Biochimica et Biophysica Acta, 637 (1981) 209–216
Elsevier/North-Holland Biomedical Press

BBA 48108

MEMBRANE POTENTIALS IN RESPIRING AND RESPIRATION-DEFICIENT YEASTS MONITORED BY A FLUORESCENT DYE

LADISLAV KOVÁČ and ĽUDOVIT VAREČKA

Institute of Animal Physiology, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji and Psychiatric Hospital, 902 01 Pezinok (Czechoslovakia)

(Received January 27th, 1981)

Key words: Cyanine dye; Membrane potential; Respiration; Fluorescence; (S. cerevisiae)

Changes in fluorescence of 3,3′-dipropylthiodicarbocyanine iodide which had been equilibrated with suspensions of the wild-type yeast *Saccharomyces cerevisiae* and of respiration-deficient mutants were followed. The changes have been attributed to changes of yeast membrane potentials, since the fluorescence with wild-type yeast could be affected in a predictable manner by uncouplers and the pore-forming agent nystatin. As in other systems, a rise of steady-state fluorescence was ascribed to depolarization and a drop of the fluorescence to hyperpolarization. (1) A considerable rise in steady-state fluorescence was brought about by addition of antimycin A or some other mitochondrial inhibitors to respiring cells. A major part of the composite membrane potential monitored in intact yeast cells appeared to be represented by the membrane potential of mitochondria. (2) Addition of D-glucose and of other substrates of hexokinase, including non-metabolizable 2-deoxy-D-glucose, induced a two-phase response of fluorescence, indicating transient depolarization followed by repolarization. Such a response was not elicited by other sugars which had been reported to be transported into the cells by a glucose carrier or by D-galactose in galactose-adapted cells. The depolarization was explained by electrogenic ATP exit from mitochondria to replenish the ATP consumed in the hexokinase reaction and the repolarization by subsequent activation of respiration. (3) In non-respiring cells only a drop in fluorescence was induced by glucose and this was ascribed to an ATP-dependent polarization of the plasma membrane. (4) Steady-state fluorescence in suspensions of respiration-deficient mutants, lacking cytochrome *a*, cytochrome *b*, or both, was high and remained unaffected by uncouplers and nystatin. This indicates that membranes of the mutants may have been entirely depolarized. A partial polarization, apparently restricted to the plasma membrane, could be achieved by glucose addition.

Introduction

A simple unicellular organism, the yeast *Saccharomyces cerevisiae*, has always been one of the most favourite subjects in studies on metabolism, functions and structure of the eucaryotic cell. In contrast to the wealth of data on chemical and transport processes in this organism, little is known so far about its membrane potentials which represent a link between the two types of processes. Detection, estimation and continuous monitoring of the membrane potentials in the intact eucaryotic cell are difficult tasks, since the interior of the cell does not form a simple phase but consists of several compartments separated by membranes of different chemical composition and electrical properties. In addition, cells of *S. cerevisiae* are too small to allow direct measurement of the membrane potentials by microelectrode impalements and an indirect measurement by distribution of lipophilic ions has met with unexpected complications in these cells [1,2].

Abbreviations: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; diS-C_3-(5), 3,3′-dipropylthiodicarbocyanine iodide; DCCD, *N,N′*-dicyclohexylcarbodiimide.

Another indirect method involves the use of cyanine dyes, the fluorescence of which is sensitive to the membrane potential. The method was previously applied to a variety of animal cells (for a review see Ref. 3). In yeast it was used in a study on sugar transport in *Saccharomyces fragilis* [4] but was reported to fail with a respiration-deficient mutant of *S. cerevisiae* [5]. In the present study we used the fluorescence method to reveal some qualitative features of membrane potentials in wild-type cells of *S. cerevisiae* and in various mutants and to follow changes of the membrane potentials elicited by substrates, uncouplers and various inhibitors.

