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Conformational Model of Active Transport: Role of Protons

(mitochondria/cations/valinomycin/nigericin)

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ABSTRACT A conformational model of monovalent cation transport in mitochondria is described. Because it incorporates the proton-generated membrane potential and pH differential of the chemiosmotic model, the model successfully rationalizes a wide variety of mitochondrial ion-transport phenomena.

The active transport of alkali metal cations in the mitochondrion can be greatly enhanced by exogenous ionophores. Thus the very same reagents that facilitate the passive transport of alkali metal cations through biological membranes and lipid bilayers also enhance their active transport. This strongly suggests that the same mechanism of permeation applies under both active and passive transport conditions.

There are two types of ionophores, the neutral and the monocarboxylate or anionic ionophores (1, 2), and it has now been demonstrated that both types induce a form of active transport across the inner mitochondrial membrane (3, 4). The two types of ionophore induce two distinct types of active transport, each with its particular characteristics.

Montal *et al.* (4) have pointed out how the two driving forces of a transmembrane electrical potential and pH differential, predicted by Mitchell's chemiosmotic model (5), could very simply rationalize both types of active transport. Although this observation provides dramatic support for the chemiosmotic model, at least as a phenomenological model of active transport, Montal *et al.* have also suggested that an "asymmetric membrane Bohr effect" may provide an alternative explanation.

We have recently proposed a model of active transport that rests upon two fundamental assumptions: (*i*) there is a cycle of conformational change in the transduction of chemical free energy into the free energy of an ion gradient, and (*ii*) the physicochemical properties of the ionophores require that the movement of ions be spontaneously down local electrochemical potential gradients (6). However, the development of the model based on these two assumptions was very inadequate. The most obvious inadequacy was that it required the completely untenable binding of a large number of weak acid anions on the inner surface of the inner mitochondrial membrane. Furthermore, it did not involve in any intrinsic manner a *cyclical* conformational change, despite the fact that a cycle of conformational change was one of the basic postulates.

A partial account of this work was presented at the 1970 Coral Gables Symposium on Physical Principles of Neuronal and Organismic Behavior. Part 1 on this topic is ref. 6.

Despite the inadequacy of our initial effort the fundamental assumptions upon which the previous model was based remain attractive. The appeal of the conformational model extends beyond the experimental evidence that has until now been introduced in support of it (7–9). The requirement for an intermediate energy form that can be coupled with electron transfer, ATP synthesis or hydrolysis, and ion translocation requires a certain versatility for which a metastable protein conformation would appear to be singularly well suited (10, 11).

We have therefore been led to analyze more carefully the implications of the two fundamental assumptions. The conclusion of such an analysis, which has been presented elsewhere (12), is that the conformational cycle must be capable of modulating the electrochemical potential gradients of the actively transported species *within* the membrane. More specifically, we concluded that the two types of ionophore-induced active transport could be rationalized if the conformational cycle would generate: (*a*) an electrical potential difference across the membrane, negative inside the mitochondrion, and (*b*) a pH gradient across the membrane, alkaline inside. For an evaluation of the evidence in support of an energy-linked membrane potential and pH gradient we refer the reader to the recent reviews of Skulachev (13) and Greville (14). In this paper we will propose a conformational mechanism for their generation. [On the basis of microelectrode experiments, an *energy-independent* membrane potential *positive* inside the mitochondrion has been proposed (15). The validity of these results has been assessed by Skulachev (13).]

MODEL

In attempting to provide a physical mechanism to achieve our dual objectives of generating an electrical potential and a pH gradient with the required polarity across the membrane, we note that these objectives require the effective movement of protons up an electrical potential gradient. This implies that we must postulate the existence of a *nonelectrical* driving force for protons across the membrane since the protons can only move down local electrochemical potential gradients.

