Uptakes of radioactive Cl\(^-\) or I\(^-\) by gastric microsomal vesicles were stimulated 2- to 8-fold by ATP. The sensitivity of those uptakes to a Cl\(^-\) \(\rightarrow\) OH\(^-\) ionophore and to osmotic swelling suggested they were due to transport rather than to binding. The ATP effect was labile, but dithiothreitol and methanol improved its stability. The stimulation of anion transport required magnesium; GTP and UTP were less potent than ATP whereas ADP and AMP had no effect. The apparent \(K_m\) for ATP was estimated to be \(2 \times 10^{-4}\) \(M\) at \(22^\circ\)C. The rate of the ATP-dependent transport showed saturation-type kinetics, with half-maximal uptake at 10 mm for I\(^-\) and 15 mm for Cl\(^-\). Nonradioactive Cl\(^-\), I\(^-\), and SCN\(^-\) competed with \(^{125}\)I\(^-\) uptake while SO\(_4\)\(^{2-}\) did not. K\(^+\) valinomycin increased the ATP-dependent Cl\(^-\) uptake. The thermostable inhibitor of CAMP-dependent protein kinases inhibited the effect of ATP. These results suggest the existence of an anion conductance, permeant to Cl\(^-\), I\(^-\), and SCN\(^-\) and nonpermeant to SO\(_4\)\(^{2-}\), which could be linked to a CAMP-dependent protein kinase.

Gastric mucosa secretes HCl down to pH 1. The mechanisms responsible for this secretion are still debated, but it is becoming clear that H\(^+\) and Cl\(^-\) are secreted by two distinct processes (1), although Cl\(^-\) appears obligatory for H\(^+\) secretion to occur (2, 3). To account for the secretion of H\(^+\), the involvement of (H\(^+\)K\(^+\))-ATPase was proposed (4-6). This ATPase was suggested to be different from both the mitochondrial F\(_1\) ATPase and the plasma membrane (Na\(^+\)K\(^+\))-ATPase because of its lack of sensitivity to specific inhibitors such as oligomycin and ouabain, respectively. It was shown to catalyze a neutral exchange of H\(^+\) against K\(^+\) in purified gastric vesicles (1, 5), thus suggesting that it could possibly secrete H\(^+\) in the gastric lumen in vivo.

Mechanisms of gastric Cl\(^-\) secretion were mainly approached using isolated gastric mucosa mounted in Ussing chambers (2, 7, 8). Studies of ion fluxes and of transmucosal electrical parameters showed that Cl\(^-\) movement from serosal to lumen could be analysed into two components: a neutral transport and an electrogenic one. On the basis of indirect evidence, the neutral transport component was suggested to be linked to H\(^+\) secretion (1). To account for this link, a K-Cl channel has been suggested (1, 9). Thus, K\(^+\) trapped by the cell via the (H\(^+\)K\(^+\))-ATPase would be driven back to the gastric lumen by neutral co-transport of K\(^+\) and Cl\(^-\).

Electrogenic Cl\(^-\) secretion appeared to be a widely spread phenomenon. It was described in the intestine (10), the rectum (11), the cornea (12), the nerves (13), and the gills (14). In most of those tissues, Cl\(^-\) transport was hormone-sensitive. Furthermore, cyclic AMP and Ca\(^{2+}\) ionophore could mimic the effect of hormones (15-17). In none of those tissues, however, are the membranous mechanisms responsible for this Cl\(^-\) transport elucidated. In the gastric mucosa, histamine stimulates electrogenic Cl\(^-\) secretion (8) and the activity of adenylate cyclase (18, 19) and CAMP-dependent protein kinases (20). However, the implication of CAMP and protein kinases in the mediation of the secretory response has not been established. Cl\(^-\) secretion has been shown to depend upon cell metabolism and, more specifically, upon ATP synthesis (21, 22).

In previous papers (23, 24) we reported evidence for an ATP-stimulated Cl\(^-\) transport in gastric microsomal vesicles. This ATP-stimulated Cl\(^-\) transport was not inhibited by SITS, the Cl\(^-\) \(\rightarrow\) HCO\(_3\)\(^-\) channel inhibitor. Furthermore, it was sensitive neither to rutamycin nor to ouabain and vanadate which suggested it was not dependent on the activity of F\(_1\) or (Na\(^+\)K\(^+\))-ATPase. The purpose of this work was to go deeper into the characterization of this microsomal ATP-dependent anion transport. We report its sensitivity to membrane potential, and furthermore, show that its ATP dependency could be due to the involvement of CAMP-dependent protein kinase(s).