Materials and Methods

The cyanine dye diS-C_3-(5) was kindly provided by Dr. A. Waggoner (Amherst College). Antimycin A was purchased from Serva, DCCD from Ega Chemie and nystatin (Mycostatin) from Serva. Bongkrekic acid was a gift from Dr. W. Berends (Technical University, Delft), CCCP from Dr. M. Greksák (Institute of Experimental Veterinary Medicine, Ivanka pri Dunaji), oligomycin from the Upjohn Co. and valinomycin (produced by Serva) from Dr. G. Schatz (Biozentrum, Basel). Other chemicals were from Lachema.

The strains of *S. cerevisiae* employed are listed in Table I. They were usually cultured in a semi-synthetic medium containing salts, yeast extract and peptone with 2% glucose as carbon source at 30°C for 24 h aerobically on a shaker in flasks filled with medium to one-tenth of their volume. When stated, 2% galactose or 2% maltose was used as carbon source instead of glucose and the cells had been first pre-adapted to these substrates. The cells were used for experiments immediately after harvesting and washing with water, or stored for up to 3 days at 4°C, or starved of carbon source by aerating the cell suspension in water at 30°C overnight to reduce endogenous substrates. The last two treatments did not substantially affect the properties examined in this study.

Fluorescence was measured in the Hitachi-Perkin Elmer 204 spectrofluorimeter at room temperature with 622 nm excitation and 670 nm emission wavelengths. The cuvette contained yeast cells suspended in a buffer and its content was stirred continuously with an overhead stirrer placed out of the light path. Additions were made in small volumes during measurement, the first addition being a methanolic solution of diS-C_3-(5). Before each measurement the cuvette was washed with water followed by methanol and again with water.

Results

Interaction of the cyanine dye with yeast cells and evidence for membrane potentials

After diS-C_3-(5) had been added to a final concentration of 0.4 μM to the fluorimeter cuvette containing water or buffer but no cells, the fluorescence of the solution declined continuously, due to adsorption of the dye to the glass walls of the cuvette [9]. In the presence of viable yeast cells the fluorescence stabil-

TABLE I

LIST OF STRAINS

Strain	Characteristics	Origin
DT XII	diploid, wild-type	[6]
DT XIIA	diploid, cytoplasmic respiration-deficient (*rho*$^-$) mutant	[7]
DH 1	diploid, homozygous single nuclear gene (*op*1) mutant with modified mitochondrial adenine nucleotide translocase	[8]
V 97-8 A	haploid, single mitochondrial gene (*oxi*2) mutant with non-functional cytochrome oxidase	from A. Putrament
V 293	haploid, single mitochondrial gene (*oxi*3) mutant with non-functional cytochrome oxidase	from A. Putrament
G 2457	haploid, single mitochondrial gene (*box*1, *cob*1) mutant with a lesion in cytochrome *b*	from P.P. Slonimski

ized rapidly at a certain low level which, within a range, was linearly dependent on the quantity of the cells. In an osmotically stabilized solution the fluorescence reached a similar low level both with intact cells and protoplasts containing no cell wall. When the cells had been killed by boiling the suspension or by starving for 2 months the fluorescence stabilized at a much higher level than with living cells (Fig. 1). The fluorescence of the dye also remained high if, in the absence of cells, a solution of serum albumin was present in the cuvette.

It could be concluded that serum albumin and cell proteins prevented adsorption of the dye to glass, probably by binding the dye without substantially quenching the fluorescence. In analogy with observations on animal cells [3,9], it could be further supposed that the lower fluorescence level attained with viable cells than with killed cells was not due to less binding of the dye to viable cells but rather to partition of the dye across the cell membranes of living cells in such a way that part of it formed non-fluorescent aggregates.

In erythrocytes such a partition was shown to be governed by the magnitude of the membrane potential [9]. The more negative the electric potential of the cell interior, the larger the quantity of the dye that entered the cells and aggregated into the non-fluorescent form. The same appeared to be true with yeast. As shown in Fig. 2, the low fluorescence of the solution containing yeast cells and diS-C_3-(5) was considerably raised by the uncouplers CCCP or azide which should have collapsed the membrane potential in respiring cells. The pore-forming agent nystatin, which apparently impaired the membrane potential by allowing equilibration of ion concentrations across membranes, also raised the fluorescence.

