conditions. These and other differences in protein composition are reflected in the results of polyacrylamide-gel electrophoresis of the two fractions.

On analysis, mesosomal membranes are found to contain only half the amount of phospholipid by weight that is found in the plasma membrane, but approximately the same amount of protein. Appreciable quantitative differences in lipid carbohydrate, carotenoid and menaquinone are also observed. With respect to the composition of the phospholipid fraction, there is no significant difference between the two membrane types. In both cases this is diphosphatidylglycerol (67%), phosphatidylglycerol (27%) and phosphatidylinositol (6%). The fatty acid compositions of the two fractions are also similar, with a C₁₅ branched-chain fatty acid constituting more than 90% of the total.

Experiments were carried out to investigate the possibility that the bacterial mesosome represents the primary site of membrane lipid synthesis. When exponentially growing cultures of *M. lysodeikticus* and *B. megaterium* KM were given short pulses of [³²P]P_i and [¹⁴C]acetate, the specific radioactivities of total lipid and individual phospholipids in both plasma membrane and mesosome membrane were very similar. The same result was obtained with organisms growing at different rates and also when the pulse was followed by a period of growth in non-radioactive medium. These results indicate that the mesosome is not a preferential site for phospholipid synthesis in bacteria, as has been suggested (Fitz-James, 1967).

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The Bactoprenol Content of Plasma and Mesosome Membranes from *Lactobacillus casei*

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Protoplasts can be prepared from *Lactobacillus casei* (A.T.C.C. 7469) with lysozyme provided that trypsin and 4.5mM-EDTA are present (Barker &

Thorne, 1970). The presence of EDTA in the 'protoplasting' medium prevents the liberation of mesosomes from the protoplasts by controlling the Mg²⁺ concentration, as described by Reaveley & Rogers (1969) for *Bacillus licheniformis*. It has now been found possible to obtain protoplasts of *L. casei* by pretreating the cells for 10min at 37°C with 0.2mM-EDTA in 0.12M-tris-HCl at pH 7.6, and then incubating them with lysozyme (0.08mg/ml) in 5mM-sodium phosphate, pH 7, 0.6M-sucrose and concentrations of magnesium chloride from 0 to 20mM, for 60min at 37°C. Trypsin is not required. The EDTA pretreatment releases protein from the bacteria (2mg of protein/100mg dry wt.)

Electron microscopy of the protoplasts and of material released from the protoplasts showed that mesosomal vesicles were liberated when protoplasts were made without magnesium chloride but not when made in 10mM-magnesium chloride. In the absence of magnesium chloride 42% of the cell C₅₅ isoprenoid alcohol, bactoprenol (Thorne & Kodicek, 1966), was released with the mesosomes, and in 10mM-magnesium chloride only 5%. About 46% of the mesosome membrane, as measured by bactoprenol recovery, could be obtained as a pellet by centrifuging at 100 000g for 2h the material released when protoplasts were made in the absence of magnesium chloride. Plasma membrane was prepared from protoplasts made in the absence of magnesium chloride by disrupting them in water and collection at 38 000g for 30min. The membranes were washed once with water and freed of attached lysozyme by gel filtration through columns of Sepharose 2B in water. This plasma membrane contained 36% of the cell bactoprenol. From 100mg dry wt. of cells 2.2mg of mesosome membrane protein was recovered, at an efficiency of 46%, and 4.2mg of plasma membrane protein. Although the bactoprenol was about equally distributed between mesosome and plasma membrane its concentration was also the same. By growing the bacteria with saturating concentrations of [2-¹⁴C]mevalonate (20 µg/ml; Thorne & Kodicek, 1962) concentrations of 2.5 µg of bactoprenol/mg of protein and 2.6 µg of bactoprenol/mg of protein were obtained for the mesosome and plasma membrane respectively.

Evidence was obtained that the biosynthesis of bactoprenol occurs in the mesosome and plasma membranes simultaneously. After a 10min incubation with [2-¹⁴C]mevalonate the concentration of radioactive bactoprenol was 114 c.p.m./mg of protein in the mesosomes and 113 c.p.m./mg of protein in the plasma membrane, showing that the ratio of the concentrations of newly synthesized bactoprenol did not differ from the ratio of the total amount in the membranes. When cells containing [¹⁴C]-bactoprenol were incubated for 2h with unlabelled

mevalonate (20 $\mu\text{g/ml}$) 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, $\text{NADH} \leq 1.10$) 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: Mg^{2+} -stimulated adenosine monophosphatase, adenosine diphosphatase and uridine diphosphatase; Ca^{2+} -stimulated inosine diphosphatase and adenosine triphosphatase. The presence of a Ca^{2+} -stimulated adenosine triphosphatase in the microsomal fraction