Biochimica et Biophysica Acta, 460 (1977) 445–454

BBA 47309

EFFECTS OF ETHACRYNIC ACID AND FUROSEMIDE ON PHOSPHORYLATION REACTIONS OF KIDNEY MITOCHONDRIA

INHIBITION OF THE ADENINE NUCLEOTIDE TRANSLOCASE*

M. ARIFIE MANUEL and MICHAEL W. WEINER**

Department of Medicine, V. A. Hospital, and Institute for Enzyme Research, University of Wisconsin, Madison, Wisc. 53706 (U.S.A.)

(Received December 12th, 1976)

SUMMARY

Previous reports that ethacrynic acid and furosemide diminish mitochondrial P : O ratios and reduce $(Na^+ + K^+)$-ATPase activity suggested that these diuretics may inhibit mitochondrial phosphorylation reactions. This possibility was initially studied by determining the effects of ethacrynic acid and furosemide on [^{32}P]ATP exchange activity of rat kidney mitochondria. Concentrations of both drugs at 10^{-4} M or greater, significantly inhibited [^{32}P]ATP exchange. To investigate the mechanism of this inhibition, the effects of ethacrynic acid and furosemide on the ATPase activity of intact mitochondria and sonicated submitochondrial particles were determined. Both diuretics inhibited ATPase activity of intact mitochondria at 10^{-4} M. In contrast, ATPase of submitochondrial particles was significantly less susceptible to inhibition by the diuretics. These results suggested that ethacrynic acid and furosemide inhibit adenine nucleotide transport across the mitochondrial membrane. This was directly tested by determining the effects of the diuretics on the mitochondrial adenine nucleotide translocase. At $5 \cdot 10^{-4}$ M, both ethacrynic acid and furosemide significantly inhibited adenine nucleotide transport. These findings suggest that ethacrynic acid and furosemide may diminish renal tubular solute reabsorption by direct inhibition of adenine nucleotide transport across the mitochondrial inner membrane.

INTRODUCTION

Ethacrynic acid and furosemide are potent diuretic drugs which inhibit active chloride transport in the thick ascending limb of Henle's loop [5, 6]. Their subcellular

* Preliminary accounts of this work have been reported in abstracts [1–4].

** Present address: Stanford University Services, Veterans Administration Hospital, 3801 Miranda Avenue (111R), Palo Alto, Calif. 94304, U.S.A. to whom reprint requests should be addressed.

mechanism of action is unknown although a variety of effects on kidney slices [7–12], mitochondria [13–19], and enzymes [20–33] have been reported.

During an investigation concerning the effects of ethacrynic acid and furosemide on isolated kidney mitochondria, a decrease of the P : O ratio was repeatedly produced by these diuretics [22]. Previous reports of the inhibitory action of both ethacrynic acid and furosemide on $(Na^+ + K^+)$-ATPase [24–33], suggested the possibility that these compounds interfere with mitochondrial reactions involving the phosphorylation or dephosphorylation of ATP. In abstracts, Quintanilla, Levin and Lewy [34, 35] reported that furosemide inhibits ADP uptake by dog kidney mitochondria. The purpose of the present study was to investigate further the effects of ethacrynic acid and furosemide on mitochondrial phosphorylation reactions.

METHODS

All studies utilized 150–200 g male Sprague-Dawley rats. Isolated mitochondria were prepared from renal cortex by an adaptation [36] of the method of Johnson and Lardy [37]. The final pellet was suspended in mannitol/sucrose to give a protein concentration between 15 and 20 mg/ml. For some experiments, the mitochondria were sonicated in an ice bath with a Branson Sonicator at an intensity setting of 7 for 3–5 periods of 30 s each. The suspension was allowed to cool for 30 s between each sonication. Submitochondrial particles were prepared for ATPase studies by centrifuging the sonicated mitochondria at $15\,000 \times g$ for 10 min at 4 °C. The supernatant was centrifuged at $100\,000 \times g$ for 1 h at 4° C and the pellet was resuspended in mannitol/sucrose to achieve a protein concentration of 5 mg/ml.

