

ADP for the  $P_i \rightleftharpoons HOH$  exchange of mitochondrial oxidative phosphorylation has been established by Jones and Boyer (41). The pronounced stimulation of the  $P_i \rightleftharpoons HOH$  exchange during net photophosphorylation by adenine nucleotides, as noted by Shavit et al. (20), has been interpreted to indicate that such a requirement exists. In addition, a nucleotide requirement for the medium  $P_i \rightleftharpoons HOH$  of myosin, natural and reconstituted actomyosin and heavy meromyosin has been reported by several workers (5,30).

Results of the measurement of effects of added ADP on the  $P_i \rightleftharpoons HOH$  exchange catalyzed by a  $(Na^+, K^+)$ -ATPase preparation, which has been previously depleted by endogenous nucleotides by an ion-exchange procedure, are given in Table IV. The expected decrease in the extent of exchange due to  $Mg^{2+}$  chelation by ADP is also included. The data show that added ADP does not activate the exchange and that there is no apparent requirement of ADP for the exchange. Slight inhibition by 5 mM ADP was noted with the native preparation whereas prior treatment with the ion-exchange resin apparently sensitized the enzyme to ADP thereby producing 50% inhibition of exchange. This may be interpreted as indicating that ion-exchange treatment removed a non-inhibitory, firmly-bound anion at the ADP site thus allowing ADP entry, binding and inhibition.

Lack of  $P_i \rightleftharpoons ATP$  and  $ATP \rightleftharpoons HOH$  Exchanges. The data in Table V show the relative rates of the  $P_i \rightleftharpoons ATP$  and the  $ATP \rightleftharpoons HOH$  exchanges occurring during ATP hydrolysis compared to the rate of the  $P_i \rightleftharpoons HOH$  in the absence of added ATP. The  $(Na^+, K^+)$ -ATPase does not catalyze a  $P_i \rightleftharpoons ATP$  exchange under the conditions employed for ATP cleavage, in agreement with previous reports (42,43). In addition, the enzyme does not catalyze an  $ATP \rightleftharpoons HOH$  exchange. The enzyme will, however, catalyze an  $ADP \rightleftharpoons ATP$  exchange (2,3,43).