REFERENCES

- Böttcher, C. J. F. & van Gent, C. M. (1961). *J. Atheroscler. Res.* **1**, 36.
- Brandner, J. D. & Birkmeier, R. L. (1960). *J. Amer. Oil Chem. Soc.* **37**, 390.
- Collins, F. D. (1962). *Biochem. biophys. Res. Commun.* **9**, 289.
- de Haas, G. H. & van Deenen, L. L. M. (1961). In *The Enzymes of Lipid Metabolism*, p. 53. Ed. by Desnuelle, P. London: Pergamon Press Ltd.
- Farquhar, J. W., Insull, W., jun., Rosen, P., Stoffel, W. & Ahrens, E. H., jun. (1959). *Nutr. Rev.* **17**, suppl. 18, part 2.
- Fulco, A. J. & Mead, J. F. (1959). *J. biol. Chem.* **234**, 1411.
- Hanahan, D. J. (1960). *Lipide Chemistry*, p. 75. New York and London: John Wiley and Sons Inc.
- Hanahan, D. J., Watts, R. M. & Pappajohn, D. (1960). *J. Lipid Res.* **1**, 421.
- Lands, W. E. M. (1960). *J. biol. Chem.* **235**, 2233.
- Leat, W. M. F. (1963). *Biochem. J.* **89**, 44.
- Leat, W. M. F. (1964). *Biochem. J.* **91**, 437.
- Moore, J. H. & Williams, D. L. (1963). *Biochim. biophys. Acta*, **70**, 348.
- Rhodes, D. N. (1958). *Biochem. J.* **68**, 380.
- Robertson, A. F. & Lands, W. E. M. (1962). *Biochemistry*, **1**, 804.
- Tattrie, N. H. (1959). *J. Lipid Res.* **1**, 60.
- Woodford, F. P. & van Gent, C. M. (1960). *J. Lipid Res.* **1**, 188.

Biochem. J. (1964) **91**, 447

The Active Transport of Potassium and Sodium Ions in Respiratory-Deficient Mutants of *Saccharomyces cerevisiae*

By C. REILLY*

Department of Biochemistry, University College, Dublin, Eire

(Received 8 August 1963)

Stiehler & Flexner (1938) first clearly expressed the view that ion transport could be linked directly with the respiratory systems of the cell. Lundegårdh (1939) further developed this view in connexion with the active transport of anions through the root hairs of plants. Conway & Brady (1948) put forward a redox theory for the secretion of hydrochloric acid by the oxyntic cells of the gastric mucosa. This theory was expanded into the more general 'redox pump' theory applicable to the active transport of inorganic cations across cell membranes (Conway, 1951). The evidence for the theory is particularly strong for the active transport of K^+ and Na^+ ions in yeast. Conway & Kernan (1955) found that very small concentrations of redox dyes could exercise a considerable influence on the uptake of K^+ ions and the excretion of Na^+ ions by yeast, and on the basis of this and other findings they concluded that the active transport of these ions across the yeast cell membrane is directly concerned with redox systems acting as carriers and functioning as the immediate suppliers of the energy necessary for the transport. Considerable evidence has been presented (Conway, 1953, 1955, 1963) to show the validity of the 'redox pump' concept also in relation to skeletal muscle.

* Present address: Department of Radiation Biology, School of Medicine and Dentistry, Rochester University, Rochester 20, N.Y., U.S.A.

The use of respiratory-deficient mutants of yeast as described in the present paper has given further confirmation of the operation of such a redox mechanism for the transport of cations. The accumulation and secretion of K^+ and Na^+ ions in normal and mutant yeasts are compared, as are also the effects of redox dyes. These observations are considered in the light of the general metabolism and of the deficiency of the cytochrome system of the mutant yeast.

EXPERIMENTAL

Yeast cultures. The yeasts employed were a pure strain of brewer's yeast (Guinness 521 A) and an acriflavine-produced respiratory-deficient mutant derived from it.

The yeasts were grown in 2 l. conical flasks containing a 'natural medium' consisting of inorganic salts, yeast extract and glucose (Slonimski, 1953, p. 7). The flasks were incubated, with occasional shaking, at 30°, and growth was allowed to continue for 48–62 hr. The crop was harvested by centrifugation and was twice washed with tap water. The yield was about 15 g./l. for the normal yeast and 10 g./l. for the mutant yeast.