Mitochondrial oxygen consumption was measured with a Gilson Oxygraph and Clarke oxygen electrode in an incubation medium containing the appropriate substrate, 0.15 M KCl, 5 mM triethanolamine phosphate, 10 mM triethanolamine · HCl, 5 mM $MgCl_2$, 1 mM ethyleneglycol-bis-(β-aminoethylether)-*N*,*N*′-tetraacetic acid (EGTA), pH 7.4, at 30 °C, total volume 2.6 ml. Each experiment was started by adding 0.2–0.5 mg mitochondrial protein to the Oxygraph cell. Initially, a state 4 (resting) rate of respiration was measured for approx. 1 min. Addition of 0.5 μmol ADP increased the respiratory rate (state 3 or active respiration [38]). The experiment was continued until the respiration slowed to the state 4 rate. The respiratory control ratio was calculated by dividing the state 3 rate by the state 4 rate [38]. Mitochondria were only utilized if the control respiratory control ratio was greater than 4 with succinate as substrate. The P : O ratio was calculated by dividing the μmol of ADP added by net μmol of oxygen consumed during state 3.

[^{32}P]ATP exchange activity was measured by an adaptation of the method of Pullman [39]. The final reaction mixture contained 0.15 M sucrose, 10 mM $MgCl_2$, appropriate concentration of diuretic, 10 mM ATP, 10 mM potassium phosphate, and 60000–80000 cpm of ^{32}P in a total volume of 1.0 ml, pH 7.4.

Energy-dependent ion transport was assayed by measuring the rate of mitochondrial swelling by a spectrophotometric method [40]. The spectrophotometer cuvette contained an incubation mixture of 0.25 M sucrose, 15 mM potassium acetate, 10 mM triethanolamine · HCl, $5 \cdot 10^{-8}$ M valinomycin, appropriate concentration of diuretic, pH 7.4, 27 °C, total volume of 3.0 ml. Mitochondria (0.5 mg protein) were added, and the absorbance at 520 nm was determined with a

Gilford Recording Spectrophotometer. After a 2 min preincubation period, 3 mM ATP was added and the change in absorbance was measured for 3 min.

ATPase activity was assayed by an adaptation [36] of the method of Lardy and Wellman [41] in 0.25 M sucrose, 15 mM KCl, 6 mM ATP, 10 mM triethanolamine · HCl, appropriate concentration of diuretic, pH 7.4, in a total volume 1.0 ml. When ATPase activity of intact mitochondria was assayed, 10^{-6} M carbonyl-cyanide *m*-chlorophenylhydrazone (CCCP) was added to produce maximal stimulation of ATPase activity. When sonicated mitochondrial particles were assayed, 1.5 mM $MgCl_2$ was present in the incubation medium but no CCCP was added.

Adenine nucleotide translocase activity was measured by an adaptation of the method of Wöjtczak and Zaluska [43] in 120 mM KCl, 1 mM EGTA, 10 mM triethanolamine · HCl, 2 μM ADP, 10 000 cpm [^{14}C]ADP, appropriate concentration of diuretic, pH 7.4, total volume of 1.0 ml. The reaction was carried out in centrifuge tubes at 0 °C. After the mitochondria were preincubated for 4 min, the reaction was begun by adding the [^{14}C]ADP. After 1 min, 0.04 μg atractyloside was added and the tubes were immediately centrifuged at 15 000 $\times$ *g* (4 °C) for 10 min. The supernatant was removed and the remaining pellet was dissolved in 0.3 ml toluene. The dissolved pellet was placed in Bray's solution and the radioactivity determined in a Packard Tricarb Liquid Scintillation Counter. The rate of ADP uptake was calculated from the radioactivity incorporated into the pellet and the specific activity of the ADP. ADP uptake is expressed as μmol ADP taken up/mg mitochondrial protein in 1 min.

Mitochondrial protein was determined by the method of Lowry et al. [44]. Statistical significance was calculated using Student's *t*-test.

