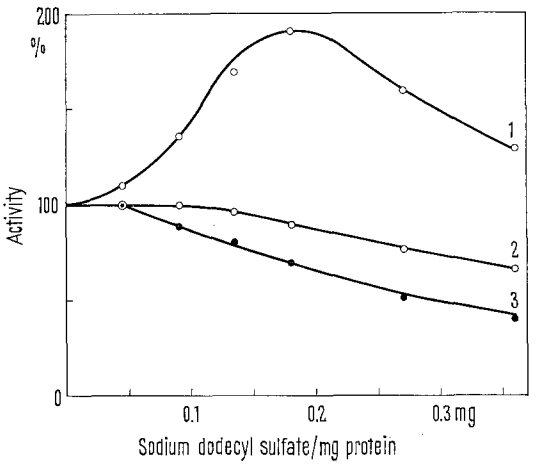
of the activity as with Triton X-100 and succinate-cytochrome c-reductase or as with SDS and NADH-cytochrome c-reductase) or they affect the correct alignment of the enzyme within the complete electron transport system.

In the presence of DOC or Triton X-100, further inactivation beyond the degree obtained by the detergent concentration employed proceeds very slowly and the stimulated activities are practically stable[1,2]. In contrast, samples containing SDS become increasingly inactivated. With a detergent/membrane protein ratio of 0.18 the activities of the Figure (which remain unchanged for 15 min) become inactivated within 3 h: NADH-cytochrome c-reductase by 25%, succinate-cytochrome c-reductase by 75% and NADH oxidase by 80%. Attempts to minimize this inactivation by dialyzing the samples (3 and 6 h against frequent changes of 0.05 *M* phosphate buffer pH 7.5 in the cold) had no effect, indicating an apparent firm bonding of the detergent with the membranes.



The effect of SDS on (1) NADH- and (2) succinate-cytochrome c-reductase and on (3) NADH oxidase of the electron transport particulate fraction of *R. rubrum*. SDS was added in the cold to electron transport particles (4.2 mg protein/ml) to obtain the desired detergent/membrane protein ratio. After 15 min at 0 °C activity was determined.

Inactivation of electron transport enzymes by SDS

mg SDS/ mg protein (min at 20 °C)	Inactivation (%)			
	NADH-cyto-reductase	Succinate-cyto-reductase	NADH dehydro-genase (DCPIP)	Succinate dehydro-genase
0.18 (10)	50	90	12	< 5
0.36 (10)	90	> 95	35	< 5
0.72 (10)	–	–	60	12
0.72 (30)	–	–	85	32

SDS was added to electron transport particles (4.2 mg protein/ml) at 20 °C and activities were determined after the stated time intervals. The values of the 30 min incubation were corrected for a slight inactivation without SDS.

The different stimulation-inactivation behaviour of NADH- and succinate-cytochrome c-reductase in the presence of Triton X-100 and SDS, besides indicating different modes of action of the 2 detergents, favours the idea that these effects are exerted on that part of the electron transport system where 2 distinct pathways exist: NADH → Coenzyme Q and succinate → Coenzyme Q. However neither is NADH dehydrogenase (DCPIP) stimulated nor does succinate dehydrogenase (phenazine methosulfate-DCPIP assay) become inactivated by appropriate concentrations of SDS. Only much higher detergent/membrane protein ratios would affect these activities. Because of the increasing insolubility of SDS at 0 °C, the experiments were carried out at 20 °C where inactivation, as to be expected, is more intensive (see NADH- and succinate-cytochrome c-reductase in the Figure and in the Table). The values of the Table demonstrate that succinate dehydrogenase is even less sensitive to SDS than NADH dehydrogenase. Provided that the determinations of the 2 activities with the artificial electron acceptors have physiological significance, this seems to exclude a direct influence of the detergent on the two flavoproteins of this part of the electron transport system. Glycerol or sucrose (0.5 *M*) which have in some instances been found to stabilize enzymatic activities in the presence of detergents[7] failed to show any protection of NADH- and succinate dehydrogenase.

Zusammenfassung. SDS hat die gleiche solubilisierende Wirkung auf das Membransystem von *Rhodospirillum rubrum* wie DOC und Triton X-100. NADH- und Succinat-Cytochrom-c-Reduktase des partikulären Elektronentransportsystems zeigen in Gegenwart von SDS jedoch ein sowohl von DOC als auch von Triton X-100 verschiedenes Aktivierungs-Inaktivierungsverhalten. Eine direkte Wirkung von SDS auf die beiden Flavoproteine NADH- und Succinat-Dehydrogenase im getrennten Anfangsbereich der Elektronentransportkette ist unwahrscheinlich.