A mechanism that incorporates these basic features is represented in Fig. 1. On the excitation phase of the cycle, which is coupled to electron transfer or ATP hydrolysis, ionizable groups on opposite sides of the membrane undergo opposite pK changes; this creates a transmembrane proton-driving force. Furthermore, it is necessary to postulate that there be a specific mechanism for rapid proton conductance through the membrane in the energized or metastable con-

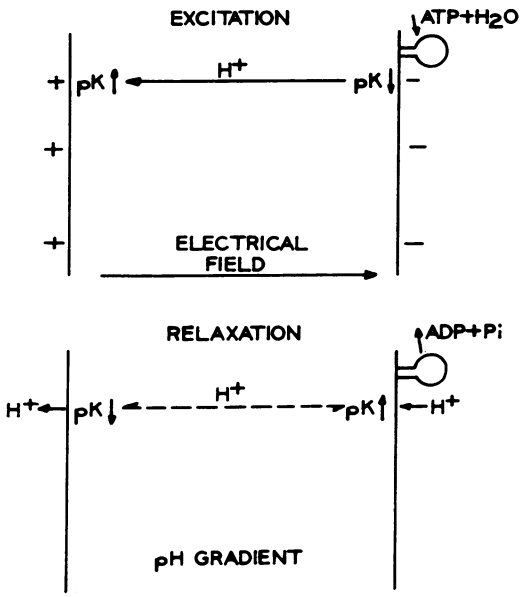

FIG. 1. Conformationally induced proton translocation as the basis for the generation of the membrane potential and pH differential. The solid line connecting the two sets of ionizable groups in the excitation phase indicates high proton conductivity, whereas the dashed line in the relaxation phase indicates low proton conductivity. The coupling of the conformational transition with a chemical reaction is represented by the adsorption of ATP and H_2O in the excitation phase and the desorption of ADP and inorganic phosphate (P_i) in the relaxation phase.

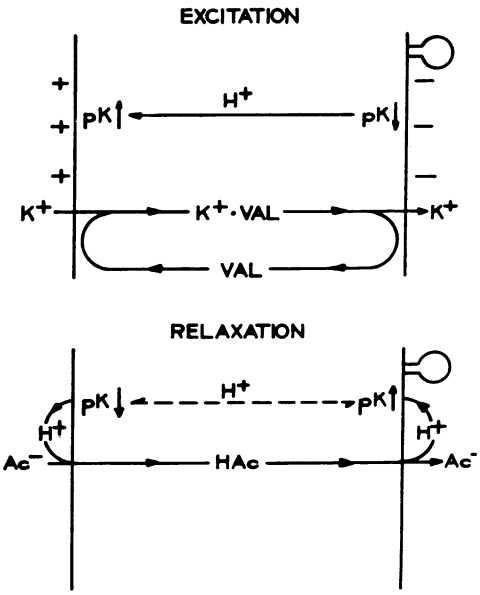

FIG. 2. Schematic representation of valinomycin-induced potassium acetate translocation driven by proton-induced membrane potential and pH gradient.

formation so as to allow for intramembrane equilibration between the two sets of ionizable groups. The essence of combining these two postulates is to obtain the effective transfer of *fixed charge* from one side of the membrane to the other, thereby generating a membrane potential.

Because of charge separation, the transfer of protons from one side of the membrane to the other is a self-inhibiting process. Thus the proton desorption–readsorption reaction cannot go to completion unless permeant ions are present that are capable of redistributing themselves within the membrane and thus attenuating the potential difference across the membrane. In this paper we will consider only the case where permeant ions are present, thereby allowing *maximal* transfer of protons during the lifetime of the metastable conformation. (The maximum number of protons transferable by this mechanism is, according to our preliminary estimate, about 3 protons per coupling site per turnover.)

Upon relaxation of the metastable conformation, the two sets of ionizable groups on the two sides of the membrane undergo the reverse of the pK changes they underwent upon excitation. Now, however, in order for these protons to generate a pH differential across the membrane, the proton conductance between these two sets of ionizable groups must be very small so as to prevent the rapid intramembrane equilibration between these two sets of ionizable groups (16). A pH gradient is thereby generated which can be rapidly dissipated by permeant proton carriers only in a nonelectrogenic manner. Because the present model involves the transfer across the membrane of bound, not free protons, the membrane potential and pH differential are generated separately, not simultaneously. There is no *electro* chemical proton gradient in the present model.

We have incorporated into this model the suggestion of Montal *et al.* (4) that concerns the "asymmetric membrane Bohr effect". We feel that this is a very appropriate term and tentatively envision the physical basis of the conformationally determined pK changes as being qualitatively similar to that applying in the case of hemoglobin (17, 18). We also envision the physical basis of the conformationally modulated proton conductivity as involving either structured water and polar side chains of amino acids along the lines proposed by Klotz (19) and Onsager (20) or an ionophoretic mechanism in which the mobility of the protonophore is conformationally determined. We must emphasize that both the asymmetric membrane Bohr effect and the conformationally modulated proton conductivity are purely *ad hoc* postulates at this point, which have been introduced solely for the sake of consistency with our basic assumptions.