If it is possible to render a cell permeable to K^+ by treatment with valinomycin, different membrane potentials, which approximate to the K^+-equilibrium potentials, can be set up by varying concentrations of K^+ in the medium. The fluorescence of diS-C_3-(5) can then be calibrated against the magnitude of the membrane potentials [9]. Unfortunately, this procedure could not be applied to yeast. Valinomycin does not prevent growth of *S. cerevisiae* on glucose but only on non-fermentable substrates, indicating that it may affect the permeability properties of the mitochondrial membrane but not of the yeast plasma membrane. Valinomycin (5 μg/ml) slowly raised the fluorescence of yeast suspensions containing diS-C_3-(5) but this was independent of K^+ concentrations in the medium. In fact, the effect of valinomycin was essentially the same in media containing 0.1 M NaCl or 0.1 M KCl.

As can be seen in Fig. 2, fluorescence of the yeast

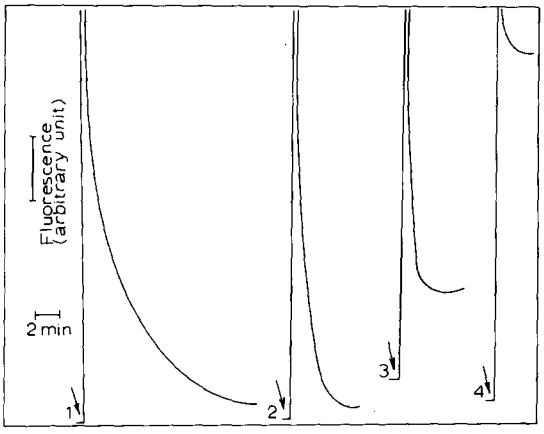

Fig. 1. Fluorescence of diS-C_3-(5) in the presence of yeast. The cuvette of the spectrofluorimeter contained in 1.3 ml: 16 mM citrate/phosphate buffer, pH 5.0, and the following amounts of wild-type yeast (dry weight): 1, none; 2, 0.35 mg; 3, 2.5 mg; 4, 1.8 mg from a suspension that had been boiled for 3 min. At arrows, 0.4 μM diS-C_3-(5) was added.

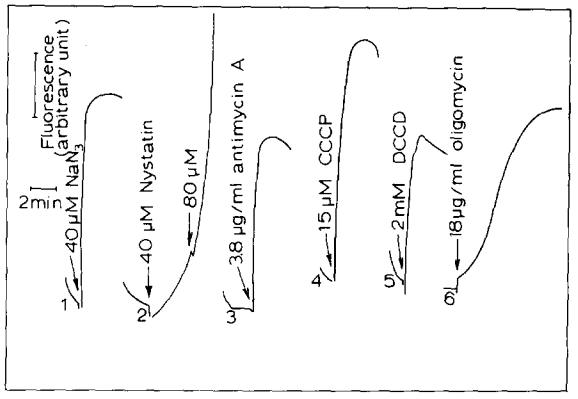

Fig. 2. Fluorescence changes induced by uncouplers, nystatin and mitochondrial inhibitors. The cuvette contained in 1.3 ml: 16 mM citrate/phosphate buffer, pH 5.0, wild-type yeast (1.9 mg dry weight in 1–3 and 2.5 mg in 4–6) and 0.4 μM diS-C_3-(5). When fluorescence reached the lowest level additions were made at arrows to final concentrations indicated in the figure.

suspension was also increased in the presence of the inhibitors of mitochondrial functions, antimycin A, DCCD and oligomycin. Each of them should have affected the membrane potential of mitochondria. It should be inferred that a large portion of membrane potentials monitored by diS-C_3-(5) must have been represented by the membrane potential of mitochondria. This inference is supported by the observation that the fluorescence of the suspension was practically independent of pH (in 16 mM citrate/phosphate buffer with pH range 3.0–7.5) and of chemical composition of the medium (being similar in water, 16 mM buffer, 0.1 M NaCl, 0.1 M KCl, 0.6 M sorbitol). This also proves that fluorescence of diS-C_3-(5) does not probe surface potentials of either the cell wall or the plasma membrane of the yeast cells.