Ethacrynic acid was provided by Dr. J. Baer of Merck, Sharpe and Dohme, Westpoint, Pa. Furosemide was provided by Dr. Leigh S. Whitlock, Hoechst, Sommerville, N. J. Carbonyl cyanide *m*-chlorophenylhydrazone was provided by Dr. Henry A. Lardy, Madison, Wisc.

RESULTS

Effects of ethacrynic acid and furosemide on [^{32}P] ATP exchange activity (Fig. 1). Our previous experiments demonstrated that both ethacrynic acid and furosemide lowered the P : 0 ratio of rat kidney mitochondria in the concentration range of 10^{-5} to 10^{-3} M [23]. In order to determine if these effects on the P : 0 ratio were due to direct interference with phosphorylation reactions, [^{32}P]ATP exchange activity was measured in the presence of the diuretics. Fig. 1 shows that both ethacrynic acid and furosemide inhibited [^{32}P]ATP exchange activity. At concentrations less than 10^{-5} M, no inhibitory effect occurred. Ethacrynic acid was a more potent inhibitor of [^{32}P]ATP exchange activity than furosemide; the inhibitory effects were significantly different for the two agents at concentrations greater than 10^{-5} M ($P < 0.01$).

Effects of ethacrynic acid and furosemide on energy-dependent mitochondrial swelling (Fig. 2). To investigate the effects of ethacrynic acid and furosemide on mitochondrial work functions supported by ATP hydrolysis, ATP-dependent ion uptake was measured. In the presence of valinomycin, mitochondria will accumulate potassium acetate using energy derived from electron transport or ATP hydrolysis

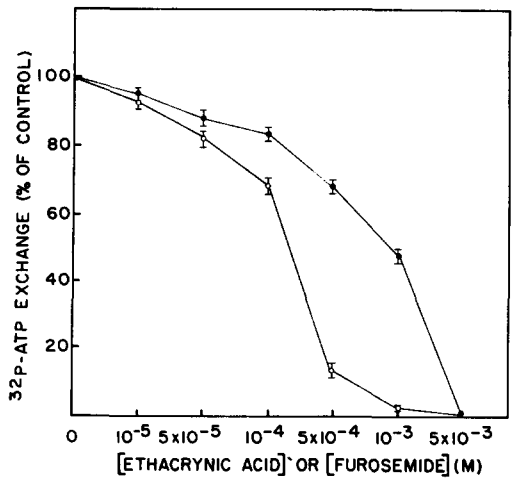

Fig. 1. Effects of ethacrynic acid (○) and furosemide (●) on [^{32}P]ATP exchange activity. Conditions are given in Methods. Data are expressed as the percent mean ±S.E. of control which was 0.028± 0.003 (S.E.) μmol P_i incorporated into ATP/mg protein per 10 min. $n = 52$, $n = 16$ for each concentration. At concentrations $> 10^{-5}$ M inhibition by ethacrynic acid was significantly greater than by furosemide ($P < 0.01$).

[45]. Under these conditions potassium uptake is facilitated by a membrane potential, and acetate uptake is supported by a pH gradient across the mitochondrial inner membrane [46]. Fig. 2 indicates that both ethacrynic acid and furosemide inhibit mitochondrial swelling which is energized by ATP hydrolysis.

Uncoupling properties of ethacrynic acid and furosemide. The findings that ethacrynic acid and furosemide diminish the P : 0 ratio, reduce [^{32}P]ATP exchange activity, and inhibit ATP-supported ion transport, have at least two possible interpretations. The first is that these compounds act as uncoupling agents. This possibility was tested by measuring the effects of ethacrynic acid and furosemide on the state 4

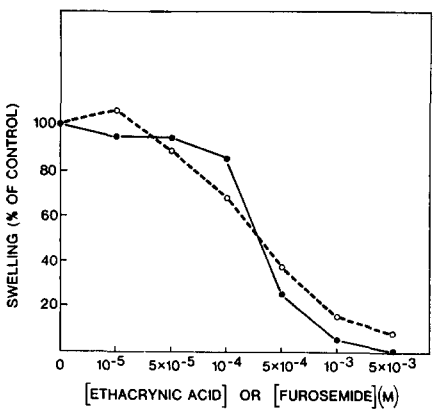

Fig. 2. Effects of ethacrynic acid (○) and furosemide (●) on ATP-dependent mitochondrial swelling. Conditions are given in Methods. Data are expressed as a percent of control which was −0.27 *A*. Each value is the mean of four determinations.

