Radiation Effects Studied with In Situ Fluorescence Spectroscopy Techniques

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Introduction

The importance of chromosomes and especially DNA as primary targets for the radiation induced inactivation of the function of cells is well recognized (1). However, it is also known that damage to irradiated cells may occur due to other processes, such as lipid peroxidation (2). This is a process to which membranes are especially susceptable resulting in the breakdown of existing structures due to reactions between unsaturated lipid, oxygen and free radicals; the latter component presumably formed by the impinging radiation. Since lipid peroxidation involves autocatalytic reactions, a minute radiation dose may have potentially drastic final effects. However, the degree to which the various radiation effects contribute to the disfunction or death of a particular cell category is in many cases not well established. This is especially true for cells of the nondividing type, such as neurons. DNA aberrations caused by irradiation may not be as critically damaging to these cells as to cells undergoing periodic mitosis. Lipid peroxidation reactions and other processes caused by irradiation may play an important role along with DNA aberrations, in determining the fate of the irradiated nondividing cells. Studies of irradiated, nondividing cells are therefore of special interest to establish the various factors responsible for cell disfunction.

Recent work by several authors has demonstrated the importance of lipid peroxidation processes for biological material exposed to radiation. Redpath and Patterson (3) have shown, for instance, that the radiosensitivity of E coli fatty acid auxotroph K - 1060 increases with the number of carbon-carbon double bonds in the fatty acid used in the growth medium. Since fatty acid double bonds are of key importance in lipid peroxidation reactions the result suggests a peroxidative process. In experiments by Konings and Drijver (4) mice which had received a diet deficient in vitamin E (a free radical scavenger) were more sensitive to X-irradiation than were normal mice. In particular the cellular membranes of the vitamin E deficient mice were more vulnerable to lipid peroxidation. It has been reported that X-rays and gamma rays as well as ultraviolet light produce hydroperoxide in lipid membranes (4).

In this paper we discuss an in situ fluorescence method designed to monitor processes related to lipid peroxidation reactions caused by irradiation of biological material. The fluorescence method does not directly register the primary steps in lipid peroxidation but detects effects due to certain compounds formed as byproducts, such as malonaldehyde. This molecule has the ability to crosslink other compounds such as available protein and form aminoiminopropane (AIP), which is a flurophore (see e.g. (5)). This compound becomes therefore an in situ crosslink. In model systems the AIP fluorophore has excitation and emission wavelengths of 400 nm and 465 nm respectively. Since the main damage to biomaterial in lipid peroxidation reactions is caused by products like malonaldehyde the fluorescence method can be regarded as a good indicator of the process (5). It is of importance in measurements of this type to note that the discussed reaction is progressing at a very slow rate. The increase in the AIP fluorophore concentration in model systems (6) has a half-time of the order of 24-48 hours. Fluorescence from AIP formed in irradiated tissue should therefore increase slowly to saturation over a period of several days.

Materials and Methods

A schematic block diagram of the in situ fluorometer used in the experiments described in this paper is shown in Figure 1. Excitation light from a conventional incandescent filament light source is selected in a Schoeffel miniature monochromator and enters a Y-shaped light guide (a Schott ultraviolet transmission guide). The diameter of the shaft of this guide is 0.3 cm. The end part of the shaft is positioned in a cannula attached to the sample to be investigated (as described below and Figure 2) which is thereby exposed to the appropriate excitation light. Fluorescence light returns through the same guide and part of this light is led through the second arm of the Y-guide to a second monochromator (a Schoeffel double monochromator, G 200) tuned to the fluorescence wavelength. The transmitted light is finally registered in a single photon counting detector (a RCA photomultiplier).