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Electrical Membrane Constants of Sartorius Muscle Fibers from the South American Frog, *Leptodactylus ocellatus*

Materials and methods. Sartorius muscles were dissected and mounted as previously described[1]. The temperature in the nerve-muscle chamber was kept at 25 °C. Bathing solutions were prepared by adding 2.5 m*M* of KCl or CsCl per l to standard K-free Ringer (112 m*M* NaCl; 1.89 m*M* $CaCl_2$, 2.5 m*M* Na_2HPO_4; 0.5 m*M* NaH_2PO_4; pH 7.2). The osmolarities of all solutions were checked cryoscopically. Each number given below for membrane potential represents an average over measurements on 40 individual muscle fibers.

Membrane electrical parameters: *Leptodactylus ocellatus*, sartorius muscle

Parameter (symbol) and units	Value in: KR	Value in: CsR	Difference %	Significance [a]
Membrane potential (V), mVolts	87.8 ± 0.8	87.7 ± 1.0	0	none
Length constant (λ), mm	1.18 ± 0.06	1.09 ± 0.05	− 8	0.001
Total effective resistance (Φ), k-ohms	294 ± 16	346 ± 21	+15	0.001
Fiber diameter (d), microns	80.0 ± 3.0	70.8 ± 3.8	−12	0.001
Specific membrane resistance (R_m), k-ohms-cm^2	1.74 ± 0.16	1.68 ± 0.10	− 4	0.001
Time constant (T_m), msec	15.4 ± 0.7	17.2 ± 0.4	+11	0.001
Membrane capacitance (C_m), μfd/cm^2	8.8 ± 0.9	10.2 ± 0.9	+16	0.001
Total No. of measurements	40	40		

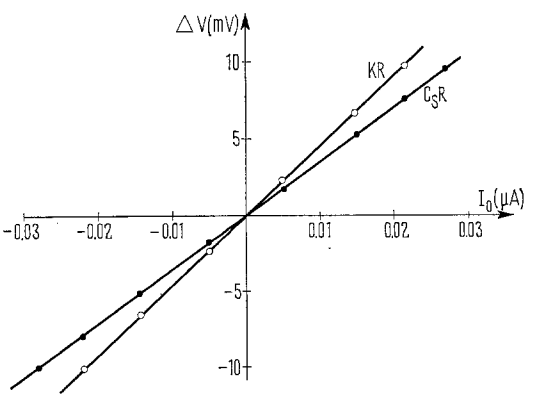
[a] In the last column is listed the calculated level of *t*-test significance of each difference.

Microelectrodes filled with 3 *M* KCl and having a resistance between 10 and 12 megohm were used. 2 microelectrodes, one for current application and the other for recording the membrane potential were inserted in an end-plate region of the muscle fiber. The criteria of HUBBARD[2] for minimal damage to the fiber membrane by impalement with the microelectrodes were observed. 4 insertions were made in each fiber, one for the recording microelectrode, the others for the current-microelectrode at 40, 400 and 1200 microns from it. All microelectrode positions were at least 1000 microns away from motor-end-plates and from the ends of the fibers.

The applied current and the membrane potential were recorded using unit gain microelectrode preamplifiers (Bak Standard Wide Band Electrometric Amplifiers) and a slave oscilloscope system (Bioelectric Instruments, Inc., USA). Membrane potential was measured before and at the end of a 200 msec current pulse by which time it had reached a steady state in every case.

The voltage-current relation, for assessing the linearity of the system, was then obtained by plotting the measured potential change against the applied rectangular current pulse amplitude (Figure). The relationship is clearly linear to a high degree.

Membrane constants were determined following the general method of FATT and KATZ[3]. Calculations were based on HODGKIN and RUSHTON's[4] analysis, with the modifications necessary for intracellular microelectrodes.



Voltage current relationships for muscle cell membrane in potassium ringer (KR) and cesium ringer (CsR). Electrode separation 60 μ.

Results and discussion. The Table summarizes the mean values determined for the various membrane parameters in the 2 solutions. It appears that the most significant difference on going from potassium to cesium Ringer is in cell size, as indicated by fiber diameter. Sartorius muscle fibers in 2.5 m*M* cesium Ringer are 12% smaller in diameter than in 2.5 m*M* potassium Ringer. This change, coupled with the corresponding increase in total effective membrane resistance, accounts for the small change in specific membrane resistance (R_m). Specific membrane capacitance (C_m) increases significantly, however, and accounts for the corresponding increase in time constant and decrease in length constant seen in the table.

The results for potassium Ringer listed here for *Leptodactylus ocellatus* are in satisfactory agreement with those reported by HUBBARD[2] for the European frog, *Rana temporaria*[6].

Résumé. Les constantes électriques de la membrane du m. sartorius, de la grenouille sudaméricaine, *Leptodactylus ocellatus*, ont été mesurées. Les résultats pour les cellules, dans du Ringer normal (2.5 m*M* potassium), s'accordent avec ceux de HUBBARD pour *Rana temporaria*. En 2.5 m*M* cesium Ringer, une diminution de diamètre de 12% et une augmentation de 16% de la capacité de la membrane ont été observées.

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