Coupling of Phosphorylation and Oxidation Processes

THE biochemical phosphorylation reactions may be classified into three main groups: (1) The hydroxyl groups in organic molecules form phosphoric esters reversibly with inorganic phosphates, in which case the free energy of the phosphate linkage is small and of the order 1,000–2,000 calories. (2) The process of esterification is coupled with an oxidation process and is also reversible. The free energy of this type of phosphate linkage is of the order of 10,000 calories. (3) Exchange occurs between the phosphate group and hydroxyl groups in the same molecule or in different molecules, either reversibly or irreversibly. The mechanism of the coupling of the oxidation and the phosphorylation in the second group of reactions has not yet been clarified, although in many instances there is fairly complete information with regard to the chemical substances and stable intermediates taking part in the reactions.

The processes belonging to the second category are presumably those for which the activation energy of the direct addition of phosphoric acid and the elimination of water is too high for the reaction to occur at measurable speeds. A coupled oxidation process is necessary in order that an alternative path be provided. This may arise if oxidation and phosphorylation occur simultaneously. If the reactant were adsorbed on the surface of a suitable substrate which provided the oxidation mechanism, then it might be so activated that it could be phosphorylated by the normal ester mechanism. There is, however, an alternative explanation by which the phosphorylation could occur freely in the aqueous medium.

In the addition of hydrobromic acid to the double bond, the normal process results in the bromine going to the least hydrogenated of the two doubly bound carbon atoms, yielding the most stable isomer. On the other hand, in the presence of hydrogen peroxide, the addition takes place so as to form the less stable isomer. Kharasch¹ explains this oxidation coupling in terms of a chain reaction in which bromine atoms formed by oxidation of hydrogen bromide attack the double bond directly. It is possible that the oxidation-phosphorylation reactions take a similar course.

Little is known about the free radicals derived from phosphoric acid; but there is considerable evidence from Backström's² work on the reactions occurring during the oxidation of sulphite and Haber's³ work on the action of light on sulphite that the $\text{HSO}_3^\cdot$ radical is present. Analogously, phosphoric acid may yield a free radical in the presence of a high-energy source of oxygen, as follows.

or

A possible source of OH and HO_2 in biochemical systems is the interaction of hydrogen peroxide with iron catalysts⁴.

The free radicals may bring about biochemical oxidation-phosphorylation reactions of the following type:

W. A. Waters⁵ has discussed this reaction (V) from the point of view of the interaction of phosphoric acid with a carbonyl radical to give a carboxyl phosphate.

The effect of the oxidative breakdown of phosphoric acid would be to permit of the transfer of some of the energy of the oxidation-reduction mechanism to the carbonyl group and its preservation in the phosphorylated carboxyl group. It would also allow of the reduction of the activation energy of phosphorylation.

The lack of evidence bearing on the existence of free radicals from phosphoric acid makes it not worth while pursuing the above theory very far. The suggestion would, however, appear to open up possible lines of investigation into oxidative phosphorylation in reactions occurring in the absence of the special characteristics of the living cell.

C. E. H. BAWN
W. E. GARNER

Department of Inorganic and Physical Chemistry,
University, Bristol 8.

¹ Mayo and Walling, *Chem. Rev.*, **27**, 351 (1940).

² Backström, *Trans. Farad. Soc.*, **24**, 601 (1928).

³ Haber and Wansborough-Jones, *Z. phys. Chem.*, **B**, **18**, 103 (1932).

⁴ Haber and Weiss, *Proc. Roy. Soc., A*, **147**, 332 (1934).

⁵ Waters, W. A., *Trans. Farad. Soc.*, **39**, 145 (1943).

Fibrillar Structure of Cellulose of Bacterial and Animal Origin

It has been demonstrated both by electron ray and by ordinary and polarized light microscopy that the cellulose cell-wall of green plants has a fibrillar structure^{1,2}. Since bacterial cellulose (*Acetobacter xylinum* zooglea) and animal cellulose (tunicin) may be assumed to be formed in conditions which differ radically from those which govern cell-wall deposition in green plants, it appeared to be of interest to investigate the microscopic structure of these natural cellulose membranes.