**Experimental Procedures**

**Materials**

1. **Reagents**—ATP (disodium and magnesium), PEP (disodium or tricyclohexylamine), hexokinase from yeast (type III), oligomycin, ouabain, bovine serum albumin Fraction V, dithiothreitol, glucose, Hepes, Dowex resin, valinomycin, and DIDS were purchased from Sigma; tributyltin was purchased from E. Merck Schuchardt (Munchen, Germany); pyruvate kinase (suspension) was purchased from Boehringer Mannheim (Meylan, France); and SITS was purchased from Polysciences. All salts were of analytical grade. Radioactive anion "Cl" (NaCl or HCl, 3 mCi/g), \(^{125}\)I\(^-\) (NaI, 200 mCi/ml), and SO\(_4\)\(^{2-}\) (KSCN, 300 mCi/g) were purchased from Amersham (Versailles, France).

2. **Sucrose Solution Used during the Work**—The solution contained 0.25 m sucrose, 50 mm Hepes, NaOH (pH 7.3), 2 mm dithiothreitol, and 10 \(\mu\)M DIDS.

3. **Protein Kinase Inhibitor**—The heat-stable protein inhibitor of CAMP-dependent protein kinases was a gift from Dr. Munari Silem (Lyon, France) and Dr. P. Mangeat (Marseille, France). This inhibitor was purified from the rat brain as previously described (25) up to the stage of the DEAE-cellulose column. It was provided at a concentra-

---

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: SITS, 4'-acetamido-4'-isothiocyanato-2,2'-disulfonic acid stilbene; PEP, phosphoenolpyruvate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DIDS, 4,4'-diisothiocyanato-2,2'-disulfonic acid stilbene.
tion of 410 μg of protein/ml in 5 mM sodium glycerophosphate, 0.2 mM EDTA, pH 7.0. The activity of the inhibitor was determined by Dr. P. Mangeat, using a purified preparation of pancreatic protein kinase (26). A unit of inhibitor was defined as the quantity which inhibits the transfer by the purified protein kinase of 1 pmol of phosphate to protein (1 mg/ml of histone) in 1 min at 30°C.

4. Dowex Columns—Commercially available chloride Dowex 1-X8 was regenerated to its formate form. This was realized by successive washings of the resin 1) with 1 N NaOH (10 liters/500 g of resin), 2) with distilled water until no trace of free C1- was detected in the washings of the resin 1) with 1 N formic acid (1 liter/500 g of resin), and 4) with distilled water until the pH of the rinsing water reached pH 4. The regenerated resin was stable to 4 months at 4°C.

Columns were set up by filling Pasteur pipets (10 × 0.55 cm) with regenerated Dowex 1-X8 (27). Before the test, each column was washed with 0.5 ml of 10% (w/v) bovine serum albumin to prevent C1- trapping by gastric microsomes. So treated, the columns could be used four times and they retained 99.9% of the radioactive anion while they allowed a good recovery of the proteins (Table I).

Methods

1. Preparation of Microsomal Fractions—Microsomes were prepared from hog, rabbit, and human fundic stomachs. Fresh hog stomachs were kindly provided by Olida’s slaughtering house (Lev- allois, France). Rabbit stomachs were taken from albino rabbits killed with nembutal. Human stomachs were fresh pieces of surgery. They were opened and washed with fresh tap water. Mucous was removed by rubbing the mucosa with a glass slide. Fundic mucosa was separated from the submucosa and cut into small pieces. These were diluted in sucrose solution at 4°C and homogenized (Ultraturrax homogenizer) for 3 min at 4°C. Unhomogenized tissues were discarded by filtration on gauze tissue. The homogenate was then centrifuged at 4°C to separate microsomal fractions: 1) the nuclear pellets were discarded after centrifugation (7 min × 25,000 rpm) (Beckman L-5-65 centrifuge, rotor 70 Ti); and 3) microsomes were pelleted by centrifugation (30 min × 20,000 rpm) and finally resuspended in sucrose solution at a concentration of 15 to 35 mg of protein/ml. Methanol was then added at a final concentration of 2.5 to 5% to improve the conservation.