After being harvested, the yeasts were usually suspended in tap water acidified to pH 5 with HCl and bubbled with oxygen for up to 24 hr., both to deplete their high K^+ ion content and to induce the development of the cytochrome system of the cells.

Sodium ion-rich yeasts. These were prepared by fermenting 1 part of yeast in 20 vol. of 0.2 M-trisodium citrate

containing 5% (w/v) of glucose for 2 hr. (Conway & Moore, 1954).

Examination of the absorption spectra of the yeasts. Identifications of the cytochromes were made with the Hartree microspectroscope attached to a microscope. Examinations were made by using solid wedges of yeast to which sodium dithionite had been added.

Manometric measurements. Examination of rates of respiration and fermentation were made by the direct Warburg method at 30°. The Q_{O_2} and Q_{CO_2} values are expressed as μ l. of oxygen or carbon dioxide/mg. dry wt./hr. For measurements of exogenous metabolism each Warburg flask contained a total volume of 2 ml. of 1% (w/v) yeast suspension buffered at pH 4.6 with 67 mM- KH_2PO_4 . Endogenous metabolism was measured with a 5% (w/v) suspension in water alone.

Fermentation rates were also measured by the Conway microdiffusion technique for estimations of carbon dioxide and ethanol (Conway, 1962). Samples for the estimation of ethanol were obtained by bubbling with nitrogen a 5% (w/v) yeast suspension containing 5% (w/v) of glucose. After 60 min. the suspension was centrifuged, and 0.1 ml. samples were taken from the supernatant and immediately transferred to Conway units for microdiffusion analysis.

Measurement of redox potentials. The technique followed was that of Conway & Kernan (1955). Measurements were made with a bright platinum-foil electrode coupled with a calomel half-cell. The yeast suspensions were contained in a stoppered 200 ml. Pyrex container with a sintered-glass base, and purified nitrogen gas was passed up through the base. This arrangement provided a fine dispersal of gas bubbles and established anaerobic conditions rapidly in the suspension. The platinum electrode and salt bridge passed through the stopper which also took a narrow-bore exit tube for the nitrogen. A 5% (w/v) suspension of yeast in 0.04 M-potassium hydrogen succinate or sodium hydrogen succinate (at pH 4.5) was placed in the fermentation vessel, and, as required, redox dyes were incorporated into the suspension at a final concentration of 0.1 mM. The relation of E_h to pH was measured by using a modification of the same apparatus, in which the stopper of the fermentation vessel was further pierced to take the tip of a burette as well as a glass electrode. This electrode and associated direct-reading pH-meter were coupled with the calomel half-cell circuit described above. To change the pH of the suspensions small volumes of N-HCl or N-NaOH were added from the burette. The acid and alkali added were kept air-free by bubbling a fine stream of nitrogen up

through the burette from a hypodermic needle that pierced the rubber joint (Conway, Duggan & Kernan, 1963).

Measurement of potassium and sodium ions. The Beckman flame photometer was used for analyses of cations. Samples taken as required from the yeast suspensions were centrifuged, washed twice with distilled water, resuspended in 10 vol. of water, boiled for 3 min. and made up to a known volume when cooled. The diluted suspensions were centrifuged and the clear supernatants used for analyses.

RESULTS

Spectroscopic examinations of the yeasts. On harvesting and subsequent oxygenation the mutant yeast showed only the absorption bands of cytochrome *c* at 550 and 520 $m\mu$, as well as the faint band of cytochrome *a*₁ at about 580 $m\mu$. Normal yeast after similar treatment showed the 'classical' spectrum of cytochromes *a*, *a*₃, *b* and *c*.

Consumption of oxygen and evolution of carbon dioxide. The results in Table 1 are typical of the results obtained. The Q_{CO_2} values for the mutant were identical under aerobic and anaerobic conditions during glucose metabolism. This absence of the Pasteur effect in the mutant resulted in vigorous fermentation under aerobic conditions. The rate of evolution of carbon dioxide by the normal yeast during the metabolism of glucose in the presence of air was considerably less than that of the mutant under identical conditions. The consumption of oxygen by the mutant was in marked contrast with that of normal yeast and clearly points to a respiratory-deficient condition. There was also a considerably decreased rate of endogenous metabolism of the mutant compared with that of normal yeast.