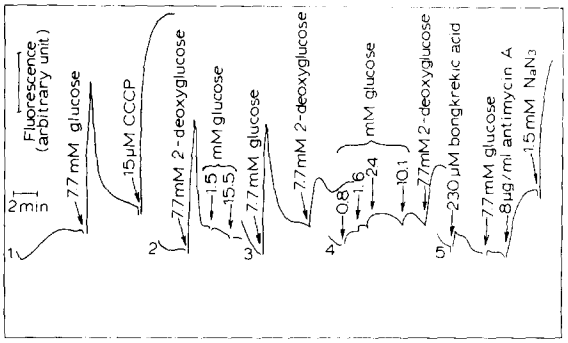

Fig. 3. Fluorescence changes in the presence of sugars. The cuvette contained in 1.3 ml: 16 mM citrate/phosphate buffer, pH 5.0, wild-type yeast (1.8 mg dry weight in 1–4 and 1.6 mg in 5) and 0.4 μM diS-C_3-(5). Additions at arrows were made to final concentrations indicated in the figure.

Changes of the fluorescence of diS-C_3-(5) upon addition of substrates

Once the fluorescence of diS-C_3-(5) added to yeast suspended in buffer reached a standard low level it remained constant for tens of minutes or rose continuously at a low rate. The rising phase was not substantially affected by depleting the cells of endogenous reserves.

The fluorescence was not changed upon addition of the non-fermentable substrates ethanol or lactate. However, a characteristic response was elicited by D-glucose: a rapid rise of fluorescence, reflecting depolarization, followed by a drop to the previous or slightly lower level. The cells could be then depolarized again by addition of uncoupler (Fig. 3). The height of the rise was dependent on glucose concentration in a saturable manner with half-saturation achieved at approx. 2 mM glucose.

A similar response was also observed upon addition of D-fructose, D-glucosamine and 2-deoxy-D-glucose, i.e., substrates of hexokinase. No changes of fluorescence accompanied addition of other monosaccharides at 7.7 mM, viz., 6-deoxyglucose, D-xylose, D-arabinose and L-sorbose. The latter sugars are known to be transported across the cell membrane of *S. cerevisiae* but are not metabolized [10–12]. D-Galactose (up to 15 mM) was inert even with cells which had been grown on it and, similarly, maltose (7.7 mM) was inactive with maltose-grown cells. With another disaccharide, sucrose, the shape of the response of fluorescence was similar to that with D-glucose, but its rate was lower, indicating that sucrose had first been hydrolyzed to glucose which was the proper active species.

It can be seen from Fig. 3 that no apparent depolarization took place when a saturating quantity of glucose was not added at once but in the form of successive small portions. When glucose was added after 2-deoxyglucose the rise of fluorescence was also usually not observed and only a drop of fluorescence was discernible. On the other hand, the addition of 2-deoxyglucose after glucose was followed by an increase of fluorescence.

Bongkrekic acid alone (0.1–0.3 mM) usually slightly depolarized the aerobic cells, often transiently. When glucose was added after bongkrekic acid the typical transient rise of fluorescence caused by glucose was lower or absent.

As mentioned in the previous section, when cells were made anaerobic by blocking their respiration with antimycin A they became largely depolarized. Fig. 4 shows that upon addition of glucose to such anaerobic cells, no additional depolarization took place but instead a considerable repolarization set in either immediately or after a lag of up to 1 min. This repolarization was not prevented by oligomycin, DCCD or bongkrekic acid, suggesting that mitochondria were not involved in the process. Similar repolarization was not induced by 2-deoxyglucose in the anaerobic cells; instead, 2-deoxyglucose enhanced the depolarization.

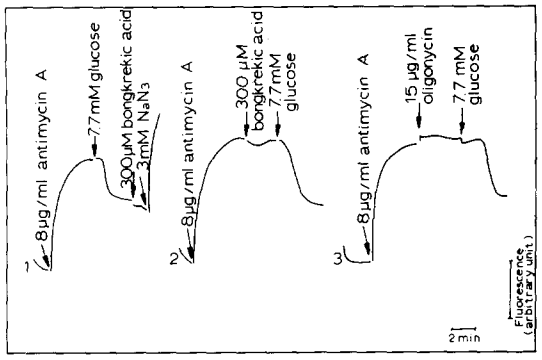

Fig. 4. Fluorescence changes in the presence of antimycin A. The cuvette contained in 1.3 ml: 16 mM citrate/phosphate buffer, pH 5.0, wild-type yeast (2.5 mg dry weight) and 0.4 μM diS-C_3-(5). Additions at arrows were made to final concentrations indicated in the figure.

