FISSION FRAGMENT TRACKS AS URANIUM TRACERS IN BIOLOGICAL ELECTRON MICROSCOPY

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ABSTRACT Fission fragment tracks from uranium-stained biological preparations have been observed in the electron microscope in a study of the feasibility of their use as tracers. Virus, DNA, and tissue sections were stained with uranium, prepared for electron microscopy, irradiated in the high thermal neutron flux of a nuclear reactor, and examined without further treatment in the electron microscope. Support films of 50 to 100 A of silicon monoxide and a conventional shadow layer of chromium revealed distinct fission fragment tracks with no other discernible damage to the specimen. Virus and DNA specimens were irradiated to 3×10^{18} n/cm^2 (thermal) and tissue sections to 3×10^{16} n/cm^2 (thermal). About ¼ of the calculated number of fissions left detectable tracks. It is estimated that the position of a uranium atom on a DNA fibril may be determined to 50 A, and a uranium atom in a virus preparation or tissue section to about ½ micron

INTRODUCTION

The damage in thin metallic films caused by passage of fission fragments has been described recently by several groups (1–3). This damage is observed in the electron microscope as an elongated region, on the order of 100 A wide, of low electron scattering power forming a "track" representing the path of the fission fragment through the film. The phenomenon has a potential application to biological electron microscopy because of the unique opportunity it affords of determining the original location of a single uranium atom by reference to the damage caused by its fission. In this regard, it is analogous to autoradiography: the difference is that here the labeling atom is a fissionable isotope of uranium, the nuclear event is induced, and it is registered directly in a thin film; whereas in autoradiography the random decay of a radioisotope produces a latent image in a photographic emulsion.

The successful application of fission fragment tracks as tracers in biological experiments has three primary requirements: (*a*) that uranium can be firmly bound to specific meaningful sites in a biological system, (*b*) that the fission fragment

tracks can be produced and observed under conditions which are compatible with effective electron microscope observation of the specimen, and (*c*) that the original site of the fissioned atom may be confidently determined with suitable accuracy (resolution) by observation of the fission fragment track. The first requirement is a chemical problem which is pertinent only to the specific problem under study and is not directly considered here. Studies of the other two requirements are described in this report.

If these requirements can be satisfied, certain other properties of the fission fragment tracks become of interest. In particular, they are image contrast, width, and minimum recognizable length. One other important consideration is the possible appearance of tracks which are not due to fission of uranium atoms specifically bound to the biological specimen as labels; these irrelevant tracks will be referred to here as "background."

It would obviously be desirable if fission fragment tracks could be observed in samples prepared for electron microscopy in conventional ways and experiments were directed towards this end. Metal films ordinarily used for shadowing would seem to be especially convenient for track registration and attention is particularly directed towards their use.

Separate experiments were performed with viruses and DNA stained with uranyl acetate. Both serve the same purpose in providing a defined source for the fission fragment; *i.e.*, location of the uranium atom. The former has an advantage in that it provides more nearly a point source, and the latter serves as a slightly better test of the preservation of the electron microscope image under the severe irradiation conditions required. The macromolecules were stained by mixing with uranyl acetate, the complex was centrifuged several times to remove free uranyl ion from the solution. Then they were deposited on the electron microscope specimen grid, shadowed, and irradiated in the high thermal neutron flux of a nuclear reactor. The irradiated grids were observed in the electron microscope without further treatment. The contrast, width, and general appearance of the fission fragment tracks, their geometrical relation to the stained macromolecules, and the preservation of detail in the image of the macromolecules were studied. Substrates of parlodion, carbon, and silicon monoxide, and shadow layers of chromium, platinum, and platinum-palladium alloy were investigated.

In addition, a method for observation of fission fragment tracks in thin tissue sections is described.