Corresponding results were obtained with Na^+ ion-rich yeasts. There was again a high Q_{CO_2} of the mutant under aerobic and anaerobic conditions and a negligible Q_{O_2} , contrasting with the quotients obtained with normal yeast under identical conditions of exogenous metabolism of glucose. Again, during endogenous metabolism, the Na^+ ion-rich mutant had almost negligible rates of consumption

Table 1. *Consumption of oxygen and production of carbon dioxide by normal and mutant yeasts, both fresh and sodium ion-rich*

Exogenous metabolism was measured with 1% (w/v) suspensions of yeast in 67 mM- KH_2PO_4 containing 5% (w/v) of glucose; 5% (w/v) suspensions in water were used for measurements of endogenous metabolism.

Yeast sample	Substrate	$Q_{O_2}^{air}$	$Q_{CO_2}^{air}$	$Q_{CO_2}^{N_2}$	R.Q.
Normal yeast	Glucose	107	162	—	1.56
Normal yeast	None	14.7	11.8	—	0.8
Mutant yeast	Glucose	4.0	217	220	—
Mutant yeast	None	1.0	2.0	—	2.0
Na^+ ion-rich normal yeast	Glucose	105	147	—	1.31
Na^+ ion-rich normal yeast	None	17.6	13.2	—	0.75
Na^+ ion-rich mutant yeast	Glucose	1.0	230	220	—
Na^+ ion-rich mutant yeast	None	0.82	1.0	—	1.2

Table 2. *Uptake of potassium ions by five samples of normal and mutant yeast during fermentation in the presence of potassium chloride*

Unbuffered 5% (w/v) suspensions of yeast in 0.1 M-KCl containing 5% (w/v) of glucose were used. Anaerobic conditions were maintained by bubbling with nitrogen gas.

Yeast sample	K ⁺ ion content (m-equiv./kg. wet wt. of yeast)					
	Mutant yeast			Normal yeast		
	Initial	After 30 min.	Change	Initial	After 30 min.	Change
1	113	133	20	123	179	40
2	122	157	35	120	150	30
3	117	140	23	106	122	16
4	99	116	17	120	144	24
5	113	131	18	139	165	26

Fig. 1. Relative rates of entrance of K⁺ ions into normal (○) and mutant (●) yeasts. The yeasts were suspended in 20 vol. of 0.1 M-KCl and bubbled with nitrogen for 10 min. Oxygen bubbling was substituted at 10 min., and at 30 min. (arrow) glucose was added to give a final concentration of 5% (w/v).

of oxygen and evolution of carbon dioxide compared with those of Na⁺ ion-rich normal yeast.

Uptake of potassium ions during fermentation. Little difference was observed between the ability of normal and mutant yeasts to take up K⁺ ions when supplied with an exogenous source of energy (glucose) in the presence of potassium chloride under either aerobic or anaerobic conditions. Table 2 summarizes the results of a series of experiments in which the initial content and uptake of K⁺ ions during 30 min. of fermentation by corresponding samples of normal and mutant yeast were compared. They indicate the absence of significant difference between the abilities of the two types of yeast to accumulate K⁺ ions.

The results in Fig. 1 confirm the above conclusion while also showing that the mutant differs from normal yeast in that it accumulates very little K⁺ ion in the absence of exogenous glucose under aerobic conditions. Both normal and mutant yeasts fail to accumulate K⁺ ions when suspended in 0.1 M-potassium chloride alone under anaerobic conditions, but once oxygen bubbling is begun a rapid entry of K⁺ ions into the normal yeast takes place, whereas the mutant does not begin to

accumulate the ion to any appreciable extent until glucose is added.

Changes in pH during fermentation in the presence of potassium chloride. The mutant yeast followed the same pattern as did normal yeast with regard to the secretion of H⁺ ions during fermentation in the presence of potassium chloride. In a particular experiment, the initial pH of 5% suspensions of both normal and mutant yeasts in 5% glucose and 0.1 M-potassium chloride was 4.8. After 20 min. of anaerobic fermentation, the pH of the suspension of normal yeast had fallen to 2.8, and that of the mutant to 3.2. In a 50% (w/v) suspension under similar conditions, the normal yeast reached pH 1.9 and the mutant pH 2.1.

