the sequence of energy transfer prior to the damage by FDNB, viz. in the inhibition of the substrate chain phosphorylation. A major decrease in creatine phosphate content was associated with this type of intoxication[9]. Although the ATP content was similar in both types of poisoning, the ATP/ADP ratios differed greatly. After administration of iodoacetate, the ADP content was increased; FDNB produced a marked tendency towards decrease. This, too, suggests that the reduction of the ATP concentration by FDNB is limited to the fraction at the contractile proteins, as the adenylate kinase activity occurs here. The point of attack of iodoacetate in the breakdown of substrate was also marked by the accumulation of fructose-1,6-diphosphate and dihydroxy acetone phosphate before the glyceraldehyde phosphate dehydrogenase reaction. The quantity of phosphate bonded in these 2 metabolites explains the difference in the inorganic phosphate concentrations.

Zusammenfassung. Nach 1-Fluor-2,4-dinitrobenzol-Vergiftung von perfundierten Kaninchenherzen waren bei vollständiger Insuffizienz der ATP-Gehalt sehr signifikant und der Kreatinphosphatgehalt weniger stark vermindert. Glykogen-, Glucose- und Lactatgehalt unterschieden sich von den Kontrollwerten nicht.

G. Gercken und U. Schlette

Chemisches Staatsinstitut der Freien und Hansestadt Hamburg, Abteilung für Biochemie, 2000 Hamburg (Germany), 2 August 1967.

Reduction of Cytochrome *c* by the Mitochondrial Respiratory Chain across a Semi-Permeable Membrane

The succinate-cytochrome *c* reductase system has been defined as a fragment of the respiratory chain capable of catalysing the oxidation of succinate by external cytochrome *c*[1].

The components of such a system and their interrelations have been extensively studied[2], but it is not yet clear how the reduction of the acceptor takes place.

The purpose of this communication is to describe an experimental device that can be used to study the mode of reduction of the terminal acceptor in the succinate-cytochrome *c* reductase system.

We have found that cytochrome *c* reduction can be achieved even when the oxidized cytochrome *c* is separated from the other catalytic components of the system by a semipermeable membrane. The apparatus employed for the study of this reduction is shown in Figure 1.

Membrane was prepared from rolls of commercial brand 'Dexstar' (Visking) 0.064–0.025 mm thick. The reaction was followed by reading the increase of absorbancy at 550 nm on a Beckman DU spectrophotometer. Cyanide was used to inhibit cytochrome *c* oxidase activity. The experimental conditions are described in the caption to Figure 2. The reaction was started by adding cytochrome c in chamber B (Figure 1) after 10 min of preincubation. The submitochondrial particles used as enzyme source were prepared from beef heart mitochondria according to Crane et al.[3].

In Figure 2 cytochrome *c* reduction under normal conditions (no semipermeable membrane) is compared with the reduction that takes place in the presence of a semipermeable membrane.

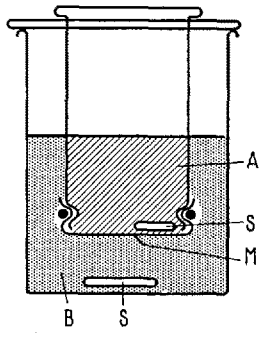

Fig. 1. Experimental device used for reduction through membrane. A, internal chamber (submitochondrial particles and substrate); B, external chamber (cytochrome *c* and substrate); S, magnetic stirrer; M, membrane (diameter 3.2 cm).

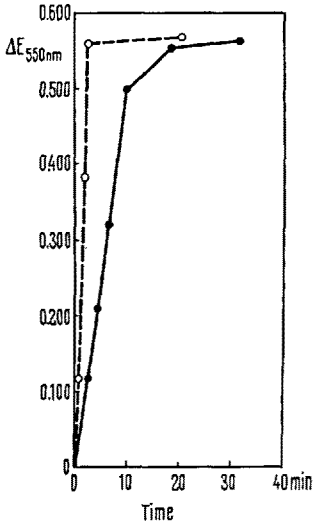

Fig. 2. Comparison of the rate of reduction of cytochrome *c* by submitochondrial particles. Incubation conditions for the reaction. Control: 0.1 *M* phosphate buffer pH 7.3; 2 m*M* succinate; 0.4 m*M* MaCN; 57 m*M* cytochrome *c*; mitochondrial protein 300 µg. Final volume 3.5 ml. In the presence of a membrane the reagent concentration was the same as for the control. Total volume in compartment A was 5.5 ml and in compartment B 12.5 ml. o----o control reaction; •——• reaction across a semipermeable membrane.

[1] T. P. Singer and E. B. Kearny, Enzymes 7, 387 (1963).
[2] S. Takemori and T. E. King, J. biol. Chem. *293*, 3546 (1964).
[3] F. L. Crane, J. L. Glenn and D. E. Green, Biochim. biophys. Acta *22*, 475 (1956).

The rate of cytochrome *c* reduction across the membrane can be controlled either by the surface area or by the thickness of the membrane. Buffer, substrate (succinate), submitochondrial particles and cyanide are required in order to produce the reduction.

