mevalonate (20 µg/ml) the concentrations of radioactive bactoprenol were again found to be in the same ratio: 6750 c.p.m./mg of protein in the mesosomes and 7700 c.p.m./mg of protein in the plasma membrane. This showed that bactoprenol is not initially synthesized in the mesosome membrane and then transferred to the plasma membrane. The same phenomenon is true for the fatty acids and phospholipids of the mesosome and plasma membranes of *Micrococcus lysodeikticus* and *Bacillus megaterium* (T. D. Thomas & D. J. Ellar, unpublished work; M. J. Daniels, unpublished work).

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Control of Respiration in *Azotobacter vinelandii* Membranes

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In spite of many previous reports that bacterial respiratory membranes catalyse oxidative phosphorylation (see Gel'man, Lukyanova & Ostrovskii, 1967), only a very few of these membrane preparations (see John & Hamilton, 1970; Pandit-Hovenkamp, 1971; Eilermann, Pandit-Hovenkamp & Kolk, 1970; Jones & Ackrell, 1971) exhibit significant ADP + P_i-dependent respiratory control as defined by Chance & Williams (1956).

As part of a general study of the energy-conservation system of the nitrogen-fixing aerobe *Azotobacter vinelandii* (Ackrell & Jones, 1969, 1971a,b) we have investigated in detail various parameters that affect the ability of partly coupled (P/O, NADH ≤ 1.10) respiratory membranes from this organism to exhibit respiratory control. We have concluded that significant respiratory control is possible only if an energy-coupling site and the rate-limiting step in electron transfer from substrate to oxygen are coincident. If they are naturally coincident, or are made coincident experimentally (see Pandit-Hovenkamp, 1971; Jones & Ackrell, 1971), the respiratory control index is directly proportional to: (1) the efficiency of energy coupling at the controlling site (assayed as the P/2e ratio) and (2) the electron pressure at the controlling site (determined from the electron-transfer rates over different segments of the respiratory chain).

Each of these parameters was varied experimentally either *in vivo* (by using membranes prepared from cells harvested at different stages of the growth curve) or *in vitro* (by exposing the isolated respiratory membranes to a variety of electron donors and acceptors, or electron-transfer inhibitors and uncouplers). By means of such experiments respiratory control was readily demonstrated at phosphorylation sites I and II, and the respiratory control index could be reproducibly manipulated to yield values between 1.00 and 1.65.

These experiments will be described in detail and the conclusions will be discussed in terms of the special role of respiration in the aerobic growth of this organism on atmospheric nitrogen.

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Membrane-Bound Enzymes in Adrenal Medulla: An Adenosine Triphosphatase Characteristic of the Golgi Apparatus

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The subcellular localization of some phosphohydrolases in the bovine adrenal medulla has been studied by centrifugation techniques and by histochemical analysis with the electron microscope.

The following enzymes were mainly recovered in the microsomal fraction of homogenates: Mg²⁺-stimulated adenosine monophosphatase, adenosine diphosphatase and uridine diphosphatase; Ca²⁺-stimulated inosine diphosphatase and adenosine triphosphatase. The presence of a Ca²⁺-stimulated adenosine triphosphatase in the microsomal fraction

has already been reported (Banks, 1965; Goz, 1967). The adenosine triphosphatase has optimum activity at pH 9–11 and pH 6.5. At a low concentration of Ca^{2+} (5mM) the activity at pH 6.5 was 30% of that at pH 9; at a higher concentration of Ca^{2+} (40mM) the activity at pH 6.5 was the same as that at pH 9. These properties are similar to those of myosin adenosine triphosphatase.

When the microsomal fraction was subjected to isopycnic density-gradient centrifugation in sucrose (0.7–1.7M) the particles containing the Ca^{2+} -stimulated adenosine triphosphatase activity could be distinguished from particles containing adenosine monophosphatase activity (presumably derived from the plasma membrane; see Wood, 1967) and from particles containing glucose 6-phosphatase activity. The distribution of the adenosine triphosphatase activity among the density-gradient fractions was, however, similar to that of thiamin pyrophosphatase, an enzyme that has been found in the Golgi apparatus of adrenal medulla by electron-microscopic histochemistry (Holtzman & Dominitz, 1968). These biochemical experiments raise the question whether the Ca^{2+} -stimulated adenosine triphosphatase is a component of the membranes of the Golgi apparatus.

To see whether this adenosine triphosphatase activity could also be demonstrated by a histochemical method, pieces of hamster adrenal medulla (fixed in 2.5% glutaraldehyde) were incubated in the same medium used for the biochemical experiments, i.e. ATP (2mM), calcium nitrate (40mM), tris (50mM) and succinic acid (50mM) titrated to pH 6.5 with sodium hydroxide, with the addition of lead nitrate (2.5mM). After incubation, the tissue was fixed in osmium tetroxide and prepared for electron microscopy. Reaction product was sometimes found in association with the plasma membrane of chromaffin cells and, more often, on the membranes of axons of the splanchnic nerve. However, in agreement with the biochemical findings, the reaction product was found most frequently between the membranes and in the vesicles of the Golgi apparatus.

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Stereochemical and Energetic Evaluation of the Phosphorylation of Histones in Chromatin

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It has been experimentally established (e.g. Stevely & Stocken, 1968) that chromosomal biosynthetic activities involve preliminary phosphorylation of the hydroxyl groups of serine/threonine. I have investigated relevant possible mechanisms using stereochemical and energetic considerations. Numerous correctly proportioned space-filling molecular models of histone-DNA (double helix) were constructed, similar to those previously reported (e.g. Lewin, 1967, 1969a,b, 1970a,b), in which the hydroxyl groups of serine/threonine were involved in hydrogen-bond donation to the hydrogen-bond acceptor groups already involved in hydrogen-bonded base-pairing (Watson & Crick, 1953; Pauling & Corey, 1956). The histone group's hydrogen-bond capacity to partially weaken the base-pairing hydrogen bonds enhances an increase in their length, thereby allowing the normally rigid double helix of DNA a 'bending latitude' of several degrees per each set of di-trinucleotides (Lewin, 1970a). Phosphorylation of the histone's hydroxyl groups has been computed to result in: (1) steric hindrance to the local adherence of histone to the base-pairing sites; (2) local repulsion away from the nearby DNA's phosphate group; (3) overall lowering of the isoelectric point of histone; (4) effective competitive attachment to a sufficiently nearby positively charged histone group initially attached to a DNA's phosphate group (because the newly phosphorylated group is likely to be double negatively charged, in contrast with the single negative charge in a DNA's phosphate group); (5) increased probability of rupture of any sufficiently near inter-histone's carboxylate-ammonium linkages by substitution of an intra-histone phosphate-ammonium linkage accompanied by liberation of the carboxylic acid groups.

All the above factors enhance local turning away of the relevant histone segment from the DNA's wide groove (and even rupturing local ribose phosphate ionic linkage with the positively charged group of the histone, thereby causing total local detachment) and exposing the originally masked area to initiation of biosynthetic processes. It is interesting that, taking into consideration the restrictions on the construction of the double-helix of the B-form of DNA (Lewin, 1969b, 1970b), the most likely groups to be unmasked in the wide groove are those of guanine, adenine and thymine.

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