Sodium ion-rich yeast. The mutant yeast accumulated Na⁺ ions by glucose-sodium citrate fermentation in the same manner as did normal yeast. However, the Na⁺ ion-rich mutant showed a marked difference in behaviour from the enriched normal yeast when suspended in potassium chloride solution under aerobic conditions. With the mutant, almost no exchange of Na⁺ ions for K⁺ ions took place until glucose was added to the suspension. The Na⁺ ion-rich normal yeast, however, exchanged its Na⁺ ions for external K⁺ ions when suspended in potassium chloride solution under aerobic conditions. Figs. 2 and 3 illustrate the behaviour of the two yeasts under such conditions. They also show that the Na⁺ ion-rich normal yeast, like the mutant, did not exchange its Na⁺ ions for K⁺ ions under anaerobic conditions.

Redox-potential measurements. Normal yeast in the resting condition, in the absence of glucose, reached a steady-state redox potential of 0–20 mv when anaerobic conditions had been established in the suspension. Even prolonged starving and very careful scrubbing of the nitrogen used to establish anaerobic conditions failed to produce such a low potential in suspensions of mutant yeast; it did not fall below 115 mv. A somewhat similar difference was found between the steady-state potentials of fermenting normal and mutant yeasts buffered at

Fig. 2. Changes in Na^+ and K^+ ion contents of Na^+ ion-rich normal yeast under aerobic and anaerobic conditions. Glucose was added (arrow) to the unbuffered 5% (w/v) suspensions containing KCl (0.1 M) at 35 min. $\circ$, Rise in K^+ ion content under aerobic conditions; $\bullet$, fall in Na^+ ion content under aerobic conditions; $\triangle$, rise in K^+ ion content under anaerobic conditions; $\square$, fall in Na^+ ion content under anaerobic conditions.

Fig. 3. Changes in Na^+ and K^+ ion contents in Na^+ ion-rich mutant yeast suspended in 0.1 M-KCl under aerobic and anaerobic conditions. Glucose was added (arrow) to the suspensions at 30 min. $\square$, K^+ ion content before the addition of glucose; $\blacksquare$, Na^+ ion content before the addition of glucose; $\triangle$, rise in K^+ ion content under aerobic conditions; $\bullet$, fall in Na^+ ion content under aerobic conditions; $\circ$, rise in K^+ ion content under anaerobic conditions; $\blacktriangle$, fall in Na^+ ion content under anaerobic conditions.

pH 4.5. The normal yeast reached a level of 190–200 mv, and the mutant 230 mv. The curves of potential with time are illustrated in Fig. 4.

As was found by Conway & Kernan (1955), the steady-state redox potential of fermenting Na^+ ion-rich normal yeast was higher than that of fresh yeast. In the present work it was also observed that the potential of Na^+ ion-rich mutant yeast during anaerobic fermentation was about 240 mv.

Effect of redox dyes on the potential developed during fermentation. With the mutant yeast, as with normal yeast, very small concentrations of redox dyes (0.1 mM) had a marked effect on the redox potentials developed by suspensions of the yeast; e.g. Nile Blue (E'_0 at pH 4.5 = 19 mv) decreased the potential of a suspension of mutant to 53 mv, whereas *o*-chlorophenol-endo-2,6-dichlorophenol (E'_0 at pH 4.5 = 385 mv) raised it to 300 mv.

Effect of redox dyes on the uptake of potassium ions during fermentation. Fig. 5 illustrates the difference in behaviour between the normal and mutant yeasts when fermenting in the presence of potassium chloride under the influence of redox dyes. Whereas dyes that increase the E'_h of the suspension above that of the control caused an increased uptake of K^+ ions by normal yeast, they had no effect on the process in suspensions of the mutant. However, dyes that lower the potential blocked the uptake of K^+ ions in both types of yeast.

Effect of redox dyes on the loss of sodium ions from sodium ion-rich yeasts. The dyes had a different effect on the extrusion of Na^+ ions from the mutant and from normal yeast, as with the uptake of K^+ ions. In normal yeast, dyes of high potential inhibited the loss of Na^+ ions, whereas those of low

Fig. 4. Changes of redox potentials of yeasts. $\bullet$, Normal yeast fermenting; $\blacktriangle$, normal yeast resting; $\circ$, mutant yeast fermenting; $\triangle$, mutant yeast resting. The suspending fluid was 0.04 M-potassium hydrogen succinate at pH 4.5; the suspending fluid for fermenting yeasts also contained 5% (w/v) of glucose. The potential curves of fermenting Na^+ ion-rich yeasts, both normal and mutant (not shown), are almost concurrent with that for mutant yeast fermenting.

