Controversy surrounds the impact of extracellular calcium on tyrosine phosphorylation during capacitation of mammalian spermatozoa, with positive (1,2) and negative (3) effects recorded in independent publications. Here we demonstrate that presence of calcium in the external medium decreases tyrosine phosphorylation in both human and mouse spermatozoa. Under these conditions, a correlative rise in intracellular pH was also noted; however, this event does not regulate tyrosine phosphorylation. The regulation of tyrosine phosphorylation in sperm incubated in calcium-depleted medium appears to lie at the level of ATP. We found that the amount of ATP in both human (57 ± 3 µg ATP/10⁶ cells) and mouse (400 ± 50 µg ATP/10⁶ cells) spermatozoa incubated in the presence of external calcium significantly changed when compared to sperm bathed in calcium-depleted conditions (human 47 ± 2 µg ATP/10⁶ cells; P < 0.05; mouse 1000 ± 100 µg ATP/10⁶ cells, P < 0.001). Furthermore, the removal of glucose, or addition of 2-deoxyglucose, decreased ATP levels within human spermatozoan populations and induced a corresponding decline in phosphotyrosine expression. The mitochondrial inhibitor rotenone had no effect on either ATP levels, or the amount of tyrosine phosphorylation. Addition of the affinity-labeling probe 8-N₃ ATP confirmed our prediction that spermatozoa have many calcium-dependent ATPases. However, quercetin, a plasma membrane ATPase inhibitor, did not increase tyrosine phosphorylation in human spermatozoa. Based on these findings, the present study indicates that extracellular calcium suppresses tyrosine phosphorylation by decreasing the availability of intracellular ATP, and not by activating tyrosine phosphatases or inhibiting tyrosine kinases as previously suggested (3).