non-infected broth cultures + glycine + EDTA). Their lytic activity was as great as, but not greater than could be accounted for on the basis of PFU count. Free lytic enzyme is apparently not released in the brucellaphage system.

Discussion. The phenomenon of 'lysis-from-without' occurs when a large number of phage particles simultaneously attack the bacterium, and the phage enzyme damages the cell wall faster than the cell can repair it[3,4]. Both EDTA[6] and glycine[7] have been used to sensitize gram-negative cells to the action of lysozyme, and EDTA was employed to sensitize *E. coli* K 12 cells to the action of endolysin from lambda phage[8]. These substances also sensitize *Brucella* cells to 'lysis-from-without' by a lytic agent associated with brucellaphage.

The similarities between this lytic agent and the lysozyme associated with other bacteriophage are: (1) its inactivation by proteolytic enzymes, (2) the kinetics of the reaction which show that the decrease in turbidity of the substrate/unit time varies directly with enzyme concentration, and (3) its specificity. The lytic agent could not be separated from the brucellaphage particle by differential centrifugation, anti-phage serum inactivation or by cesium chloride density gradient centrifugation. MURPHY and PHILIPSON[2] described a lytic enzyme which was bound to the *Bacillus megaterium* phage G particle. In contrast, other phage lysins[1,3,4,9] were separable from the phage particle[10].

Résumé. Des bactériophages de Brucella se sont montrés capables de provoquer une lyse extra-cellulaire, en cultures liquides de *B. abortus*, en présence de glycine et d'acide éthylène-diamine-tétraacétique. Des traitements au sérum anti-bactériophage, aux enzymes protéolytiques, ou la centrifugation différentielle dans le chlorure de césium n'ont pu séparer l'activité lytique de la particule infectieuse elle-même.

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Two $NADH_2$ Dehydrogenases in the Basidiomycete *Oudemansiella mucida*

During our study of oxidation-reduction systems in higher fungi, we followed enzymatic oxidation and dehydrogenation of nicotinamide adenine dinucleotide ($NADH_2$) as the first step in the respiratory chain, using the basidiomycete *Oudemansiella mucida* (Schr. ex Fr.) Höhnel.

The experiments were carried out with the acetone powders of the mycelium grown for 6 days at 25 °C in a laboratory fermenter at 350 rpm of the stirrer, the intensity of aeration being 25 mmoles O_2/1000 ml medium/h. The nutrient medium contained glucose 5.0%, corn-steep liquor (50% dry weight) 1.5%, $MgSO_4$ cryst. 0.15%, dissolved in tap-water, initial pH 5.5

The first results showed a very slow course of the $NADH_2$ oxidation in the presence of aerial oxygen. It was considered that the acetone treatment could remove some lipophilic limiting factors necessary for the undisturbed transfer of electrons to oxygen. In order to test this possible explanation, the enzymatic dehydrogenation of $NADH_2$ was followed using 2 artificial electron acceptors – 2,6-dichlorophenol-indophenol (DIF) and 2,3,5-triphenyltetrazolium chloride (TTC).

For the estimation of $NADH_2$ dehydrogenase activity the following system was adopted: $NADH_2$ 0.31 μmoles, phosphate buffer 150 μmoles, DIF 1.5 μmoles or TTC 3.0 μmoles, aqueous suspension of the acetone powder or its cell-free extract, total volume 4.0 ml. As the enzyme source, a homogenized suspension of 80 mg acetone powder in 5.0 ml distilled water (preparation A) was used, or the respective cell-free extract prepared by eluting 80 mg acetone powder with 5.0 ml of distilled water for 3 h at 4 °C and centrifuging 2 min at 800 *g* (preparation B), or cell-free extract B diluted 1:5 with distilled water (preparation C). The period of incubation at 27 °C depended on the sort of terminal electron acceptor used and on the concentration of the respective enzyme preparation. It is specified in the Tables.

Continual colorimetry at 600 nm was used for the estimation of DIF reduction, whereas the amount of the red formazane formed by the reduction of TTC was followed using the colorimetry at 480 nm according to a modification of the procedure introduced by LINDEMANN[1].

The results of experiments dealing with $NADH_2$-DIF reductase have shown considerable activity of this enzyme which could be readily eluted from the acetone powder by distilled water (Table I). Its optimal pH was

Table I. Elution of the $NADH_2$-dichlorophenol-indophenol reductase from the acetone powder of *O. mucida*

Enzyme preparation (0.2 ml)	Protein content mg	Extinction at 600 nm after the incubation period (sec)					
		0	1	15	30	45	60
A (suspension)	1.203	0.25	0	0	0	0	0
B (extract)	0.455	0.25	0.15	0.05	0	0	0
C (diluted extract)	0.091	0.25	0.25	0.22	0.19	0.16	0.14

[1] J. LINDEMANN, Schweiz. Z. allg. Path. Bakt. *17*, 311 (1954).

in the range of 6.2 (Table II). Enzyme preparations A and B were so active that it was impossible to follow the reaction course by the method used; DIF was reduced to its leuco-form in a few seconds.