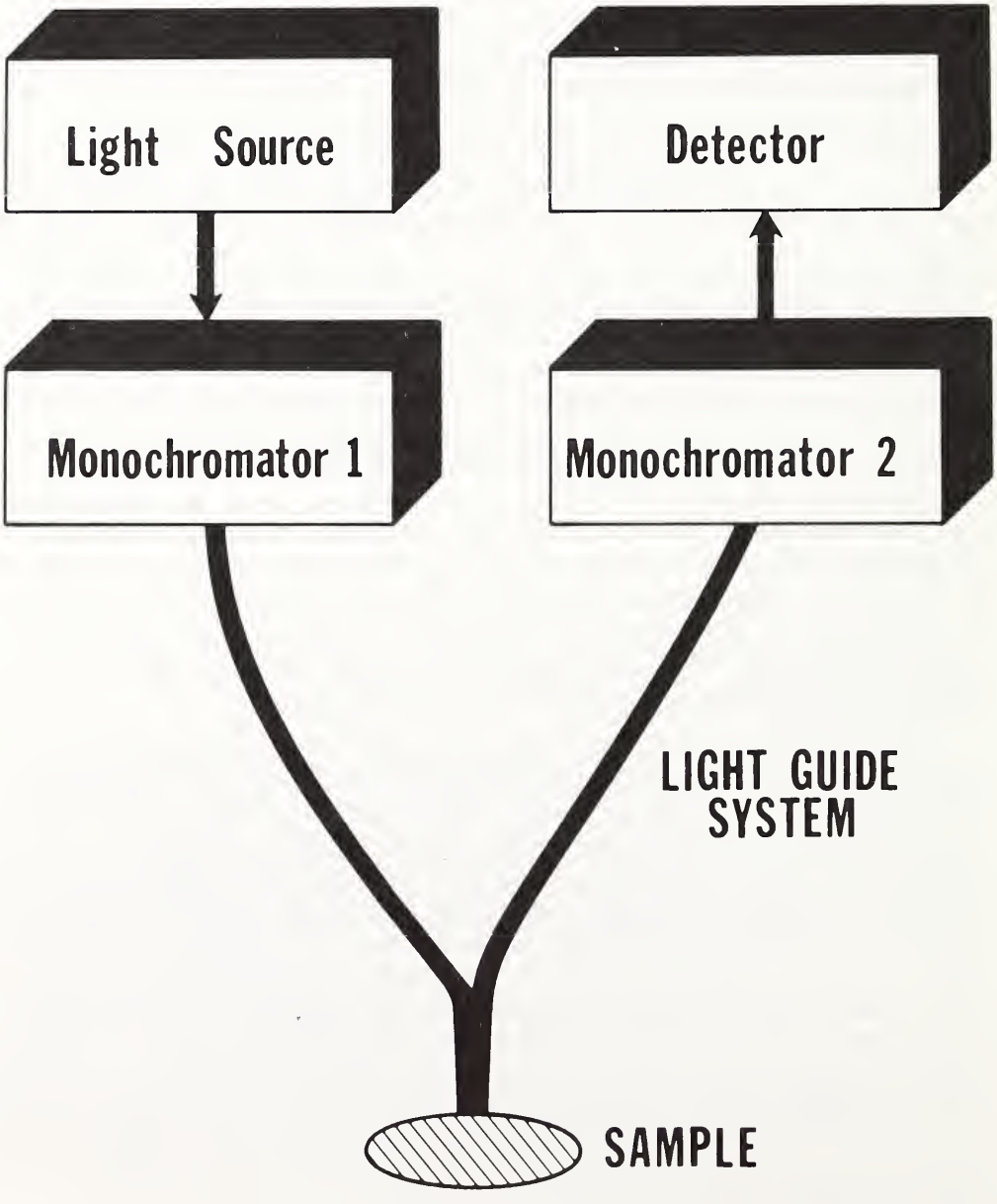

FIGURE 1 *Block diagram of in situ fluorometer.*

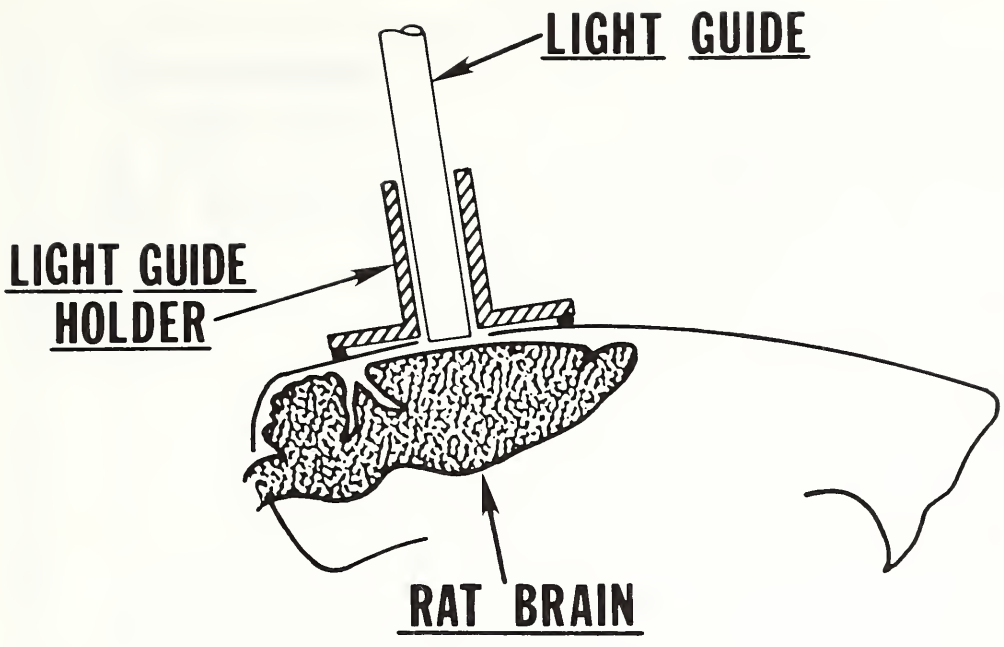

FIGURE 2 *Cross section diagram of a rat skull showing approximate positioning of the light guide.*

To observe fluorescence from the surface of a rat brain over an extended period of time, which was necessary in the present investigation, a chronically implanted cannula was introduced to hold the light guide in position (see Figure 2). A hole, approximately 0.5 cm in diameter was drilled in the parietal bone over the brain area of interest. The cannula was then fixed in place over the hole by acrylic dental cement. This arrangement has been tested in this laboratory and shown to be operative for several days.

Male Wistar rats in the age range 3-6 months were exposed to 1000 rad of gamma rays without anesthesia in wholebrain irradiations from a ^{60} Co source. The source-to-skin distance was 1 m. All parts except the head of the rats were shielded by 4 inches of lead. Prior to irradiation the light guide cannula was implanted surgically in all rats using ether as the anesthetic. A fluorometric measurement at excitation and emission wavelengths 400 nm and 465 nm were performed. After irradiation periodic scans at this fluorometric setting were done for five to seven days. After this time period the cannula arrangement started to deteriorate and was not reliable for accurate measurements. The animals were kept on regular diets during the entire experiment. The instrument was periodically calibrated with a standard quinine sulfate sample.

Results and Discussion

Figure 3 shows a representative fluorescence spectrum of rat brain tissue recorded at excitation wavelength 400 nm and prior to irradiation (time 0) and four days after irradiation (time 96 h) using the procedure discussed in the previous section. A more detailed graph of the time course of the change in the fluorescence peak at wavelength 465 nm is given in Figure 4. Data points in this figure represent the integrated area under the fluorescence emission peak corrected for light scattering effects. The slight decrease of the fluorescence intensity at time approximately seven days is probably due to repair processes in the tissue. Control experiments with non irradiated animals