EXPERIMENTAL METHODS AND MATERIALS

Staining. Dilute solutions of purified southern bean mosaic virus (SBMV) were mixed with uranyl acetate (enriched to 93 per cent U^{235}) in 0.01 M NaCl at pH 6. The strained virus was twice washed by high speed centrifugation. T2 coliphage DNA was treated similarly except that the salt concentration was 0.05 M, and natural uranium

was used for the extended irradiations. Uranium assay was by proportional counting of the natural alpha activity.

Under the conditions cited, the ratio of uranium atoms to SBMV particles was about 3:1 as measured by ultraviolet absorption and alpha counting. This ratio was preserved through further centrifugation and resuspension, suggesting that the uranium was quite strongly bound, and that a negligible amount was free in the solution. Uranium was bound to the DNA in the ratio of about 0.1 uranium per nucleotide.

Coxsackie A9 virus was stained with enriched uranium but because of impurities in the virus preparation, no reliable uranium binding assay could be made.

Specimen Preparation. Specimen grids in most cases were 200 mesh nickel; titanium and copper grids were also used. Nickel and titanium offer the advantage of significantly less radioactivation than copper, and thus may be handled safely with a minimum of delay after irradiation. The support films were carbon or silicon monoxide evaporated on parlodion. Carbon films had a distinct tendency to tear up during irradiation, silicon monoxide films of 50 to 100 A were stable, however, and were used for all the micrographs presented here.

The virus samples were deposited on the support films as a droplet, the excess liquid drained, and the grid was then shadowed.

For DNA, glass microscope slides were coated with parlodion and then silicon monoxide, the DNA was deposited as a drop and allowed to run down as the slide was tipped almost vertical, polystyrene latex spheres of average diameter about 880 A were sprayed over the DNA and the slide was then shadowed. Scored squares were floated off the slide onto water and picked up on the specimen grids.

Thin sections were cut from monkey kidney tissue culture cells fixed in osmium tetroxide, embedded in epoxy resin, and stained with depleted[1] uranyl acetate. They were shadowed at normal incidence with about 50 A of silicon monoxide and 10 A of chromium.

Thin films for which the thickness is specified were formed by complete evaporation of a weighed amount from a shallow boat. Uniform evaporation over 2 π steradians and bulk density are assumed.

Neutron Irradiation. Samples were irradiated in the Naval Research Laboratory's swimming pool reactor. Samples for maximum neutron exposure were hung in the water next to the fuel core in a nominal thermal flux of 8×10^{12} n/cm^2 sec., the water temperature was about 80°F. Up to 64 specimen grids could be irradiated simultaneously in the sample holder shown in Fig. 1. This consists of an aluminum core (*b*) with holes drilled to receive the specimen grids; the core fits snugly into the outer capsule (*a*) with clearance small enough that the grids will not slip from their individual holes. The capsule cap (*d*) screws down firmly on a pure lead gasket to make a water-tight seal. The center of the core is hollow and accepts a polyethylene cylinder (*c*) to reduce the size of the "hole" in the moderator. All the aluminum used in construction is grade 1100 to keep radioactivation to a minimum; even so, a delay of approximately 5 days is necessary after irradiation before the radioactivity of the cylinder has decayed enough for safe and convenient removal of the specimen grids.

The reactor is run at its full power of 1 Mw for a total of approximately 50 hours a

[1] The amount of U^{235} has been reduced from 0.7 per cent (natural isotopic fraction) to 0.2 per cent. The uranium in uranium compounds normally delivered by some chemical suppliers is depleted.

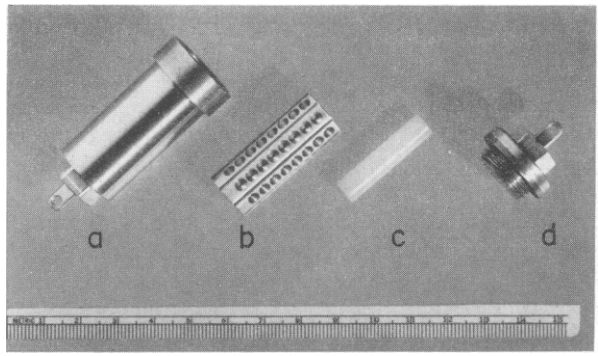

FIGURE 1 Holder for high thermal neutron flux irradiation of electron microscope grids.

week, most of the samples described here were irradiated for 2 weeks, giving an integrated thermal neutron flux of about 3×10^{18} nvt (thermal neutrons/cm^2). Using a thermal neutron fission cross-section of 580 barns, the probability that a U^{235} nucleus will fission during this irradiation is calculated to be about 2×10^{-3} (product of the cross-section and integrated flux).