2. Protein Determination—Proteins were estimated according to Lowry et al. (28) using bovine serum albumin as standard.

3. Anion Uptake Measurements—Anion uptake was measured as the radioactivity eluted from the Dowex columns prepared as reported under “Materials.” Incubations were started by adding microsomes (100 μl) to 250 μl of sucrose solution containing 0.4 to 1 mM NaCl or NaAc and 2 to 10 × 10^−3 cm M of 36Cl⁻ or 35Cl⁻. The activity of ATP was tested by adding 1 to 3 mM ATP, 1 to 3 mM MgSO₄, 4 to 16 mM PEP, and 2 to 4 units of pyruvate kinase or, in the controls, 10 to 30 mM glucose, and 1 unit of hexokinase. Incubations were run for 3 min at 20–30°C, and 3 samples of 100 μl each were applied to columns and eluted with 2 ml of sucrose solution containing 0.1% bovine serum albumin. The whole volume of the eluates was counted for the determination of trapped radioactivity.

4. ATPase Activity—ATPase activity was measured in the presence of 1 mM ATP, 1 mM MgSO₄, 10 μg/ml of oligomycin, 1 mM ouabain, 4 mM PEP, and 2 units/ml of pyruvate kinase. The incubations were run for 20 min at 30°C at pH 8.0 (50 mM Tris/acetate). Inorganic phosphate was measured as described (29).

RESULTS

1. Basal Uptake of Anions by Gastric Microsomes

Gastric microsomal vesicles were prepared in the presence of 250 μM SITS or 10 μM DIDS. These vesicles took up Cl⁻ with an initial rate of 220 pmol of Cl⁻ trapped/mg of protein/min using a 4 mM Cl⁻ gradient (i.e. [Cl⁻]₀ = 4 mM with [Cl⁻]ₐ = 0 mM). Vesicles also took up iodide at a comparable rate. In the absence of SITS, an inhibitor of the HCO₃⁻ → Cl⁻ interexchange, initial rates of uptakes were increased by 30 to 40%. Uptakes reached steady states within 20 to 100 min depending on the anion concentration. Steady state uptakes (180 min) showed a linear relationship to the anion concentrations (0.45 to 50 mM Cl⁻ or I⁻). No saturation was observed.

2. Uptake of Anions in the Presence of ATP

ATP increased both the rate and the apparent extent of 36Cl⁻ trapping by gastric microsomes of rabbit, hog, and man. It had no effect on Cl⁻ uptake by liver microsomes similarly prepared (one experiment). The stimulation of the rate of Cl⁻ influx was 2- to 4-fold over the basal rate. ATP also stimulated 3- to 8-fold the rate of 125I⁻ uptake (Fig. 1). The ATP-stimulated uptakes (i.e. uptakes in the presence of ATP minus control) were proportional to the concentration of microsomal protein (Fig. 2). By contrast, ATP did not stimulate the rate of 35S⁻ efflux from hog microsomes. Effluxes were measured using vesicles loaded with radioactive anion for 24 to 72 h at 0–4°C.

Effect of Ageing—Ageing the microsomes in 0.25 mM sucrose, 50 mM Hepes (pH 7.3) at 0–4°C for 1 day resulted in a full loss of the ATP effect. Conservation at 0–4°C was greatly improved by 1 to 2 mM dithiothreitol and, in a less obligatory way, by methanol (2.5 to 5% final concentration). In those conditions, ATP stimulation was maintained up to a week.

Characterization of the Uptake—I⁻ and Cl⁻ uptakes were assumed to represent transport events because 1) they were reversible by 5 × 10⁻³ to 10⁻² M of the Cl⁻ → OH⁻ ionophore, tributyltin (30) and 2) the magnitude of the ATP-induced uptakes correlated the volume of the microsomal vesicles as monitored by the osmolarity of the suspension medium (Fig. 3). Furthermore, uptakes extrapolated almost to zero for infinite osmolarity suggesting a low contribution of adsorption phenomena.

ATP Specificity—ATP stimulation required Mg²⁺. GTP and UTP could partially mimic the ATP effect, while AMP and ADP, i.e. ATP in the presence of glucose and hexokinase, had no effect (Table II). The apparent Kₘ for ATP was estimated to be 1.8 and 2.8 × 10⁻⁴ M (2 different microsomal preparations) at 22°C in the presence of the ATP-regenerating system, 1 mM Mg²⁺, and 4 mM I⁻.