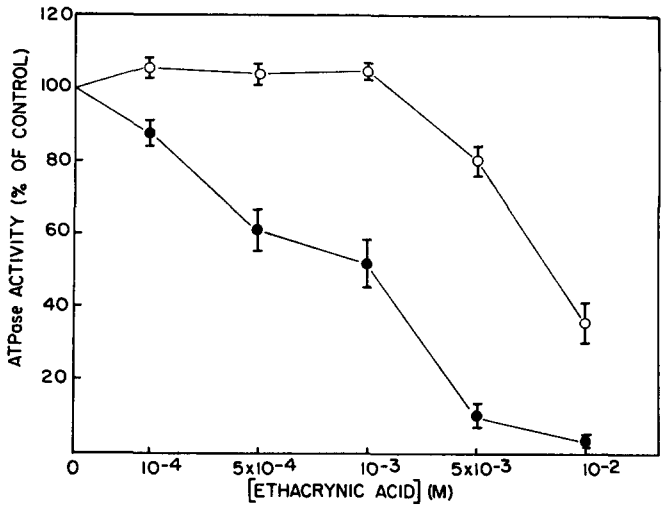

Fig. 3. Effects of ethacrynic acid on the ATPase activity of rat kidney mitochondria (●) and submitochondrial particles (○). Conditions are given in Methods. Activity is expressed as percent mean ± S.E. of the following control values; 2.83 (mitochondria) and 2.77 (submitochondrial particles) μmol P_i/mg protein per 10 min incubation. $n = 16$ for each concentration.

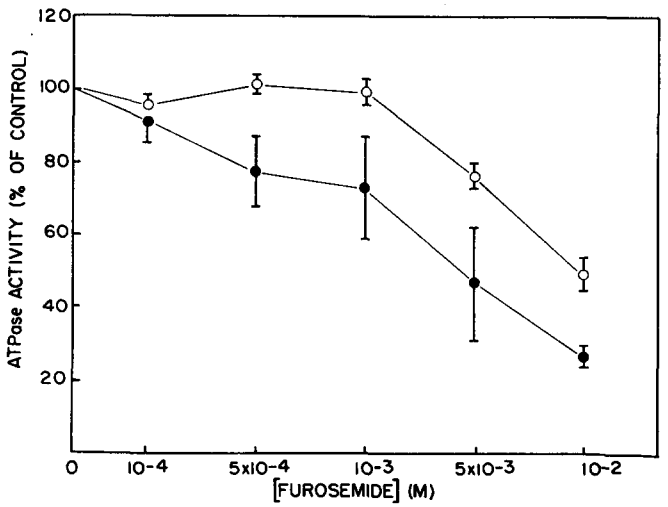

Fig. 4. Effects of furosemide on the ATPase activity of mitochondria (●) and submitochondrial particles (○) from rat kidney. Conditions are given in Methods. Activity is expressed as percent mean ± S.E. of the following control values: 2.83 (mitochondria) and 2.77 (submitochondrial particles) μmol P_i/mg protein per 10 min incubation. $n = 16$ for each concentration.

respiratory rate of well-coupled kidney mitochondria. In concentrations of 10^{-6}–$5 \cdot 10^{-3}$ M, stimulation of state 4 respiration did not occur; in fact, some respiratory inhibition was noted [23]. These findings are not consistent with an uncoupler-like action. Because uncoupling agents stimulate ATPase activity [41], the effects of ethacrynic acid and furosemide on the ATPase of well-coupled mitochondria were investigated. Neither ethacrynic acid nor furosemide (in concentration from 10^{-8} to 10^{-3} M) produced a stimulation of ATPase activity. These findings are further evidence against an uncoupling effect of the diuretics.