RESULTS

Registration Films. Shadowing films of pure platinum and 80 per cent platinum—20 per cent palladium underwent granulation during irradiation to the extent that the viruses and nucleic acid were completely obscured. Although the tracks were readily visible, their width was wider than desirable (~300 A). For these reasons, platinum is regarded as unsatisfactory for the present purpose. Chromium shadowing films survived the irradiation with no apparent generalized damage.

Tracks. The fission fragment tracks in chromium shadowed preparations are easily recognizable in the electron microscope viewing screen as well as on the photographic plate. Their width is usually about 100 A, but, depending on the nature of the film, have been observed from 50 to 200 A.

Examples of tracks from T2 DNA stained with natural uranium and irradiated to 3×10^{18} nvt are shown in Fig. 2. Comparison with an unirradiated control grid shown in Fig. 3 shows no apparent degradation in the electron microscope image. The track indicated by an arrow in Fig. 2*b* is about 800 A long, tracks shorter than this are difficult to identify with confidence in films of this sort; a track shorter than half this length would probably be impossible to discern because of the natural granularity of films shadowed at this acute angle. The longer track in Fig. 2*b* does not register clearly within about 0.1 μ of the DNA fibril. This is frequently observed, especially with the longer tracks, and is the result of the fission fragment originating above the registration layer at a small angle to the surface, so that it travels a short

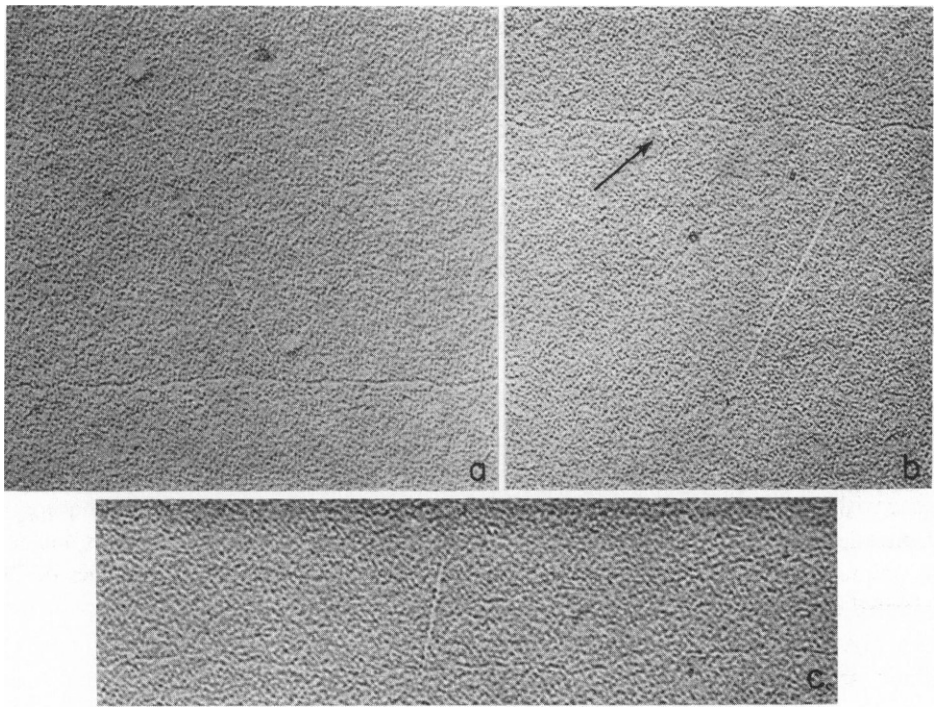

FIGURE 2 Fission fragment tracks from uranium stained T2 DNA irradiated to 3×10^{18} nvt. × 65,000.