Fluorescence responses of diS-C_3-(5) in yeast mutants

Fig. 5 depicts the changes of fluorescence measured with a non-respiring yeast mutant. In a suspension of respiration-deficient mutant DT XIIA the fluorescence of diS-C_3-(5) stabilized much more slowly and at a considerably higher level than it did in a suspension containing the same quantity of respiring wild-type cells. This stabilized level of fluorescence remained unchanged upon addition of CCCP, azide, DCCD or nystatin. Thus, in the absence of exogenous substrate the respiration-deficient mutant cells appeared to be entirely depolarized.

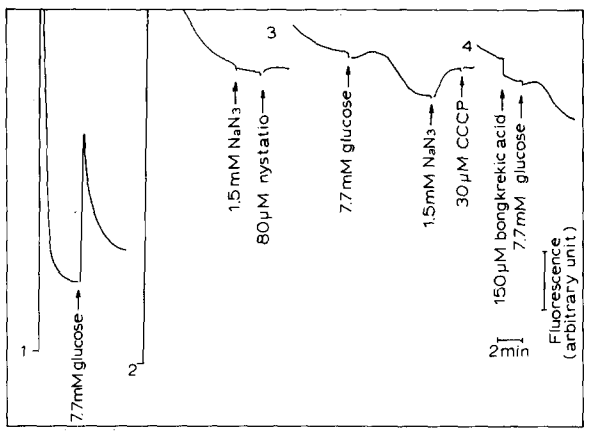

Fig. 5. Fluorescence changes in suspensions of a respiration-deficient mutant. The cuvette contained in 1.3 ml: 16 mM citrate/phosphate buffer, pH 5.0, 0.4 μM diS-C_3(5) and, in 2–4, respiration-deficient mutant DT XIIA (1.9 mg dry weight). For comparison, trace 1 shows a response of a similar suspension of wild-type yeast (1.8 mg dry weight). Additions were made at arrows. 1 and 2 are complete traces started shortly before addition of diS-C_3-(5) (note that in 2 the trace remained for 3 min out of the recorder scale), while 3 and 4 depict only the later stages when the fluorescence had already almost stabilized.

A polarization set in upon addition of glucose, but only after a lag which may lasted for several minutes. As with wild-type cells preincubated with antimycin A, the polarization was not prevented by mitochondrial inhibitors, indicating that the mitochondrial membrane remained in the depolarized state. Once the cells had become polarized they could be depolarized again upon addition of uncoupler or nystatin.

Properties exactly similar to those of the *rho*$^-$ mutant were exhibited also by mitochondrial mutants V 97-8A and V 293 carrying deficiency in cytochrome oxidase (*oxi*2 and *oxi*3, respectively) and also G 2457 deficient in cytochrome *b* (*box*1, *cob*1); they were depolarized in the absence of substrate and partly repolarized upon addition of glucose.

On the other hand, mutant DH 1 with a modified mitochondrial adenine nucleotide translocase [8] did not essentially differ in its fluorescence response from the wild-type strain DT XII.

Discussion

The nature of membrane potentials monitored by diS-C_3-(5)

It has been assumed throughout this study that the fluorescence response of diS-C_3-(5), equilibrated with yeast cells, reflected changes in membrane potentials of the cells, in analogy with observations on liposomes and animal cells [3,9]. Unfortunately, it was not possible to calibrate the fluorescence responses against known membrane potentials and therefore all the reasonings which follow remain qualitative. In this respect, our knowledge of the yeast cells falls short of that of some other eucaryotic systems, such as hepatocytes [13], neuroblastoma cells [14] or synaptosomes [15], in which quantitative values of membrane potentials across mitochondrial and plasma membranes have been estimated by other methods.