Fig. 5. Effect of redox dyes on the uptake of K^+ ions during the fermentation of normal (A) and mutant (B) yeasts, buffered by 0.04 M-potassium hydrogen succinate at pH 4.5. The dyes employed were: $\square$, none (control suspension, containing water in place of dyes); $\bullet$, Nile Blue; $\blacktriangle$, Safranin T; $\boxtimes$, Janus Green; $\circ$, *o*-cresol-indo-2,6-dichlorophenol; ∇ , phenol-indo-2,6-dichlorophenol; $\blacksquare$, *o*-chlorophenol-indo-2,6-dichlorophenol; $\odot$, *o*-chlorophenol-indophenol. The dyes were used at a concentration of 0.1 mM.

potential caused a stimulation. With the Na^+ ion-rich mutant, however, dyes of high potential had little effect, whereas those of low potential caused a very marked stimulation of the loss. Fig. 6 illustrates the behaviour of the dye-treated yeasts.

Effect of redox dyes on the fermentation rate of mutant yeast. Conway & Kernan (1955) showed that redox dyes at the concentrations employed in the present work have no effect on the fermentation rates of normal yeast. A corresponding absence of effect was demonstrated in the mutant yeast during the present study. The production of both ethanol and carbon dioxide during anaerobic fermentation was measured with corresponding suspensions of dye-treated and untreated yeast. No significant effect of the dyes on either aspect of fermentation was observed. The seven dyes listed in Fig. 5 were examined. They cover an E'_h range at pH 4.5 of +396 to -170 mv.

Relationship of redox potential to pH in suspensions of fermenting mutant yeast. Conway *et al.* (1963) reported that the effect of change of pH on the redox potential of fermenting normal yeast is 8 mv per unit change of pH within the pH range 4–6. A somewhat similar result was obtained with the mutant yeast. Between pH 3 and 6 the change in potential was 11 mv per unit pH change, but between pH 2 and 3 it was 18 mv.

DISCUSSION

Although the exact mechanism of the induction of respiratory deficiency in yeast is still unknown, it is becoming clear that more is involved in such

Fig. 6. Effect of redox dyes on the loss of Na^+ ions from Na^+ ion-rich normal (A) and mutant (B) yeasts during fermentation in 0.04 M-sodium hydrogen succinate-buffered suspension at pH 4.5. The conditions and symbols were as given in Fig. 5.

mutations than the blocking of synthesis of part of the cytochrome system of the cell. The involvement of other enzyme systems (Slonimski, 1953, p. 112) and of other types of syntheses (Parks & Starr, 1963), as well as morphological changes in mitochondria (Linnane, Vitols & Nowland, 1962), has been shown. It now also appears that the mutation leads to an interference with some of the ion-transport mechanisms of the cell.

It is not, apparently, the normal process of accumulation of K^+ ions and excretion of H^+ ions by yeast during glucose fermentation that is affected by the mutation, but rather the ability of the yeast when enriched with Na^+ ions to excrete Na^+ ions and replace them with K^+ ions. Conway, Ryan & Carton (1954) showed that normal yeast is capable of actively and specifically excreting Na^+ ions and that this excretory power is cyanide-sensitive. They also showed, as has been confirmed in the present work, that, in Na^+ ion-rich normal yeast suspended in potassium chloride solution under aerobic conditions, the extrusion of Na^+ ions and the balancing intake of K^+ ions proceeded until the distribution of ions was once more that found in fresh yeast. With Na^+ ion-rich mutant yeast only a very limited extrusion of Na^+ ions or uptake

of K^+ ions took place in the absence of glucose. The rate of endogenous metabolism of the mutant was extremely low, but on the addition of glucose vigorous fermentation took place, and the exchange of Na^+ ions for K^+ ions across the cell membrane proceeded. Thus metabolic energy is an essential element in the transport of K^+ and Na^+ ions.

Conway *et al.* (1963) considered the theoretical relationship of redox potential to pH change in normal yeast and concluded that in the yeast cell the active carrier is a metal catalyst of the cytochrome type. Such a system would pass on electrons and not H^+ ions down the metabolic chain of catalysts and would not be affected by changes of pH. Conway *et al.* (1963) reported a small change of 8 mv per unit change of pH, whereas if one H^+ ion were involved in the redox system transfers a change of 30 mv per unit pH would be expected. In the present work with the mutant yeast, a change of 11 mv was found per unit pH change in the pH range 3–6, and this result can also be interpreted as pointing to the involvement of electron-transfer redox systems of the metal catalyst type.