Let us now examine how this conformational cycle can account for the basic observations of active transport. In Fig. 2 we have considered an example of active transport induced by a neutral ionophore (1, 2). Upon excitation the electrical potential difference provides a driving force for the valinomycin-facilitated movement of potassium. During the relaxation phase of the cycle the pH differential drives the movement of acetic acid. (We are not distinguishing here between acetate crossing via a symport mechanism with a proton or via an antiport mechanism with a hydroxyl ion, since they are equivalent for present purposes. See also ref. 21.) One potassium acetate is transported per proton cycled. The separate driving forces for anions and cations in this model requires that under passive transport conditions the anions and cations move independently through the membrane. This has been directly demonstrated for potassium acetate by exchange diffusion studies (22).

In Fig. 3 we have indicated how this model can also account for the active transport induced by the anionic ionophores (3, 4). In the excitation phase of the cycle the electrical potential gradient drives the transport of the nitrate ion. In

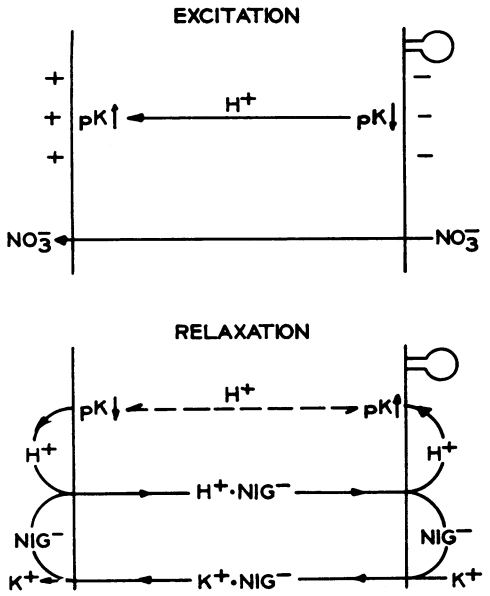

FIG. 3. Schematic representation of nigericin-induced potassium nitrate translocation driven by proton-induced membrane potential and pH gradient.

the relaxation phase of the cycle the pH gradient drives the nigericin-facilitated transport of potassium. One potassium nitrate is transported per proton cycle.

Not all active transport of ions in mitochondria falls into the two categories of active *salt* transport that we have considered until now. The distinguishing feature of these two categories is that there is no net movement of protons. However, as one can see from Figs. 2 and 3, if a permeant ion capable of responding to the proton-generated membrane potential is present, but no ion or ionophore capable of responding to a pH differential is present, then the conformational cycle would involve a net movement of protons across the membrane. For example, valinomycin in a KCl medium induces an energy-dependent proton ejection and potassium uptake in mitochondria (23). The proton to potassium ratio is decreased by the addition of an anion such as acetate, although potassium uptake is stimulated (24, 25). Thus the present model would rationalize those cases of ion movements involving net proton movements as being partial reactions of the full cycle proposed above for salt transport.

TABLE 1. *Conditions for passive swelling of mitochondria in 0.15 M salt solution*

Additions	mg water per mg protein	
	CH_3COOK	KNO_3
None	4.1	4.5
Valinomycin	4.9	14.3
Nigericin	10.7	4.3
m-Cl-CCP (carbonylcyanide *m*-chlorophenylhydrazone)	4.2	4.8
Valinomycin + *m*-Cl-CCP	12.3	—
Nigericin + *m*-Cl-CCP	—	12.7

Heavy mitochondria from beef heart (0.5 ml of a suspension containing 12 mg of mitochondrial protein) were added to 11.5 ml of incubation medium containing either potassium acetate or potassium nitrate at a final concentration of 0.15 M. The incubation media also contained Tris–nitrate or Tris–acetate (60 μmol), pH 7.4, together with antimycin and rotenone, each at a final concentration of 2.0 μg/mg protein. The concentrations of added reagents were: valinomycin (0.2 μg/mg P), *m*-Cl-CCP (2×10^{-7} M), and nigericin (0.1 μg/mg P). Incubations were carried out for 15 min at 25°C; duplicate 5.0-ml aliquots were centrifuged and the pellets were analyzed gravimetrically as described elsewhere (22).