Temperature Sensitivity—Increasing the temperature of the incubation increased the stimulation by ATP (apparent Eₘ = 10.4 kcal/mol). By contrast, the basal transports of CI⁻
rate was stopped by applying three samples of ATP columns. This plot is the mean started by adding 100 mg of protein/ml to 2.52 ml of sucrose solution to 260 pl of microsomes. The reaction was started by adding 1.25 units of hexokinase. After incubation for 2.5 min at 23°C and containing 4 mM NaI, 5.6 X 10⁶ cpm of ¹²⁵I- and 20 mM glucose, and 1 unit of hexokinase. The osmolarity was monitored by the concentration of sucrose; all other constituents were maintained constant. Incubations were run for 13 min at 30°C and 0.1-ml samples were applied to the columns. The results are the difference of the I- uptake in the presence of ATP minus I- uptake in the control. They are the mean of two triplicate experiments.

TABLE I

| Addition                  | I- trapped pmol/mg protein/min |
|---------------------------|-------------------------------|
| None                      | 220                           |
| Mg + PK-PEP               | 153                           |
| ATP + Mg + PK-PEP         | 1516                          |
| GTP + Mg + PK-PEP         | 660                           |
| UTP + Mg + PK-PEP         | 451                           |
| AMP + Mg                  | 228                           |
| ATP + Mg + hexokinase/glucose | 180                      |

Estimated to be 1.33 to 2 nmol of I- transported/mg of protein/min in two different microsomal preparations. With a rabbit microsomal preparation, half-maximal Cl- uptake was obtained at 15 mM.

Anion Selectivity—Separate experiments showed that the influx of ³⁶Cl-, ¹²⁵I-, and ³⁴CN- were all stimulated by ATP. Competition studies were carried out in the presence of ATP, using ¹²⁵I- as tracer (Fig. 6). I-, CI-, and SCN- competed with ¹²⁵I-, while SO₄²⁻ up to 80 mM did not. These observations suggested that Cl-, I-, and SCN- might be transported by the same ATP-dependent mechanism while SO₄²⁻ is not.

Influence of Membrane Potential—ATP-dependent Cl- transport was markedly increased by the addition of K+ and valinomycin (Fig. 7). The addition of valinomycin or potassium alone had no effect. The K+ concentration dependence of the K+-valinomycin effect was consistent with the hypothesis of a K+-generated potential according to the Nernst equation. The magnitude of the effect was furthermore dependent upon Cl- concentration (being higher for high [Cl-]) which suggested it could be due to attenuation of a self-created potential that limits Cl- uptake.

Mechanism of ATP Involvement—The presence of an anion-sensitive ATPase was investigated. In the presence of 10
Fig. 4. pH dependency of the uptake of I\textsuperscript{-} by hog gastric microsomes. The reaction was started by adding 50 \mu l of microsomes (37.2 mg of protein/ml) to 330 \mu l of sucrose solution containing 0.5 mM NaI, 1.3 \times 10\textsuperscript{5} cpm of I\textsuperscript{131} and, in the series with ATP (O——O), 2.2 mM MgATP, 16 mM PEP, and 4 units of pyruvate kinase, or, in the controls (●——●), 2.2 mM MgSO\textsubscript{4}, 33 mM glucose, and 1 unit of hexokinase. pH was monitored using 50 mM Hepes/NaOH or 50 mM Tris/Hepes. The incubations were run for 3 min at 30°C and 0.1-ml samples were applied to the columns. The results plotted are the mean of two triplicate experiments.

Fig. 5. Effect of I\textsuperscript{-} concentration on the rate of I\textsuperscript{-} uptake by hog gastric microsomes. The reaction was started by adding 250 \mu l of microsomes to 380 \mu l of sucrose solution containing 8 \times 10\textsuperscript{5} cpm of I\textsuperscript{131} and either 7 mM MgATP, 20 mM PEP, and 2.4 units of pyruvate kinase, or, in the controls, 7 mM MgSO\textsubscript{4}. Incubations were run for 2.5 min at 22°C in the presence of various concentrations of NaI and 0.1-ml samples were applied to the columns. The results represent the uptake of I\textsuperscript{-} in the presence of ATP minus the uptake of I\textsuperscript{-} in the controls in two different microsomal preparations: 12.5 mg of protein/ml (O——O) and 31 mg of protein/ml (●——●). Each point is the mean of two experiments with triplicate determinations.