Besides the impossibility of quantifying the membrane potential two additional drawbacks are inherent in applying the fluorescence method with diS-C_3-(5) to yeast. First, the dye was shown not to

be innocouous to yeast: it affected mitochondria in intact cells, exerting a discernible uncoupling effect even at very low concentrations and inducing respiration-deficient mutants at higher concentrations [16]. To minimize this adverse effect the lowest possible concentration of the dye was employed in this study. A continuous slow rise of fluorescence which usually took place in yeast suspensions equilibrated with the dye may still have been due to slight uncoupling action. Second, as implied by the mitochondrial effect of diS-C_3-(5), the dye is partitioned not only across the plasma membrane but also across other cellular membranes. Accordingly, the membrane potential monitored by the dye in intact cells represents a composite quantity consisting of the membrane potentials of all cellular membranes.

The electric potential differences across the nuclear membrane may not be appreciable [17]. Little is known so far about the properties of the tonoplast, the membrane of the vacuole, to allow assessment of its membrane potential. The magnitude of the membrane potential of the yeast plasma membrane may fall anywhere within the limits of about −40 mV found in animal cells [13–15,18] and −200 mV found in *Neurospora crassa* [19]. Yet, the fact that a pH difference of several units exists across the yeast plasma membrane [20], created probably by an H^+-pumping ATPase [5,21], strongly suggests that the major portion of the proton-motive force across the plasma membrane is represented by the concentration gradient of protons so that the electrical component would be rather small. On the other hand, the proton-motive force across the mitochondrial membrane in respiring cells should be high and, as in mitochondria from other sources, mainly in the form of a membrane potential, generally over −150 mV [22].

The data obtained in this work by means of inhibitors and mutants leave little doubt that the major part of the membrane potential monitored by diS-C_3-(5) in respiring cells corresponds to a membrane potential of mitochondria.

Membrane potentials in respiration-deficient mutants

Membranes in respiration-deficient mutants, of both *rho*$^-$ and *mit*$^-$ types [24], seemed to be entirely depolarized, showing no changes of diS-C_3-(5) fluorescence upon addition of uncoupler or nystatin. This appears to be true not only for the mitochondrial membrane but also for all cell membranes.

In wild-type cells the elimination of respiration by antimycin A may have also entirely depolarized the mitochondrial membrane, as indicated by the rise of diS-C_3-(5) fluorescence. However, some membrane potential, probably set up across the plasma membrane, remained preserved since a further rise of fluorescence was induced by uncouplers and nystatin. Accordingly, non-respiring wild-type cells are not equivalent to respiration-deficient mutants as far as the electrical phenomena in the cells are concerned. It has been shown that wild-type cells transferred from aerobic to anaerobic conditions maintained a fairly high concentration of intracellular ATP [25] while negligible amounts of ATP or none at all could be detected in respiration-deficient mutants, even under aerobic conditions, unless exogenous substrate was present [5,26]. In the absence of exogenous substrate the ATP may have accumulated in wild-type cells during the aerobic phase at the expense of degradation of endogenous reserves; this degradation proceeds only in respiring cells [27] and does not take place in respiration-deficient mutants [28]. Thus, intracellular ATP is available, at least for some tens of minutes as followed in this study, to maintain a potential difference across the plasma membrane in non-respiring wild-type cells but not in respiration-deficient mutants.

Theoretically, ATP formed in respiration-deficient mutants upon addition of glucose may enter mitochondria in exchange for ADP and create a membrane potential across the mitochondrial membrane of the same orientation as is created by respiration in wild-type cells [29,30]. Such a process would be prevented by bongkrekic acid. However, no effect of bongkrekic acid was observed in experiments with the respiration-deficient mutants, although bongkrekic acid-sensitive nucleotide translocase is present in *rho*$^-$ mitochondria [31,32]. Since the level of exchangeable adenine nucleotide pool in *rho*$^-$ mitochondria is exceedingly low [31], the exchange process may be too slow to enable creation of a discernible potential difference across the mitochondrial membrane in these cells.