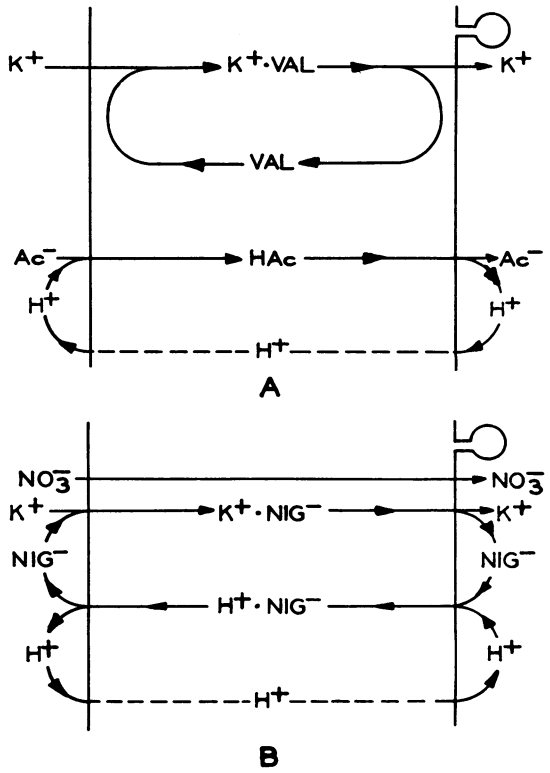

Fig. 4. Proposed mechanism of passive salt permeation for: (*A*) valinomycin plus potassium acetate, and (*B*) nigericin plus potassium nitrate.

From an analysis similar to that shown in Figs. 2 and 3, one can readily see that the combinations valinomycin plus potassium nitrate and nigericin plus potassium acetate would not yield active transport under appropriate conditions because such combinations tend to move cations and anions in opposite directions. Similarly, one can see that the two types of ionophores tend to move cations in opposite directions. This explains, at least partially, why the two types of ionophores act effectively as competitive inhibitors of one another's active transport-inducing activities (1, 2, 4).

SALT PERMEABILITY

The modulation of electrochemical potentials as proposed in the previous section is a necessary but not a sufficient condition for the efficient coupling of a chemical reaction and a *net* ion flux. Since active transport involves the transport of some species from a medium of lower to a medium of higher electrochemical potential, the active process must always compete with the passive *reverse* process. Furthermore, the present model assumes that the mechanism of permeation

is identical for active and passive transport. How, then, can the active process compete effectively with the passive process so as to yield a net ion flux?

The answer is that the active process can compete effectively with the passive process only if the *energy-linked step* in the active process is at the *rate-limiting step* of the passive process. Since the energy-linked step in the present model is the transmembrane movement of protons, self-consistency requires that the rate-limiting step of salt permeation for those salts that can be actively concentrated must be proton permeation. We will refer to the salt plus ionophore combinations that yield active transport under appropriate conditions as "active" combinations.

Table 1 presents the pellet water values for mitochondria suspended in 0.15 M salt solutions and incubated for 15 min. We note that those systems without ionophore or the two active combinations do not swell, whereas the inactive combinations do swell. As shown in Fig. 4, both of the active combinations involve permeation mechanisms in which *one and only one* of the ions crosses either with a proton or in exchange for a proton. Thus a net flux of either salt across the membrane, without a corresponding *net* flux of protons, involves the unfacilitated transport of protons back across the membrane. If we assume that the membrane is relatively impermeable to protons compared to the ions of the salt (16), proton permeation would be the rate-limiting step in salt permeation for these two active combinations.

Furthermore, swelling occurs when an uncoupler of oxidative phosphorylation, such as carbonylcyanide *m*-chlorophenylhydrazone (*m*-Cl-CCP) is added to an active combination (Table 1). Uncouplers have been shown to facilitate proton permeability across both the inner mitochondrial membrane (16) and the lipid bilayers (27). Similarly, if one analyzes the inactive combinations, (*a*) valinomycin plus potassium nitrate and (*b*) nigericin plus potassium acetate as in Fig. 4, one finds that they do not involve unfacilitated proton transport, and, consequently, cause swelling.