\[ \text{\mu g/ml of oligomycin and 1 mM ouabain that inhibited 20 to 35\% of the ATPase activity of the microsomes, the activity was estimated to be 90 nmol P, liberated/min/mg of protein at 30°C. Cl\textsuperscript{-} and I\textsuperscript{-} (4 to 20 mM) stimulated basal activity (0 to 23\%). } \text{HCO}_3\textsuperscript{-}, which is known to stimulate an ATPase in gastric microsomes (31, 32), had no effect on our preparations. More consistent is the finding that the thermostable inhibitor of cAMP-dependent protein kinases (25) prevented the stimulation of the rate of I\textsuperscript{-} influx by ATP (Fig. 8). Half-maximal inhibition was obtained with a concentration as low as 35 units of protein kinase inhibitor/mg of microsomal protein. One hundred units/mg gave an almost complete inhibition of the ATP effect.}

DISCUSSION

Gastric membrane vesicles have been used by Sachs et al. (5) to study the active transport of H\textsuperscript{+} by (H\textsuperscript{+},K\textsuperscript{-})-ATPase.
These vesicles, when fresh, showed a low transport capacity for Cl⁻ in contrast to the high transport capacity of gastric microsomes. Microsomes (25 mg of protein/ml) contained 10 μg/ml of oligomycin and 1 mM ouabain. Reactions were started by adding 100 μl of microsomes to a medium containing 1) 220 μl of various dilutions of protein kinase inhibitor buffer (see "Experimental Procedures") to keep the osmolarity constant; 2) 50 μl of 4 mM NaI, 3 × 10⁶ cpn of ¹⁵⁷T, 10 mM MgSO₄, and, in the series with ATP, 10 mM Na⁺ATP, 20 mM PEP, and 2 units of pyruvate kinase, or, in the controls, 50 mM glucose and 1 unit of hexokinase. Incubations were run for 3 min at 30 °C and 0.1 ml samples were applied to the columns three times. The inhibitor concentration was expressed as unit (defined in "Materials") per mg of microsomal protein in the assay. Results and expressed as the I⁻ uptakes in the presence of ATP (○) and in the controls (●). They are the mean of three experiments with triplicate determinations.

The electrical nature of the ATP-dependent Cl⁻ transport in vesicles was suggested by its capacity to respond to the rate of synthesis of pyruvate by the pyruvate kinase. This inhibition did not appear to be due to the glyceral phosphate buffer in which the agent was provided. In Fig. 8, showing the dose-response curve to the inhibitor, buffer concentration was kept constant in all the samples, including those without inhibitor. Neither was the inhibition due to an effect on the ATP-regenerating system (phosphoenolpyruvate; pyruvate kinase) because we found that it did not alter the rate of synthesis of pyruvate by the pyruvate kinase. Synthesis of pyruvate was measured by the oxidation of βNADH + H⁺ catalyzed by lactic dehydrogenase in excess. Our result does suggest that the catalytic subunit of a cAMP-dependent protein kinase might be implicated in the regulation of the gastric microsomal anion transport. This finding brings on a new interest in the mechanism of Cl⁻ transport in gastric mucosa as well as in other epithelia. Further studies are, however, needed to characterize this protein kinase dependency.