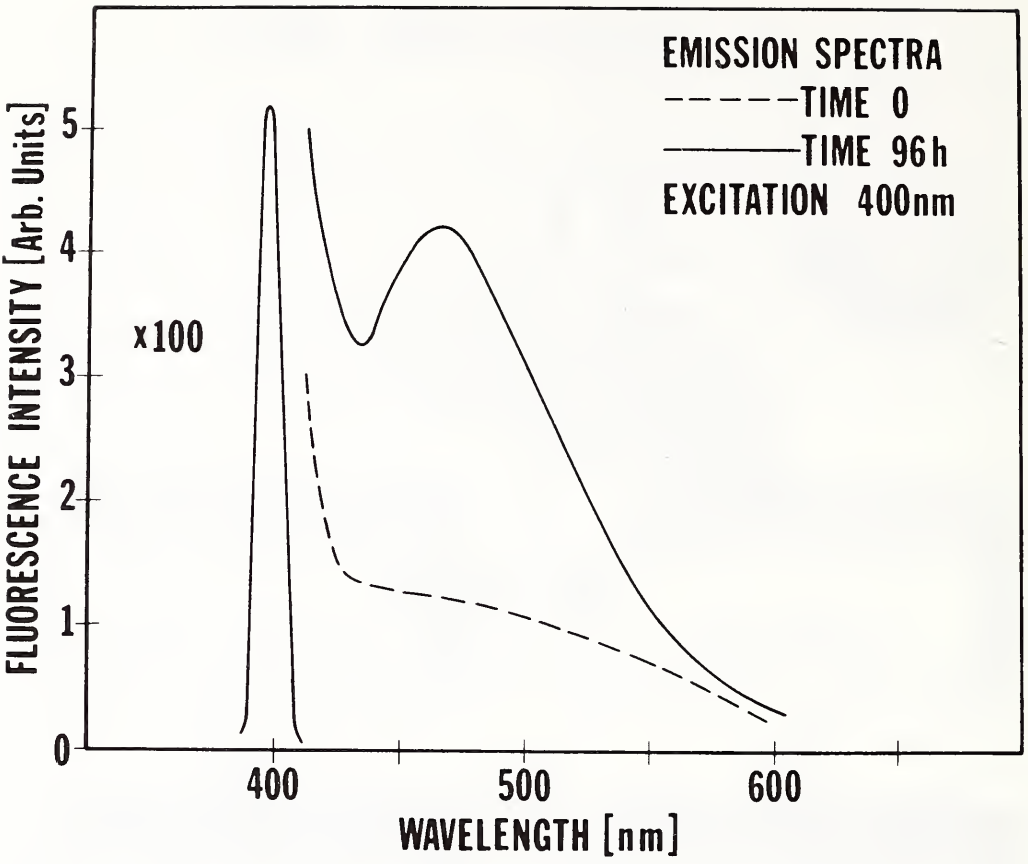

FIGURE 3 *Representative fluorescence emission spectra of irradiated tissue as measured by the in situ light guide probe shown in Figure 1 and Figure 2.*

did not show any significant change in fluorescence at the appropriate wavelengths during the time interval of interest.

The interpretation of the fluorescence peak as being due to AIP formed in lipid peroxidation processes is based partly on previous results showing that such reactions do occur in the irradiated tissue (7,8) and partly on experiments with model systems in which proteins and other cell components have been incubated with the compound malonaldehyde, a known product in lipid peroxidation reactions (6). In the model system a series of experiments with rat liver mitochondrial ghosts as well as bovine serum albumin were reacted with malonaldehyde. In both cases two main fluorescence changes were detected. Protein fluorescence as observed at excitation and emission wavelengths of 295 nm and 340 nm respectively decreased with half-time of approximately two hours and was completely quenched after a period of ten hours. A newly formed fluorophore interpreted to be AIP, observed at excitation and emission wavelengths of 400 nm and 465 nm respectively reached a maximum with a much slower rate; the half-time was approximately 24 hours for pure protein and 48 hours for mitochondrial ghosts. These experiments support the hypothesis that a two step reaction takes place. First malonaldehyde reacts with protein quenching its flurorescence and then fluorescent interprotein AIP crosslinks are formed.

