$P_i \rightleftarrows$ HOH Exchange during ATP Cleavage. Hydrolytic cleavage of ATP to form ADP and P_i must result in the incorporation of at least one oxygen atom into the ADP or P_i; all incorporation appears to be in the P_i. Data shown in Fig. 1 indicate that there is an appreciable, relatively constant incorporation of ^{18}O from $H^{18}OH$ into P_i in excess of that expected for ATP hydrolysis. Figure 2 shows that this extra incorporation is linear with ATP hydrolysis and shows no lag period.

It was previously noted in Table I that Na^+, at a concentration optimal for (Na^+,K^+)-ATPase activity and in the absence of added ATP, severely inhibited the medullar $P_i \rightleftarrows$ HOH exchange. Table VI demonstrates that the exchange is inhibited by Na^+ and ouabain and that an extra incorporation of ^{18}O occurs during the course of ATP hydrolysis. This extra incorporation represents either apparent reversal of Na^+ inhibition by ATP with restoral of the medium exchange, or it represents a new exchange which arises solely during ATP cleavage.

The extra exchange of H_2O oxygen into phosphate during ATP cleavage by myosin and actomyosin has been previously recognized to be of two types--that occurring preferentially with P_i formed from cleavage of ATP, designated as "intermediate exchange," and that occurring with P_i of the reaction medium, designated as "medium exchange" (4). Results from experiments designed to assess whether the ATP-dependent exchange was medium or intermediate exchange are presented in Table VI. Measurements were made of the comparative amount of exchange as determined by the loss of ^{18}O from ^{18}O-labeled P_i and the gain of ^{18}O into P_i from ^{18}O-labeled water. Exchange occurring with a phosphoryl group, with P_i of the medium, or with P_i released from ATP would result in incorporation of ^{18}O from water into P_i. In contrast,