A second possible explanation for the decrease of the P : O ratio, ATP-supported ion transport, and [^{32}P]ATP exchange by ethacrynic acid and furosemide, is a direct inhibition of mitochondrial phosphorylation reactions. This possibility is considered below.

Effects of ethacrynic acid on ATPase activity of intact kidney mitochondria and submitochondrial particles (Fig. 3). In order to study the effects of ethacrynic acid on mitochondrial ATPase, the uncoupling agent CCCP was used to stimulate ATPase activity. Ethacrynic acid was a potent inhibitor of mitochondrial ATPase activity in concentrations from 10^{-4} to 10^{-2} M (Fig. 3). In order to determine if ethacrynic acid inhibited transport of the adenine nucleotides or phosphate across the mitochondrial membrane, the effects of the diuretic on the ATPase activity of sonicated submitochondrial particles were compared with its effects on intact mitochondria. Fig. 3 demonstrates that the sonicated submitochondrial particles were much less sensitive to inhibition of ATPase by ethacrynic acid ($P < 0.05$ at 10^{-4} M, $p < 0.01$ at $5 \cdot 10^{-4}$ M, and $P < 0.001$ at concentrations greater than 10^{-3} M).

Effects of furosemide on ATPase activity of intact kidney mitochondria and submitochondrial particles (Fig. 4). Similar to the effects of ethacrynic acid, furosemide inhibited the ATPase activity of intact kidney mitochondria induced by CCCP. At a concentration greater than 10^{-3} M, furosemide also inhibited ATPase activity of submitochondrial particles, but the inhibition of intact mitochondria and submitochondrial particles was not significantly different. Ethacrynic acid appeared to be a more potent inhibitor of ATPase activity than furosemide, but there was no significant difference between these diuretics. Furthermore, there was no difference between effects of ethacrynic acid and furosemide on the ATPase activity of kidney mitochondria compared with those obtained from liver (data not shown). This indicates that kidney mitochondria are not especially sensitive to these diuretic actions.

The effects of pH on inhibition of ATPase by ethacrynic acid and furosemide (Fig. 5). The effects of pH on the ATPase-inhibiting properties of ethacrynic acid and furosemide were studied using intact kidney mitochondria. The inhibition of ATPase by furosemide was not affected by alterations of pH. In contrast, the inhibitory action of ethacrynic acid was strongly influenced by pH; the greatest inhibitory action of ethacrynic acid occurred between pH 7.0 and 7.5.

Effects of ethacrynic acid and furosemide on the adenine nucleotide translocase (Fig. 6). The observation that ethacrynic acid had a significantly greater inhibitory action on the ATPase of intact mitochondria compared with sonicated submitochondrial particles, suggested that this agent might interfere with the transport of the substrates and/or products of the reaction across the mitochondrial inner membrane. To investigate this possibility, the effects of ethacrynic acid and furosemide on the

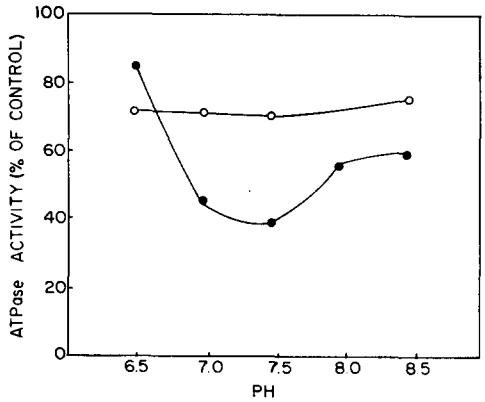

Fig. 5. The effects of pH on inhibition of rat kidney mitochondrial ATPase activity produced by 10^{-3} M ethacrynic acid (●) and 10^{-3} M furosemide (○). Conditions are given in Methods. Activity is expressed as a percent mean of the control for each pH, $n = 4$ for each concentration.