REFERENCES

1. Sachs, G., Spenney, J. G., and Lewin, M. (1978) Physiol. Rev. 58, 106-173
2. Rehm, W. S. (1965) Fed. Proc. 24, 1387-1395
3. Berglind, T. (1977) Gastroenterology 73, 574-580
4. Lee, J., Sispam, G., and Scholes, F. (1974) Biochem. Biophys. Res. Commun. 68, 226-232
5. Sachs, G., Chang, F. H., Rabon, E., Schackman, R., Lewin, M., and Saccomani, G. (1976) J. Biol. Chem. 251, 7690-7698
6. Saccomani, G., Stewart, H. B., Shaw, D., Lewin, M., and Sachs, G. (1977) Biochim. Biophys. Acta 465, 311-330
7. Hudson, C. M. (1951) Proc. Natl. Acad. Sci. U. S. A. 23, 399-395
8. Forte, J. G., and Machen, T. E. (1975) J. Physiol. (Lond.) 244, 33-51
9. Sachs, G., Chang, H., Rabon, E., Schackman, R., Sarau, H. M., and Saccomani, G. (1977) Gastroenterology 73, 931-940
10. Frier, R. A. (1977) J. Membr. Biol. 35, 175-187
11. Stoff, J. S., Rosa, R., Hallac, R., Silva, P., and Epstein, F. M. (1979) Am. J. Physiol. 237, F135-F144
12. Candel, O. A., Montenegro, R., and Podos, S. M. (1977) Am. J. Physiol. 233, F94-F101
13. Takeshi, H., Watanabe, K., and Tamura, H. (1978) Comp. Biochem. Physiol. 61, 309-315
14. Degnan, K. J., Karnaky, K. J., Jr., and Zadunaisky, J. A. (1977) J. Physiol. (Lond.) 277, 155-191
15. Zadunaisky, J. A. (1979) in Mechanisms of Intestinal Secretion (Binder, H. J., ed) pp. 53-64, Alan R. Liss, New York
16. Berridge, M. J. (1979) in *Mechanisms of Intestinal Secretion* (Binder, H. J., ed) pp. 65–81, Alan R. Liss, New York
17. Field, M. (1979) in *Mechanisms of Intestinal Secretion* (Binder, H. J., ed) pp. 83–91, Alan R. Liss, New York
18. Lewin, M. J. M., Grelac, F., Cheret, A. M., René, E., and Bonfils, S. (1979) in *Hormone Receptors in Digestion and Nutrition* (Rosselin, G., Fromageot, P., Bonfils, S., eds), pp. 383–390, Elsevier/North Holland, Amsterdam
19. Batzri, S., and Gardner, J. D. (1979) *Mol. Pharmacol.* 16, 406–416
20. Mangeat, P., Marchis-Mouren, G., Cheret, A. M., and Lewin, M. J. M. (1980) *Biochim. Biophys. Acta* 629, 604–608
21. Hersey, S. J. (1977) *Biochim. Biophys. Acta* 496, 359–366
22. Mandel, L. J., and Riddle, T. G. (1979) *Am. J. Physiol.* 236, E301–E308
23. Soumarmon, A., and Racker, E. (1978) in *Frontiers of Biological Energetics* (Dutton, P. L., ed) Vol. 1, pp. 555–562, Academic Press, New York
24. Soumarmon, A., Ghesquier, D., and Lewin, M. J. M. (1979) in *Hormone Receptors in Digestion and Nutrition* (Rosselin, G., Fromageot, P., Bonfils, S., ed) pp. 349–354, Elsevier/North Holland, Amsterdam
25. Walsh, D. A., Ashby, C. D., Gonzalez, C., Calkins, D., Fischer, E., H., and Krebs, E. G. (1971) *J. Biol. Chem.* 246, 1977–1985
26. Mangeat, P. H., Chabiniak, H., and Marchis-Mourems, G. J. (1978) *Biochimie (Paris)* 60, 777–785
27. Gasko, O. D., Knowles, A. F., Shertzzer, H. G., Suolinna, E. M., and Racker, E. (1976) *Anal. Biochem.* 72, 57–64
28. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* 193, 265–275
29. Soumarmon, A., Lewin, M., Cheret, A. M., and Bonfils, S. (1974) *Biochim Biophys. Acta* 339, 403–414
30. Selwyn, M. J., Dawson, A. P., Stockdale, M., and Gains, N. (1970) *Eur. J. Biochem.* 14, 120–126
31. Sachs, G., Wiebelhaus, V. D., Blum, A. L., and Hirschowitz, R. I. (1972) in *Gastric Secretion* (Sachs, G., Heinz, E., Ulrich, K. J., eds) pp. 321–343, Academic Press, New York
32. De Renzis, G., Bornancin, M. (1977) *Biochim. Biophys. Acta* 467, 192–207
33. Rabon, E., Kajdos, I., and Sachs, G. (1979) *Biochim. Biophys. Acta* 556, 469–478
34. Lewin, M. J. M., Saccomani, G., Schackman, R., and Sachs, G. (1977) *J. Membr. Biol.* 32, 301–318
35. Racker, E. (1975) in *Electron Transfer Chains and Oxidative Phosphorylation* (Quagliariello, E., ed) pp. 401–406, North Holland, Amsterdam