In contrast to the TTC reductase, the activity of the DIF reductase was not influenced by the addition of menadione. Our results revealed that the enzymatic reduction of TTC depends on the presence of catalytic amounts of menadione (0.006 μmoles) which evidently serves as an intermediary electron transferring agent. The other differences of the respective enzyme from the one reducing DIF include its substantially lower activity, optimal pH in the region of 7.4 (Table III) and the lack of evidence for its elution from the acetone powder.

Table II. Effect of pH on the $NADH_2$-dichlorophenol-indophenol reductase activity in *O. mucida*

Enzyme preparation (0.2 ml)	pH	μmoles of reduced DIF/mg protein after 2 min incubation
C	6.2	12.9
	7.4	8.0
	7.9	4.1

Table III. Effect of pH and menadione on the $NADH_2$-triphenyltetrazolium chloride reductase activity in *O. mucida*

Enzyme preparation (0.5 ml)	Menadione 0.006 μmoles	pH	μmoles of reduced TTC/mg protein after the incubation period (min)	
			30	60
A	+	6.2	0	0.02
	+	7.4	0.05	0.15
	+	7.9	0.006	0.06
	−	7.4	0	0

These results suggest that the reduction of DIF and TTC in the basidiomycete *O. mucida* is mediated by 2 enzyme systems differing in their activity, optimal pH, requirements for menadione and by their solubility. The fact that the dehydrogenation of $NADH_2$ with both artificial electron acceptors was faster than its oxidation with aerial oxygen as terminal acceptor supports our view about the removal of some lipophilic cofactors, necessary for the transfer of electrons to oxygen, during the acetone treatment of the mycelium. This view is in accord with the results of LESTER and FLEISCHER[2], KLUGE et al.[3], DOWNEY[4] and other authors.

The low activity of the $NADH_2$-TTC reductase even in the presence of menadione could be caused by the not fully adequate function of this compound in the respective enzymatic process in *O. mucida*; other compounds of the quinone type (e.g. CoQ, other K-vitamins etc.) were not tested. On the other hand, the positive effect of menadione on enzymatic dehydrogenation of $NADH_2$ was observed also in *Puccinia graminis*[5].

Zusammenfassung. In der Basidiomycete *Oudemansiella mucida* (Schr. ex Fr.) Höhnel wurde eine $NADH_2$-Dichlorophenolindophenol-Dehydrogenase und eine $NADH_2$-Menadion-Triphenyltetrazoliumchlorid-Dehydrogenase gefunden. Beide Enzyme unterscheiden sich durch ihre pH-Abhängigkeit, Aktivität und Eluierbarkeit.

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Effect of Silica Dusts on Macrophage Permeability Studied with $^{86}RbCl$

The intracellular ion environment seems to influence greatly the activity of many apparently regulatory reactions in the cell[1]. Alterations in ion distribution in cells by impaired plasma membrane permeability have been suggested to imbalance the cell metabolism and to underlie the initial stages of cell necrosis[2,3]. Movements of Na^+ in and K^+ out occur in injured cells, and increased intracellular Ca^{2+} concentrations interfere with Na^+ and K^+ transport[4,5]. Evidence for altered plasma membrane permeability is possibly the appearance of cell enzymes in the extracellular fluids[2,3].

This study investigates whether increased permeability phenomena may occur in the toxic action of silica on macrophages. Silica has been shown to release enzymes from phagocytes[6,7] and to cause erythrocyte lysis[8–10]. This might render silica very similar in its action to a fat-active or surface-active agent. Electron microscopy indicates defects in the osmotic control by phagocytes in this condition[11]. Inhibition of macrophage metabolism[12] might so be initiated by ion shifts[13,14].

The $^{86}Rb^+$ efflux from peritoneal macrophages previously loaded with this isotope and incubated with different preparations of silica dust[7] has been studied. Rb^+ is known to behave like K^+, being electrochemically similar and having similar affinities to its carrier[15–19]. Peritoneal macrophages were obtained and handled as previously indicated[7]. Cells were counted and incubated in siliconized flasks with $^{86}RbCl$ $3.7 \times 10^{-6} M$ (Sorin, Saluggia, Italy: specific activity 0.16 mc/mg Rb^+) for 2.5 h at 37 °C in a modified Ringer-*Tris* medium[7] containing $1.5 \times 10^{-3} M$ glucose. Following centrifugation at 1800 *g* for 10 min at 2 °C, washing and recentrifugation, the final cell pellet was suspended to the appropriate concentration in Ringer-*Tris* medium[7] and incubated in 25 ml siliconized flasks in metabolic shaking incubator at 37 °C. Portions of the incubate were withdrawn from