The effect of redox dyes on the transport of K^+ and Na^+ ions in the two types of yeast suggests the same conclusion. In both cases dyes in minute amounts exercised considerable influence on the potentials of the cell suspensions. In normal yeast, however, such changes led to either decreased or increased extrusion of Na^+ ions or uptake of K^+ ions, whereas in the mutant it was only when the potential was lowered that an effect, the increased excretion of Na^+ ions or decreased uptake of K^+ ions, was observed. If the behaviour of the mutant alone were considered it might seem that the overall effect of the dyes was to decrease the selective permeability of the cell membrane, e.g. by the oxidation or reduction of thiol groups in the membrane structure (Passow, Rothstein & Lowenstein, 1959), but when the stimulatory effect of some dyes on the uptake of K^+ ions in normal yeast is also considered it is seen that this is at least not the complete explanation. Moreover, if the influence of the dyes were solely on such groups in the cell membrane, it would be difficult to account for the lack of uniformity in their effects on the different types of yeast. When the results presented above are considered in relation to the deficiency of the cytochrome system and corresponding absence of cyanide-sensitive respiration in mutant yeasts (Slonimski, 1953, p. 52) it is seen that they fit in well with a theory of active transport, e.g. Conway's 'redox pump' theory, that postulates a direct connexion between the redox catalysts of the cell and the processes of cation transfer and accumulation.

SUMMARY

1. The electrolyte permeability of respiratory-deficient mutants of yeast cells was investigated.

2. The mutant yeast, unlike normal yeast, could not excrete Na^+ ions in exchange for K^+ ions in the absence of glucose.

3. Redox dyes, at concentrations that did not affect fermentation rates, could alter the steady-state potentials of suspensions of both types of yeast to the same extent, but their effects on the transport of K^+ and Na^+ ions were different. The uptake of K^+ ions was stimulated by increased potential in normal but not in mutant yeast. The excretion of Na^+ ions was inhibited by increased potential in normal but not in mutant yeast. Decreased potential blocked the uptake of K^+ ions and stimulated the excretion of Na^+ ions in both yeasts.

4. The alteration of E_h with pH in a suspension of the mutant was 11 mv per unit pH change between pH 3 and 6.

My thanks are due to Professor E. J. Conway, F.R.S., for his encouragement and helpful discussion, and to the Medical Research Council of Ireland for Grants-in-Aid. The investigations were supported in part, also, by a Research Grant (H-4860) from the Physiology Division, U.S. Public Health Service, National Institutes of Health, Bethesda 14, Md., U.S.A. Acknowledgement is also made of assistance received from Arthur Guinness, Son and Co., Dublin, who supplied the original stock of normal and mutant brewer's yeast.

REFERENCES

- Conway, E. J. (1951). *Science*, **113**, 270.
- Conway, E. J. (1953). *Int. Rev. Cytol.* **2**, 419.
- Conway, E. J. (1955). *Int. Rev. Cytol.* **4**, 377.
- Conway, E. J. (1962). *Microdiffusion Analysis and Volumetric Error*, 5th ed., pp. 201, 248. London: Crosby Lockwood Ltd.
- Conway, E. J. (1963). *Nature, Lond.*, **198**, 760.
- Conway, E. J. & Brady, T. (1948). *Nature, Lond.*, **162**, 456.
- Conway, E. J., Duggan, P. F. & Kernan, R. P. (1963). *Proc. R. Irish Acad.* **63**, sect. B, no. 6.
- Conway, E. J. & Kernan, R. P. (1955). *Biochem. J.* **61**, 32.
- Conway, E. J. & Moore, P. T. (1954). *Biochem. J.* **57**, 523.
- Conway, E. J., Ryan, H. & Carton, E. (1954). *Biochem. J.* **58**, 158.
- Linnane, A. W., Vitols, E. & Nowland, P. G. (1962). *J. cell. Biol.* **13**, 345.
- Lundegårdh, H. (1939). *Nature, Lond.*, **143**, 203.
- Parks, L. W. & Starr, P. R. (1963). *J. cell. comp. Physiol.* **61**, 61.
- Passow, H., Rothstein, A. & Lowenstein, B. (1959). *J. gen. Physiol.* **43**, 97.
- Slonimski, P. (1953). *La Formation des Enzymes Respiratoires chez la Levure*. Paris: Masson et Cie.
- Stiehler, R. D. & Flexner, L. B. (1938). *J. biol. Chem.* **126**, 603.