Direct microscopic examination in ordinary light of a thin cellulose zooglea formed by *Acetobacter xylinum* on the surface of a suitable culture medium³ reveals a matrix which seems to be optically empty and within which micro-organisms in random or chain arrangements may be discerned. The picture is indeed very suggestive of that exhibited by a lëvan- or dextran-forming organism when it is cultivated on a sucrose medium. The *xylinum* cellulose zooglea was found to have a definite substructure, however, when it was examined in suitable conditions under dark-field illumination as described below.

A membrane freshly taken from a culture was cut by frozen section technique either parallel or normal to the membrane surface in slices about 10μ thick. The sections were immediately mounted in water and examined with a high dry lens using a cardioid dark-field condenser. With this illumination, the material is revealed as a web of numerous slender discrete fibrils among which individual bacteria can easily be discerned as separate entities.

An alternative method of revealing the fibrillar structure of bacterial cellulose is the following: an undried membrane is treated in several volumes of water for a few minutes in a Waring Blender. The foil is rapidly disintegrated by this treatment and an opalescent fluid which presents properties of a stable colloidal solution results. The fluid is actually, however, a pulp of floating large floccules which are revealed by the dark-field microscope as tangled masses of long fine tenuous fibrils and bacteria.

Animal cellulose (tunicin) was disintegrated in a Waring Blender and examined with the same technique. It was thus shown that this membrane, too, consists of microscopic fibrils.

The microscopic fibril found in the zooglea of *Acetobacter xylinum* gives the typical chemical and physical tests of cellulose. In the dark-field microscope, the fibrils appear to have a uniform width. Their length is difficult to determine as their identity is lost within tangles, but protruding ends up to 20μ in length have been encountered. The width of the fibrils is much less than that of a *xylinum* organism.

The technique which we have described reveals a novel orientation of fibrils in relation to the individual bacteria. Very thin and transparent parts of a membrane in incipient formation have been found to be a favourable material for the study of this aspect. In such material, a small number of fibrils is discernible in the field of the dark-ground microscope and the identity of individual fibrils can be followed for a considerable part of their entire length. They are usually straight and form an irregular net the mesh of which is enormous in comparison with the dimensions of the bacterium. The arrangement of the bacteria in the field is irregular and they show no spatial orientation in relation to the fibrils. Furthermore, bacteria separated by mechanical means from the cellulose mesh of a *xylinum* membrane are never seen to carry microscopically visible fibrillar appendages. These observations seem to exclude the possibility that the fibril in *Acetobacter xylinum* zooglea is a hair-like outgrowth of the cell-wall. They support the conception that the fibril is formed by an extracellular process of cellulose crystallization.

It may be remarked that the X-ray diffraction analysis of bacterial cellulose accords well with its microscopically fibrillar nature⁴. The demonstration of fibrils as the basis of structure of the *xylinum* zooglea suggests an explanation for specific chemical and physical properties of this cellulose. Furthermore, it affords a new approach to the problem of the mechanics of fibril formation.

A detailed report of these findings is in preparation.

M. ASCHNER

Department of Hygiene and Bacteriology,

S. HESTRIN

Hormone Research Laboratory,
Hebrew University,
Jerusalem,
March 7.

¹ Compare review in "High Polymers", vol. 5, edited by E. Ott (Interscience Publishers, Inc., New York, 1943).

² Barnes, R., and Burton, C., *Ind. Eng. Chem.*, **35**, 120 (1943).

³ Aschner, M., *J. Bact.*, **33**, 249 (1937).

⁴ Sisson, W., *J. Phys. Chem.*, **40**, 343 (1936).

Antithyroid Activity of Thiouracil Derivatives

A RECENT report by Anderson *et al.*¹ on the antithyroid activity of a series of alkyl derivatives of thiouracil shows that peak activity is reached at the *n*-propyl compound. A similar series of compounds is under investigation in this Laboratory, and some preliminary results may be of interest as confirming those of Anderson *et al.* in regard to the high activity of the *n*-propyl derivative. The substances under examination comprise methyl, ethyl, *n*-propyl, isopropyl and *n*-amyl thiouracil, and preliminary screening experiments indicated that the *n*-propyl compound was probably the most active of the series. Our experiments are not yet sufficiently complete to allot comparative values to the whole series, but they confirm the preliminary findings of the high activity of the *n*-propyl compound.

The work was carried out on inbred Wistar rats, weighing 100–200 gm., and the compounds were administered by stomach tube daily for 28 days. The rats were then killed and the thyroid gland cleanly dissected, rapidly weighed on a torsion balance and fixed in Bouin's fluid. Typical results were as follows: