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EPR spectroscopy of free radicals in the perfused heart

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Electron Paramagnetic Resonance, EPR, spectroscopy can be applied to directly measure free radicals; however, it has not be possible to measure important biological radicals in situ because conventional spectrometer designs are not suitable for the performance of measurements on large aqueous structures such as whole organs or tissues. We describe the design, construction, and application of instrumentation developed in an effort to obtain optimum performance in measuring free radicals in intact biological organs and tissues. This spectrometer consists of a 1-2 GHz microwave bridge with the source locked to the resonant frequency of a specially designed recessed gap, loop-gap resonator. The principles of resonator design and construction are analyzed and described. Using these spectrometer radical concentrations as low as 0.4 μM in aqueous solutions could be measured. Studies of isolated beating hearts involving simultaneous real time measurements of free radicals and cardiac contractile function are performed. This *in vivo* EPR technique is applied to study the kinetics of free radical uptake and metabolism in normally perfused and globally ischemic hearts. In addition it is demonstrated that this technique can be used to noninvasively measure tissue oxygen consumption. Thus, low frequency EPR spectroscopy offers great promise in the study of *in vivo* free radical generation and the effects of this radical generation in whole biological tissues.