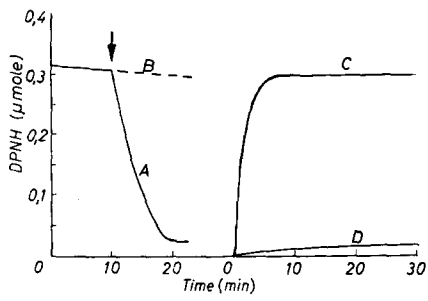

Fig. 1. Reversibility of the 5-ketogluconoreductase reaction in *Klebsiella* 5K3B extract as measured in the Beckman spectrophotometer model DU. Left part: reaction of 5-ketogluconate with DPNH. The system contained in 3 ml 0.02 *M* tris (hydroxylmethyl)aminomethane–HCl buffer, pH 7.2 : 0.3 μmole DPNH; 0.025 ml enzyme; 2 μmoles 5-ketogluconate (curve A) or water, D-fructose, L-sorbose or 2-ketogluconate (curve B) is added at the arrow. Right part: reaction of gluconate with DPN. The system contains in 3 ml 0.02 *M* glycocoll buffer, pH 10; 50 μmoles sodium gluconate; 0.3 μmole DPN and 0.1 ml enzyme (curve C). Curve D: without gluconate.

However, there is still another pathway for 5-ketogluconate metabolism. By the manometric method of COLOWICK AND KALCKAR[10], a 5-ketogluconokinase was detected. 5-Ketogluconate did not disappear in the absence of ATP. The kinase was present in greatest activity in the *Klebsiella*'s. It could hardly be detected in the other strains. Concomittant analysis of 5-ketogluconate disappearance and CO_2 formation during the phosphorylation showed that about 2 moles of CO_2 are liberated per mole substrate metabolized. The reaction thus proceeds beyond the primary phosphorylation, which we assume to be the formation of 5-keto-6-phosphogluconate. A calculation of the relative importance of either pathway, based on the enzyme activities in the extracts, showed that 5-ketogluconate is metabolized about 300 times as fast by the reductive pathway as by the phosphorylative. That the former pathway is physiologically the more important in our bacteria follows also from the above mentioned experiments on the sequential induction pattern of resting cells. Work on the mechanism of the phosphorolytic pathway is in progress.

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The path of electrons in the respiratory chain of enzymes

Kinetic studies of the respiratory enzymes of whole wheat roots and baker's yeast[1,2,3] have shown that the main enzymes, viz. cytochromes a_3, *c* and *b*, flavoprotein (FP) and diphosphopyridine nucleotide (DPN), are reduced in a characteristic time order, if the cells or tissues are exposed to anaerobiosis. One experiment with yeast yielded the following figures of the start times following a shift from air to N_2:

a_3	*c*	*b*	FP	DPN
0.5	1.0	8.2	7.4	24.7 sec

The reoxidation (shift from N_2 to air) runs considerably faster. It could be shown, however, that the time order is now reversed, *i.e.* cytochrome *b* is more rapidly oxidized than cytochrome *c*, etc. As shown from continuous records of the spectral bands at increasing reduction after anaerobiosis, the group cytochromes a_3–*c* is nearly reduced before the group cytochrome *b*–FP starts reduction, and DPN starts reduction about simultaneously with the completed reduction of cytochrome *b*–FP.

This rhythmic procession of the reduction cannot easily be explained on the basis of free combination of the reacting enzymes. A possible explanation is, however, an interaction of multimolecular groups. The well-known fact that some of the enzymes, in particular cytochromes a_3 and b, are intimately attached to the structure of the protoplasm, supports this idea. Organization means a more or less crystal-like arrangement of molecules and it is reasonable to assume that such a tendency prevails during the formation of the structures. Each one of the groups cytochrome a_3–c and cytochrome b–FP may thus form multimolecular groups joined by a reaction centre which is composed of a much smaller group of a transferring enzyme, *viz.* a "factor". Because cytochromes a_3 and b are decidedly particle-bound, whereas cytochrome c and FP partly go into the supernatant, if the cells are homogenized[4], it can be pictured that cytochromes a_3 and c, and cytochrome b and FP, respectively, form "mixed crystals", *viz.* molecular groups in which the structural orientation of the molecules is determined by the particle-bound enzymes.

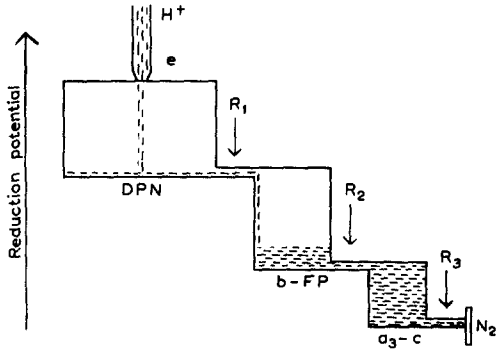

Fig. 1

The operation of an electron ladder composed by multimolecular groups joined by reaction centres is visualized by the scheme in Fig. 1. The enzyme groups are represented by a series of communicating vessels, the height of which corresponds to the oxidation–reduction potentials. The connecting tubes represent the reacting centres (R), or "factors", the flowing water the stream of electrons. If the outflow from cytochromes a_3–c is closed (= shift from air to N_2) this vessel will be filled (= reduced) before the level of cytochrome b–FP can start rising, etc. At reoxidation (= shift from N_2 to air) the outflow from cytochromes a_3–c is first noticed as a sinking level of DPN. In the steady state of continuous flow (= normal respiration) the level in each vessel will depend upon the relative volume of the vessels (= number of enzyme molecules), the intensity of the flow (= the actual electronic activity of the total system), and the turnover capacity of the reaction centres (= the effectivity of the factors).

The existence or non-existence of multimolecular groups of respiratory enzymes can presumably be tested by means of electron microscopy. Of special interest is to know if the molecules are arranged in layers or in more crystal-like structures. As to the proposed "reaction centres" attention is called to results obtained from studies of photosynthesis[5,6]. These have shown that large groups of chlorophyll molecules are probably transmitting light energy *via* reaction centres to other biochemical systems which are using this energy for reduction of carbon dioxide.

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