Changes of membrane potentials in the presence of substrates of hexokinase

The changes of membrane potential induced by

sugars in non-respiring cells are consistent with the view that the addition of glucose to non-respiring cells increases the potential difference across the plasma membrane with no effect upon the membrane potential of mitochondria. It has long been known that yeast cells supplied with glucose start to secret H^+ into the medium [20,21] and this has been later interpreted as active pumping driven by an H^+-ATPase located in the plasma membrane [5,21]. It is understandable that the secretion of positively charged H^+ should increase the potential difference across the plasma membrane even though it is largely compensated by a simultaneous exit of anions and influx of cations [20,21]. 2-Deoxyglucose, which is phosphorylated by ATP but not further metabolized, must have been exhausting intracellular ATP necessary for maintaining the plasma membrane potential and this is why it potentiated the depolarization elicited in wild-type cells by respiratory inhibitors.

More complicated were changes of the membrane potential induced by sugars in respiring cells, indicating a depolarization followed by repolarization. Similar changes of membrane potential were observed to be induced by glucose in a number of other eucaryotic systems [4,19,33–35]. While the repolarization phase has been concordantly interpreted as being due to an increase in ATP concentration formed by glucose metabolism, different explanations have been raised to account for the preceding depolarization phase. In the case of brush border membranes the depolarization was ascribed to a neutralization of negative charges in the interior of the membrane vesicles by Na^+ symported with glucose [33]. Symport of H^+ with glucose was held responsible for depolarization in cells of *N. crassa* [19] and *Rhodotorula gracilis* [34]. A similar explanation was proposed to account for depolarization, monitored by cyanine dye fluorescence, induced in *S. fragilis* by lactose [4]. In respiring ascites tumor cells a transient glucose-induced depolarization was attributed to a decrease of intracellular ATP consumed in phosphorylation of the sugar [35].

In *S. cerevisiae* glucose is generally supposed to enter the cell by facilitated diffusion [10–12] (see, however, Ref. 36) and there is no evidence for its symport with H^+ [21,37]. Influx of H^+ along with glucose would thus not account for the transient depolarization. Also, non-metabolizable sugars which are supposed to be transported into the yeast cells by glucose carrier did not induce depolarization. 2-Deoxyglucose, which is also non-metabolizable but is a substrate of hexokinase, did depolarize.

The following course of events would be compatible with the data obtained. The first step of sugar utilization, transport into the yeast cell, is not linked to changes of membrane potential. Once present in the cell a substrate of hexokinase is phosphorylated at the expense of ATP available in the cytosol close to mitochondria. This ATP is rapidly replenished by mitochondrial ATP which exchanges for cytosolic ADP, carrying with it surplus negative charges into the cytosol and thus producing depolarization of the mitochondrial membrane [29,30]. Bongkrekic acid inhibits the ATP-ADP exchange and thus the depolarization. In the *op*1 mutant, which possesses a modified adenine nucleotide translocase [8], the exchange is not entirely prevented as a suit of the mutation and the depolarization takes place as in the wild-type strain. The original membrane potential of mitochondria is later re-established by increased respiration which is made possible by oxidation of metabolites produced from glucose or, in the case of non-metabolizable 2-deoxyglucose, by activation of endogenous metabolism. The latter conclusion is inescapable to account for the repolarization phase observed in the presence of this non-metabolizable sugar. In fact, activation of endogenous metabolism by 2-deoxyglucose has been described [38].

When sub-saturating amounts of glucose were successively added to the cells the depolarization-repolarization process was not observed. The rate and extent of glucose phosphorylation may have been low enough to be matched with simultaneous replenishment of ATP and re-establishment of the mitochondrial membrane potential. It is remarkable that phosphorylation of galactose in galactose-adapted cells, which proceeds by a mechanism independent of hexokinase, was not linked to depolarization. Hexokinase may be more intimately functionally linked to mitochonria than is galactokinase and this link may have a bearing upon the Pasteur efect in yeast, a process which is still far from being completely understood.