If the model system experiments were directly applicable to the in vivo situation studied here a change in the protein fluorescence would also be expected. Measurements of the protein fluorescence in the present system indicated a slight decrease in intensity,

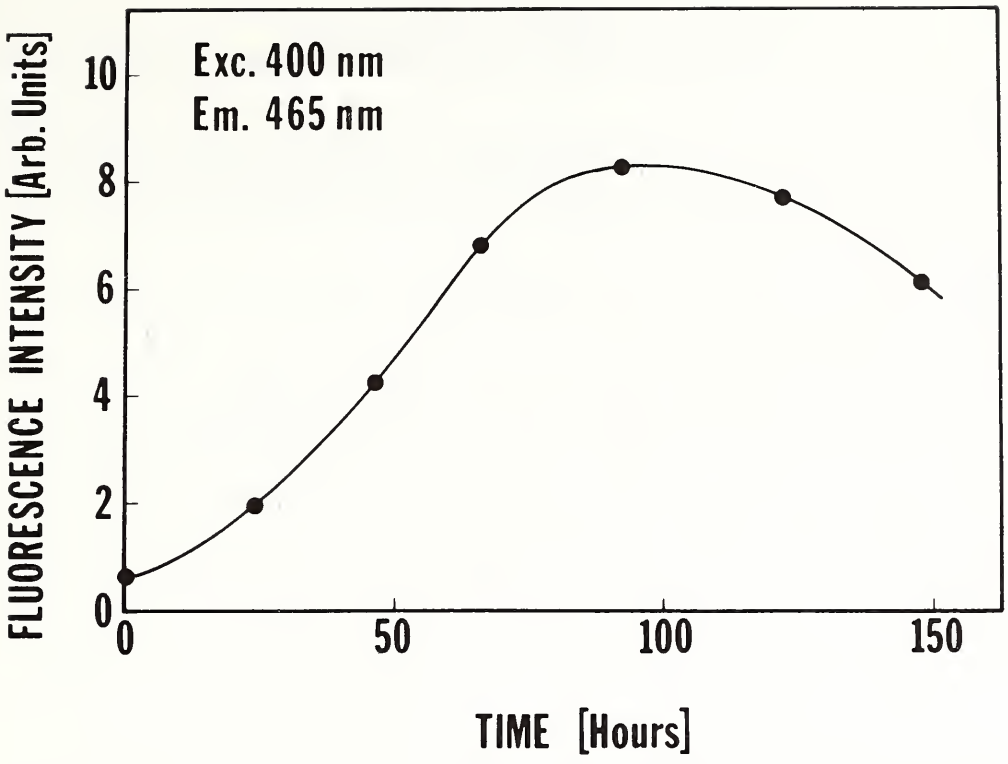

Figure 4 *Time course of the fluorescence light at emission wavelength 465 nm of irradiated tissue. Data points represent the integrated area under the fluorescence emission peak shown in Figure 3.*

but no complete quenching took place as in the model system. This is not surprising since it is reasonable to assume that only a fraction of the brain tissue is affected by the irradiation and a total disappearing of protein fluorescence is therefore not likely. An accurate masurement of this change could therefore be used to estimate the amount of affected irradiated tissue. Such a measurement may be developed into an assay method of lipid peroxidation destruction processes if indeed it is shown that it is an importnat contributing factor in injury of irradiated tissue.

As demonstrtaed in this work the in situ technique allows the registration of the time course of radiation effects in single animals. This offers some advantages over conventional methods. An example is repair of irradiated tissue. The data in Figure 4 seem to indicate that the intensity of fluorescence of AIP decrease after a certain time period. This probably means that affected tissue is being repaired with subsequent removal of AIP compounds. A detailed in situ study with somewhat improved technique may allow observation of the time course of this effect over an extended period of time with repeated exposure of radiation and subsequent repair in individual animals. The in situ method also makes it possible to avoid certain artifacts that may be introduced by preparation of samples from sacrificed animals. This is often of importance in lipid peroxidation investigations since tissue removed from its natural environment with its defense systems often show an enhanced lipid peroxidation effect.

Acknowledgment

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