrastructure of nerve cell membranes and problems of cell interaction and indicated the existence of a wide variety of interneural relations in the central nervous systems of vertebrates. Pappas discussed the various morphological criteria that have proved useful in correlating structure and function.

Eduardo De Robertis (Universidad de Buenos Aires) reviewed synaptic complexes and synaptic vesicles as structural and biochemical units of the central nervous system and described a technique of osmotic shock which permits isolation of synaptic vesicles from the nerve endings. He showed that synaptic vesicles are storage units for acetylcholine, norepinephrine, dopamine, and probably other transmitter substances in the brain.

David Nachmansohn (Columbia University), presiding over the biochemistry session, presented a paper on molecular forces which control bioelectric currents in membranes and concluded that the theory of neurohumoral transmission is no longer tenable. O. H. Lowry (Washington University School of Medicine) reviewed the chemistry of the nerve cell. R. J Rossiter (University of Western Ontario) discussed biosynthesis of phospholipids and sphingolipids in the nervous system, concluding that in brain and nerve, as in most other tissues, phospholipids and sphingolipids are formed *in situ* from appropriate smaller molecules; he pointed out that the brain of a young animal, taken at the time of rapid myelin deposition, serves as an excellent source of many of the enzymes responsible for the biosynthesis of complex lipids.

Hans Weil-Malherbe (St. Elizabeth's Hospital, Washington, D. C.) spoke on storage and metabolism of neurotransmitters, and Eugene Roberts (City of Hope Medical Center, Duarte, Calif.)