A combination of the two types of ionophores would also render the membrane permeable to any of the salts considered here. Thus excessive salt permeability is another factor in explaining why the two types of ionophores act as competitive inhibitors of one another's active transport-inducing activities. Excessive salt permeability also provides a sufficient explanation for the uncoupling of ion transport from electron transfer by *m*-Cl-CCP.

We recognize that salt permeability is only a necessary but not a sufficient condition for swelling since permeation of and by itself does not generate an osmotic driving force. Thus the swelling data presented here are consistent with but do not conclusively confirm our interpretation based on proton permeability. Nonetheless, in the absence of an equally compelling interpretation, we feel that these results provide rather strong support for our interpretation.

If we tentatively accept this interpretation, we can see the great significance of modulating proton conductivity in the present model. The crux of the present model lies in the separate mechanisms by which the individual ions of the actively transported salt cross the membrane—one ion via an electrogenic mechanism and one ion via an electrically neutral, pH-driven mechanism. As shown in Figs. 2 and 3, the excitation phase of the conformational cycle involves the electrically driven transport of the permeant ion. The back

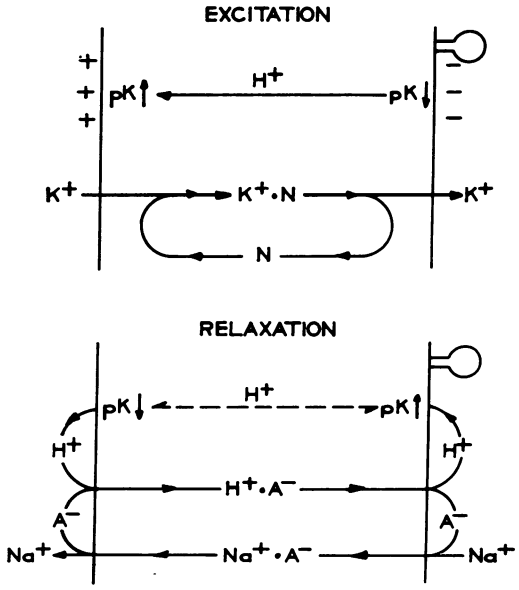

FIG. 5. Schematic representation of endogenous ionophore-induced sodium–potassium exchange in plasma membrane driven by proton-induced membrane potential and pH gradient. *N* denotes neutral ionophore and *A* denotes anionic ionophore.

diffusion of these ions would be prevented upon relaxation if the proton conductivity were very low in the relaxed or ground state. Thus, although the ions remain readily exchangeable, a *net* flux of ions back across the membrane would involve spontaneous charge separation and is therefore inhibited. The transported protons are no longer available to provide a countercurrent, as they were in the excited state. Thus the present model requires the modulation of proton conductivity not only to generate the two driving forces of a membrane potential and pH differential, but also to obtain a unidirectional flux of ions across the membrane.

GENERALITY OF MODEL

The ionophores we have considered until now are all of microbiological origin, and one may ask whether the type of active transport we have considered is a meaningful physiological phenomenon. In reply to this question we would like to point out that Blondin *et al.* have now isolated one ionophore, and are attempting to isolate another, from beef heart mitochondria (27–29). One is a neutral ionophore that exhibits little specificity for sodium versus potassium, and the other is an anionic ionophore that is apparently highly sodium-specific. Since specificity is, however, frequently dependent on the assay used, these statements must be considered tentative. See, for example, refs. 30, 31.

In Fig. 5 we have indicated how these two ionophores together might explain the familiar energy-linked sodium–potassium exchange of the plasma membrane. On the excitation phase of the cycle the electrical potential difference provides a driving force for the neutral ionophore-induced potassium transport. On the relaxation phase of the cycle the pH gradient drives the anionic ionophore-facilitated transport of sodium. Note that the polarity of the membrane potential predicted by this model is negative inside the cell, as is observed. Note also that the discussion in the previous section concerning the significance of modulating proton con-

ductivity is equally applicable to the mechanism of sodium–potassium exchange proposed here.

To suggest at the present time that the present model is applicable to active transport systems other than the mitochondrion is, of course, highly speculative. Even the mammalian origin of the two ionophores just discussed might be disputed. We would like to point out, however, that the essence of the present model would remain unchanged even if other mechanisms of facilitated transport were to be invoked as long as they were phenomenologically equivalent to the two types of ionophore-facilitated transport considered here. The basic aim of the present model has been to provide a physically reasonable mechanism for coupling a chemical reaction and an ion flux when the mechanisms of ion permeation are identical under active and passive transport conditions.