References

1 Riemersma, J.C. and Alsbach, E.J.J. (1974) Biochim. Biophys. Acta 339, 274–284
2 Barts, P.W.J.A., Hoebcrichts, J.A., Klaasen, A. and Borst-Pauwels, G.W.F.H. (1980) Biochim. Biophys. Acta 597, 125–136
3 Waggoner, A. (1976) J. Membrane Biol. 27, 317–334
4 Eddy, A.A., Philo, R., Earnshaw, P. and Brocklehurst, R. (1977) FEBS Symp. 42, 250–260
5 Serrano, R. (1980) Eur. J. Biochem. 105, 419–424
6 Kováč, L., Bednárová, H. and Greksák, M. (1968) Biochim. Biophys. Acta 153, 32–42
7 Kormančiková, V., Kováč, L. and Vidová, M. (1968) Biochim. Biophys. Acta 180, 9–17
8 Kolarov, J., Šubík, J. and Kováč, L. (1972) Biochim. Biophys. Acta 267, 465–478
9 Sims, P.J., Waggoner, A.S., Wang, C.-H. and Hoffman, J.F. (1974) Biochemistry 13, 3315–3330
10 Cirillo, V.P. (1961) Annu. Rev. Microbiol. 15, 197–218
11 Kotyk, A. (1967) Biochim. Biophys. Acta 135, 112–119
12 Heredia, C.F., Sols, A. and DelaFuente, G. (1968) Eur. J. Biochem. 5, 321–329
13 Hock, J.B., Nicholls, D.G. and Williamson, J.R. (1980) J. Biol. Chem. 255, 1458–1464
14 Deutsch, C., Erecińska, M., Werrlein, R. and Silver, I.A. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 2175–2179
15 Scott, I.D. and Nicholls, D.G. (1980) Biochem. J. 186, 21–33
16 Kováč, L. and Poliachová, V. (1981) Biochem. Int. 2, 503–507
17 Palmer, L.G. and Civan, M.M. (1977) J. Membrane Biol. 33, 41–61
18 Wondergem, R. and Harder, D.R. (1980) J. Cell. Physiol. 102, 193–197
19 Slayman, C.L. and Slayman, C.W. (1974) Proc. Natl. Acad. Sci. U.S.A. 71, 1935–1939
20 Conway, E.J. and O'Malley, E. (1946) Biochem. J. 40, 55–67
21 Eddy, A.A. (1978) Curr. Top. Membrane Transp. 10, 279–360
22 Mitchell, P. and Moyle, J. (1969) Eur. J. Biochem. 7, 471–484
23 Philo, R.D. and Eddy, A.A. (1978) Biochem. J. 174, 801–810
24 Slonimski, P.P. and Tzagoloff, A. (1976) Eur. J. Biochem. 61, 27-41
25 Holzer, H. and Freytag-Hilf, R. (1959) Hoppe Seyler's Z. Physiol. Chem. 316, 7–30
26 Solimene, R., Guerrini, A.M. and Donini, P. (1980) J. Bacteriol. 143, 710–714
27 Stier, T.J.B. and Stannard, J.N. (1936) J. Gen. Physiol. 19, 461–478
28 Talwalkar, R.T. and Lester, R.L. (1973) Biochim. Biophys. Acta 306, 412–421
29 Klingenberg, M. and Rottenberg, H. (1977) Eur. J. Biochem. 73, 125–130
30 Laris, P.L. (1977) Biochim. Biophys. Acta 459, 110–118
31 Kolarov, J., Šubík, J. and Kováč, L. (1972) Biochim. Biophys. Acta 267, 457–464
32 Šubík, J., Kolarov, J. and Kováč, L. (1974) Biochim. Biophys. Acta 357, 453–456
33 Beck, J.C. and Sacktor, B. (1978) J. Biol. Chem. 253, 7158–7162
34 Hauer, R. and Höfer, M. (1978) J. Membrane Biol. 43, 335–349
35 Laris, P.C., Pershadsingh, H.A. and Johnstone, R.M. (1976) Biochim. Biophys. Acta 436, 475–488
36 Van Steveninck, J. and Rothstein, A. (1965) J. Gen. Physiol. 49, 235–246
37 Eddy, A.A. and Nowacki, J.A. (1971) Biochem. J. 122, 701–711
38 Scharff, T.G. and Montgomery, O.L. (1965) Life Sci. 4, 1049–1053