SW-480 cell line was cultivated on two microtiter-plates of 48 wells, by using the routine culture methods and all what needed from culture media and reagents. One microtiter-plate of SW-480 cell line was exposed to 0.5 IU/L of neutral insulin for 10 hours of incubation, followed by serial double dilutions of 5FU starting from 80 µg/mL to 1.25 µg/mL of 5FU for 12 hours incubation. The second cultured plate was exposed to same 5FU serial dilution but without proceeding with insulin. Gas chromatographic analysis was performed to determine the residual 5FU concentration in the culture medium of SW-480 cell line wells in both cultured plates. There was significant differences (P<0.05) in the residual 5FU between them. In conclusion: insulin increases the uptake of 5FU into human colonic cancer SW-480 cell line significantly.