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**CREATINE KINASE ISOENZYME BB IS ASSOCIATED WITH THE ESTROGEN RECEPTOR IN BREAST CANCER**

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The creatine kinase isoenzyme CK-BB is produced by many tumors but its clinical usefulness has not as yet been established. In order to examine the value of CK-BB isoenzyme as a prognostic indicator in breast cancer, we have initially developed a highly sensitive immunofluorometric procedure, based on enzymatically amplified time-resolved fluorometry with Tb<sup>3+</sup>-chelates as labels and two monoclonal antibodies. One antibody was immobilized in polystyrene microtiter wells and the other was biotinylated. The immunocomplex was detected with a streptavidin-alkaline phosphatase conjugate using diflunisal phosphate as substrate. The dephosphorylated diflunisal forms highly fluorescent complexes with Tb<sup>3+</sup>-EDTA which can be quantified with time-resolved fluorometry. The developed assay takes 2h to complete, is precise (CV's < 10%) and highly sensitive, with a detection limit of < 0.05 µg/L. Comparison with an enzymatic CK-BB assay for 166 breast tumor cytosols (determination of CK-B subunit after immunoinhibition of CK-M subunit) gave the following equation: y (TR-FIA method) = 0.71 x (enzymatic method) + 5.8, r = 0.83. Cross-reactivity of the CK-BB TR-FIA assay by CK-MM was not detectable at concentrations as high as 1000 µg/L. The cross-reactivity of CK-MB isoenzyme was 5% at 100 µg/L of CK-MB. The method was used to measure CK-BB activity in 166 breast tumor cytosols which were also measured for (ER) and progesterone (PR) receptors. Using a cutoff value of 10 fmol/mg protein for the receptors and 26 µg/L for CK-BB, we examined the possible association between receptors and CK-BB. We found a strong association between CK-BB and ER (P=0.001, chi-square test) and a weak association between CK-BB and PR (P=0.054). We conclude that CK-BB production by breast tumors is mediated by the action of the estrogen receptor.