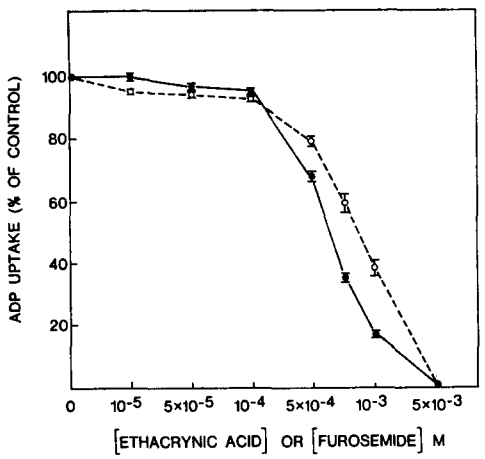

Fig. 6. Effects of ethacrynic acid (●) and furosemide (○) on ADP uptake. All data are expressed as percent mean ± S.E. of control ADP uptake ($n = 58$) which was 0.276±0.005 nmol ADP (equivalent to 7378 cpm [^{14}C]ADP) taken up/mg protein in 1 min. $n > 12$ for each concentration.

adenine nucleotide translocase activity of intact mitochondria were determined. At concentrations greater than 10^{-4} M, both ethacrynic acid and furosemide inhibited uptake of ADP by kidney mitochondria. Ethacrynic acid was a more potent inhibitor of the adenine nucleotide translocase than furosemide ($P < 0.05$ at $5 \cdot 10^{-4}$ M – 10^{-3} M).

DISCUSSION

The previous reports that diuretics may inhibit $(Na^+ + K^+)$-ATPase activity and diminish the P : O ratio suggested an action on mitochondrial phosphorylation reactions. The inhibition of the [^{32}P]-ATP exchange reaction and ATP-supported ion

transport was consistent with this view, although an action to uncouple oxidative phosphorylation would have the same effect. An uncoupler-like action was ruled out by the failure of ethacrynic acid or furosemide to stimulate state 4 respiration and their inability to induce an ATPase activity of well-coupled mitochondria. On the contrary, Figs. 3 and 4 indicate that both diuretics inhibit ATPase activity.

Two classes of compounds produce inhibition of mitochondrial ATPase: agents which directly act on the ATPase enzyme (e.g. oligomycin [47] and aurovertin [48]) and agents which impair transport of adenine nucleotides across the mitochondrial inner membrane (atractyloside [49] and bongkrekic acid [50]). In addition, inhibition of inorganic phosphate transport across the mitochondrial membrane by sulfhydryl group reagents [51] will also inhibit ATPase activity. The studies comparing the effects of ethacrynic acid (Fig. 3) and furosemide (Fig. 4) on intact mitochondria with those on submitochondrial particles were designed to distinguish between these possibilities. The greater sensitivity of intact mitochondria to ATPase inhibition suggested that ethacrynic acid and furosemide may impair phosphate transport or the adenine nucleotide translocase. The latter action was established by direct measurement of the rate of [^{14}C]ADP uptake by intact kidney mitochondria (Fig. 6), and confirmed the findings of Quintanilla et al. [34, 35].

The adenine nucleotide translocase functions as a carrier system to transport ADP into mitochondria in exchange for ATP transported out. This vital system allows export of ATP produced by oxidative phosphorylation for the various work demands of the cell. The adenine nucleotide translocase has been suggested to be a rate-limiting, and therefore regulatory, reaction of oxidative metabolism [52]. Impairment of this process would be expected to have profoundly deleterious effect on cellular functions.

The mechanism by which ethacrynic acid and furosemide inhibit the adenine nucleotide translocase is unknown. Agents which bind to sulfhydryl groups appear to inhibit adenine nucleotide transport [53]. Therefore, the well-described sulfhydryl group reactivity of ethacrynic acid might account for its action on mitochondrial adenine nucleotide translocase. Furosemide is a derivative of the thiazide class of diuretics; it is a sulfonamide with weak carbonic anhydrase inhibitory properties. No mechanistic explanation for its action on adenine nucleotide transport across mitochondria is available.

