Serum starvation induces binucleation in HeLa cells, but the effects of serum starvation on mitosis and the significance of binucleation remain unknown. We investigated the effect of serum starvation on mitosis and analyzed the growth of binucleated cells. The frequency of binucleation caused by cytokinesis failure in DMEM without FBS (0% medium) was higher than that in DMEM with FBS (10% medium). In 0% medium, the metaphase spindle location was off-center, and RhoA localization significantly lacked symmetry. The frequency of the extension of intercellular bridge with the midbody in 0% medium was significantly higher than that in 10% medium. Moreover, all mononucleated mitotic cells caused bipolar mitosis and produced only mononucleated daughter cells, but binucleated cells produced various nucleated cells by multipolar mitosis in 0% medium. These results suggest that serum starvation may have various effects on mitosis, and binucleated cells may be related to formation of aneuploidy.