In addition to reduction of cytochrome *c* across the membrane, a number of phenomena have been found to be associated with this reaction: (a) calcium ions increase the rate of the reaction only in the presence of a semipermeable membrane; (b) pH changes occur on both sides of the membrane and follow the kinetics of cytochrome *c* reduction; (c) gramicidin, a specific membrane potential-collapsing reagent[4], has an inhibitory effect on the reduction of cytochrome *c*. No inhibition has been observed in normal reduction.

In order to explain the possible mechanism of this reduction across the membrane, we have investigated the possibility of some soluble and dialysable component that could be released from the particles in our conditions. In fact a soluble factor capable of reducing cytochrome *c* has been detected after dialysis of the particles in the same conditions used for the assay, and its features are now under extensive investigation.

It is possible that such a soluble dialysable factor might be responsible for a shuttle transport of electrons across the membrane.

Otherwise the succinate-cytochrome *c* reductase activity in the presence of a semipermeable membrane seems to share certain features of a chemioosmotic process such as the one postulated by MITCHELL[5], namely the proton-translocating respiratory chain and the exchange-diffusion system coupling proton translocation to that of anions and cations.

Further experiments are in progress to utilize extensively this apparatus and system in order to evaluate the basic transfer of electrons in the respiratory chain and to investigate the characteristics of dialysable soluble factors that could play a fundamental role in such a process.

Riassunto. La riduzione del citocromo *c* può aver luogo anche quando sia separato dalla catena respiratoria mitocondriale da una membrana. L'azione del calcio e della gramicidina sono caratteristiche distintive tra la riduzione attraverso la membrana e la riduzione diretta.

L. GALZIGNA and L. SARTORELLI

Istituto di Chimica Biologica, Università di Padova (Italy), 31 July 1967.

[4] J. B. CHAPPELL and A. R. CROFTS, Biochem. J. *95*, 393 (1965).
[5] P. MITCHELL, Publ. No. 66/1 of Glynn Res. Ltd. (1966).

A Lytic Factor Associated with Brucellaphage Causing 'Lysis-from-without'

Concentrations of *Brucella abortus* phage, greater than the routine test dilution, cause lysis or inhibitory reactions on lawns of *Brucella suis*, *Brucella neotomae*, and some *Brucella melitensis* cultures, although the phage does not replicate on these cultures. An attempt was made to associate this phenomenon with a the activity of a lytic enzyme causing 'lysis-from-without'[1–4]. The present report describes the tube-test developed with *B. abortus* cells and demonstrates that lytic activity is bound to the infective phage particle. Studies relating the specificity of the lytic factor with phage typing reactions on other *Brucella* species will be reported elsewhere.

Materials and methods. The source and characteristics of the brucellaphage and its host propagating strain *B. abortus* R19 have been described previously[5]. Trypticase soy broth (BBL) was employed for growth of cells. High titer phage was obtained by centrifuging phage infected broth cultures before lysis occurred, resuspending cells in a small volume of *Tris* buffer and allowing lysis to proceed. The phage used in this study had a count of 2×10^{12} plaque-forming units (PFU)/ml and was stored at -20 °C. For demonstration of the lytic reaction, early log phase broth cultures incubated at 37 °C on a shaker were used within a few min of removal from the shaker. Glycine and ethylenediamine tetra acetic acid (EDTA) were added at final concentrations of 0.3 *M* and 0.0013 *M* respectively. The reaction mixture consisted of 1.6 ml broth culture ($2–3 \times 10^9$ cells/ml), 0.3 ml of 3 *M* glycine, 0.1 ml of 15% EDTA, 1 ml phage dilution in 0.1 *M Tris* buffer, pH 8. The change in optical density (O.D.) was measured in a spectrophotometer at intervals during incubation without shaking in a 37 °C waterbath. The % of the initial O.D. was determined at each time interval and plotted on semi-log paper.

Results. Figure 1 shows the decrease in O.D. with time, observed with various ingredients of the reaction mixture singly and in combination. Glycine and phage, or EDTA and phage do not cause as great a decrease as glycine, EDTA and phage.

Citrate (0.3 *M*) could be substituted for EDTA, and phosphate buffered saline (0.1 *M*, pH 8) could be substituted for *Tris* buffer. The rate of the reaction was greater at pH 7.8–8 than at pH 6.5–7, and at 37–40 °C than at 25–32 °C. No reaction occurred at 5 °C. Cells killed by toluene, heat, acetone, ether or formalin were not lysed. Chloramphenicol treated cells (25 µg/ml broth culture for 30 min at 37 °C) were lysed. Other genera (*Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureas* and *Micrococcus lysodeikticus*) were not lysed.

Figure 2 shows the kinetics of the reaction using constant substrate and varying amounts of phage. One hundred PFU/cell caused 50% drop in O.D. in about 25 min. An excess of 400 PFU/cell did not speed the reaction time. If fewer than 25 PFU/cell were employed, the time required for lysis approached the latent period of the bacterium-phage system (e.g. 100 min). At least 5×10^{10}

[1] J. S. MURPHY, Virology *4*, 563 (1957).
[2] J. S. MURPHY and L. PHILIPSON, J. gen. Physiol. *45*, 155 (1962).
[3] H. STOLP and M. P. STARR, A. Rev. Microbiol. *19*, 79 (1965).
[4] W. WEIDEL, A. Rev. Microbiol. *12*, 27 (1958).
[5] C. R. MCDUFF, L. M. JONES and J. B. WILSON, J. Bact. *83*, 324 (1962).