The in vivo pharmacological application of these in vitro observations is not clear. Concentrations of 10^{-4} M or greater of the diuretics were usually necessary to demonstrate significant inhibitory effects. The interpretation of the present results is further hampered by a lack of knowledge of the concentration of the diuretics in the renal tubular cell. Burg and coworkers [5, 6] found a significant inhibition of active transport when 10^{-6} M was perfused through the tubular lumen, but since these agents are concentrated by the cells [54] the concentration of drug to which the mitochondria are exposed in vivo remains unknown.

Although the present results do not prove that the diuretic properties of ethacrynic acid and furosemide are due to an inhibition of adenine nucleotide translocase, this may be a possible mechanism of diuretic action. Furthermore, ethacrynic acid and furosemide should now be included in the class of agents which have been shown to impair adenine nucleotide transport by mitochondria.

ACKNOWLEDGEMENTS

The technical assistance of Stephanie Schultz, John Straughn, and Jonelle Hecker are gratefully acknowledged. The authors appreciate the suggestions made by Drs. Austin Shug, Rex L. Jamison, and Roy H. Maffly. This work was partly supported by the Medical Research Service of the Veterans Administration, N.I.H. Grant AM 15512 and a grant from the Wisconsin Heart Association. Michael W. Weiner is a V.A. Clinical Investigator.