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1. Pressman, B. C., E. J. Harris, W. S. Jagger, and J. H. Johnson, *Proc. Nat. Acad. Sci. USA*, **58**, 1949 (1967).
2. Lardy, H. A., S. N. Graven, and S. Estrada-O, *Fed. Proc.*, **26**, 1355 (1967).
3. Cockrell, R. S., and E. Racker, *Biochem. Biophys. Res. Commun.*, **35**, 414 (1959).
4. Montal, M., B. Chance, and C. P. Lee, *J. Membrane Biol.*, **2**, 201 (1970).
5. Mitchell, P., *Biol. Rev. Cambridge Phil. Soc.*, **41**, 445 (1966).
6. Young, J. H., G. A. Blondin, G. Vanderkooi, and D. E. Green, *Proc. Nat. Acad. Sci. USA*, **67**, 550 (1970).
7. Penniston, J. T., R. A. Harris, J. Asai, and D. E. Green, *Proc. Nat. Acad. Sci. USA*, **59**, 624 (1968).
8. Harris, R. A., J. T. Penniston, J. Asai, and D. E. Green, *Proc. Nat. Acad. Sci. USA*, **59**, 830 (1968).
9. Williams, C. H., W. J. Vail, R. A. Harris, M. Caldwell, D. E. Green, and E. Valdivia, *J. Bioenergetics*, **1**, 147 (1970).
10. Green, D. E., *Proc. Nat. Acad. Sci. USA*, **67**, 544 (1970).
11. Green, D. E., and J. H. Young, *Amer. Sci.*, **59**, 92 (1971).
12. Young, J. H., G. A. Blondin, and D. E. Green, in *Physical Principles of Neuronal and Organismic Behavior* (Gordon and Breach Science Publishers, Inc., New York, 1971), in press.
13. Skulachev, V. P., *FEBS Lett.*, **11**, 301 (1970).
14. Greville, G. D., in *Current Topics in Bioenergetics*, ed. D. R. Sanadi (Academic Press, New York, London, 1969), Vol. 3, p. 1.
15. Tupper, J. H., and H. Tedeschi, *Proc. Nat. Acad. Sci. USA*, **63**, 370, 713 (1969).
16. Mitchell, P., and J. Moyle, *Biochem. J.*, **104**, 588 (1967).
17. Perutz, M. F., *Nature*, **228**, 726 (1970).
18. Orttung, W. H., *Biochemistry*, **9**, 2394 (1970).
19. Klotz, L. M., in *Horizons in Biochemistry*, ed. M. Kasha and B. Pullman (Academic Press, New York, London, 1962), p. 523.
20. Onsager, L., *Science*, **166**, 1359 (1969).
21. Chappell, J. B., and K. N. Haarhoff, in *Biochemistry of Mitochondria*, ed. E. C. Slater, Z. Kaninga, and L Wojtezak (Academic Press, New York, London, 1967), p. 75.
22. Blondin, G. A., and D. E. Green, *J. Bioenergetics*, **1**, 479 (1970).
23. Moore, C., and B. C. Pressman, *Biochem. Biophys. Res. Commun.*, **15**, 562 (1964).
24. Harris, E. J., R. Cockrell, and B. C. Pressman, *Biochem. J.*, **99**, 200 (1966).
25. Blondin, G. A., and D. E. Green, *Arch. Biochem. Biophys.*, **132**, 509 (1969).
26. Hopfer, U., A. L. Lehninger, and T. E. Thompson, *Proc. Nat. Acad. Sci. USA*, **59**, 484 (1968).
27. Blondin, G. A., *Biophys. Abst.*, **10**, 194a (1970).
28. Blondin, G. A., A. F. DeCastro, and A. E. Senior, *Biochem. Biophys. Res. Commun.*, **43**, 28 (1971).
29. Blondin, G. A., and R. M. Hull, *Biophys. Abst.*, **11**, 120a (1971).
30. Pressman, B. C., and M. J. Heeb, *Biophys. Abst.*, **11**, 310a (1971).
31. Goodall, M. C., *Biochim. Biophys. Acta*, **203**, 28 (1970).