REFERENCES

1 Manuel, M. A. and Weiner, M. W. (1974) Fed. Proc. 33, 1292
2 Manuel, M. A., Cunarro, J. A. and Weiner, M. W. (1974) Clin. Res. 22, 537A
3 Weiner, M. W., Cunarro, J. A. and Manuel, M. A. (1974) Proc. Int. Union Physiol. Sci. 11, 111
4 Cunarro, J. A., Manuel, M. A. and Weiner, M. W. (1974) Circulation 50, 3–136
5 Burg, M. and Green, N. (1973) Kidney Int. 4, 301–308
6 Burg, M., Stoner, L., Cardinal, J., et al. (1973) Am. J. Physiol. 225, 119–124
7 Epstein, R. W. (1972) Biochim. Biophys. Acta 274, 128–139
8 Jones, V. D. and Landon, E. J. (1967) Biochem. Pharmacol. 16, 2163–2169
9 Macknight, A. D. C. (1969) Biochim. Biophys. Acta 173, 223–233
10 Case, D. B., Gunther, S. J. and Cannon, P. J. (1973) Am. J. Physiol. 224, 769–780
11 Macknight, A. D. C. (1971) Proc. Univ. Otago Med. Sch. 49, 51–53
12 Herms, W. and Kerstin, F. (1969) in Progress in Nephrology (Peters, G. and Roch-Ramel, F., eds.), pp. 290–295, Springer Verlag, New York
13 Gaudemer, Y. and Foucher, B. (1967) Biochim. Biophys. Acta 131, 255–264
14 Foucher, B., Geyssant, A., Goldschmidt, D. and Gaudemer, Y. (1969) Eur. J. Biochem. 9, 63–69
15 Goldschmidt, D., Sabadie-Pialoux, N., Morelis, R., et al. (1972) C. R. Acad. Sci. 275, 2767–2770
16 Goldschmidt, D., Morelis, R., Gaudemer, Y., et al. (1970) Bull. Soc. Chem. Biol. 52, 523–529
17 Yoshida, A., Yamada, T. and Koshikawa, S. (1971) Biochem. Pharmacol. 20, 1933–1942
18 Gordon, E. E. (1968) Biochem. Pharmacol. 17, 1237–1242
19 Francois, C. (1972) Arch. Int. Physiol. Biochim. 80, 799–806
20 Janata, V. and Lege, L. (1972) Int. J. Clin. Pharmacol. 63, 214–217
21 Zesch, A., Senft, G. and Losert, W. (1969) in Progress in Nephrology (Peters, G. and Roch-Ramel, F., eds.), pp. 275–280, Springer Verlag, New York
22 Manual, M. A. and Weiner, M. W. (1976) J. Pharm. Exp. Ther 198, 209–221
23 Barnes, L. D., Jui, Y. S. F. and Dousa, T. P. (1973) Clin. Res. 21, 853
24 Duggan, D. E. and Noll, R. M. (1965) Arch. Biochem. Biophys. 109, 388–396
25 Hook, J. R. and Williamson, H. E. (1965) Proc. Soc. Exp. Biol. Med. 120, 358–360
26 Nechay, B. R., Palmer, R. F., Chinoy, D. A. and Posey, V. A. (1967) J. Pharm. Exp. Ther. 157, 599–617
27 Davis, P. W. (1970) Biochem. Pharmacol. 19, 1983–1989
28 Banerjee, S. P., Khanna, V. K. and Sen, A. K. (1970) Mol. Pharmacol. 6, 680–690
29 Banerjee, S. P., Khanna, V. K. and Sen, A. K. (1971) Biochem. Pharmacol. 20, 1649–1660
30 Daniel, E. E., Kidwai, A. M., Robinson, D., et al. (1970) J. Pharm. Exp. Ther. 186, 563–579
31 Nechay, B. R. and Contreras, R. R. (1972) J. Pharm. Exp. Ther. 183, 127–136
32 Ebel, H., Ehrich, J., De Santo, N. G., et al. (1972) Pflugers Arch. 335, 224–234
33 Duggan, D. E. and Noll, R. M. (1972) Proc. Soc. Exp. Biol. Med. 139, 762–767
34 Levin, N., Quintanilla, A. and Lewy, P. (1972) Clin. Res. 20, 601 (Abstract)
35 Quintanilla, A., Lewy, P. and Levin, N. W. (1972) Fifth International Congress of Nephrology, p. 33 (Abstract)
36 Weiner, M. W. (1975) Am. J. Physiol. 228, 815–820
37 Johnson, D. and Lardy, H. A. (1967) Methods Enzymol. 10, 94–95
38 Chance, B. (1959) in Ciba Foundation Symposium on the Regulation of Cell Metabolism, pp. 99–129, Little Brown
39 Pullman, M. E. (1966) Methods Enzymol. 10, 57–59

40 Cunarro, J. and Weiner, M. W. (1975) Biochim. Biophys. Acta 387, 234–240
41 Lardy, H. A. and Wellman, H. (1953) J. Biol. Chem. 201, 357–370
42 Summer, J. B. (1944) Science 100, 413–414
43 Wöjtczak, L. and Zaluska, H. (1967) Biochem. Biophys. Res. Commun. 28, 76–81
44 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
45 Cockrell, R., Harris, E. and Pressman, B. (1966) Biochemistry 5, 2326–2335
46 Mitchell, P. (1968) Chemiosmotic Coupling and Energy Transduction, Glynn Research Ltd., Bodmin Cornwall, England
47 Lardy, H. A., Johnson, D. and McMurray, W. C. (1958) Arch. Biochem. Biophys. 78, 587–597
48 Lardy, H. A., Connelly, J. L. and Johnson, D. (1964) Biochemistry 3, 1961–1968
49 Vignais, P. V., Duee, E. D., Vignais, P. M. and Huet, J. (1966) Biochim. Biophys. Acta 118, 465–483
50 Henderson, P. J. F. and Lardy, H. A. (1970) J. Biol. Chem. 245, 1319–1326
51 Tyler, D. D. (1969) Biochem. J. 111, 665
52 Klingenberg, M. (1970) Essays Biochem. 6, 119–159
53 Vignais, P. V. and Vignais, P. M. (1972) FEBS Lett. 26, 27–31
54 Charnock, J. S. and Almeida, A. F. (1972) Biochem. Pharmacol. 21, 647–655