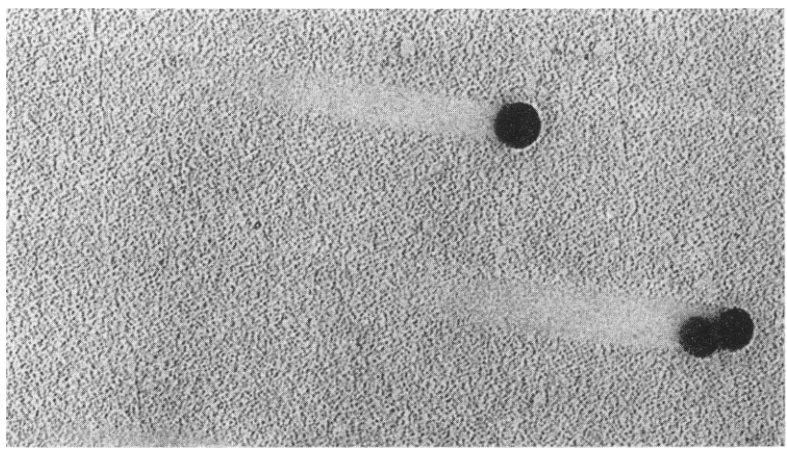

FIGURE 3 T2 DNA before irradiation, with 880 A average diameter polystyrene latex spheres. × 65,000.

distance in air before it enters the film; the effect is seen more frequently in virus preparations because of the greater possible height above the film of the uranium atom.

Examples of tracks from SBMV irradiated to 3×10^{18} nvt are indicated by the arrows in Fig. 4. The chromium is 10 A thick and is deposited almost vertically. The tracks in this case are about 100 A wide, the track on the right in Fig. 4 is about 300 A long and probably represents the shortest track which can be identified as such with confidence. Less distinct tracks, such as that seen on the left of Fig. 4, are encountered occasionally. They are tracks from which the chromium has apparently not been completely evaporated.

Figs. 5 and 6 represent tracks from Coxsackie A9 virus which was shadowed almost vertically with a very light (less than 5 A) deposit of chromium. The tracks are significantly wider than those of the thicker chromium film used for the SBMV film. In Fig. 6, an arrow indicates a very faint track about 500 A wide which is collinear with an ordinary track. Such tracks are frequently observed in lightly shadowed preparations and probably are formed at the silicon monoxide–parlodion or parlodion–air surface as the fission fragment leaves the film. Tracks were rarely observed more than ½ micron from a virus particle.

In Fig. 7, the appearance of tracks in thin (about 500 A) sections of monkey kidney cells, shadowed vertically with 50 A of silicon monoxide and 10 A of chromium, and irradiated to 3×10^{16} nvt, are shown. Tracks similar to those observed in virus and DNA preparations are clearly seen; although, because of the greater thickness and complexity of the specimen, short tracks are not as easily recognizable. In addition to the typical light contrast tracks, intermittent regions of high electron scattering power, forming a dark contrast track are seen; an example is shown between the arrows in Fig. 7*b*. The appearance of the cell components is not significantly altered in these examples, but in similar samples irradiated to 1×10^{18} nvt, some degradation was noticed.

Efficiency of Detection. The detection efficiency (fraction of nuclear fissions which lead to detectable tracks) was estimated from samples of SBMV sprayed on the grids. Counting of viruses and droplets was facilitated, in this case, by the opportunity to observe large numbers of virus particles in a relatively small area. Approximately 11,200 SBMV particles were counted, and 27 fission fragment tracks were observed to be associated with them. On the basis of three uranium atoms per virus and a fission probability of 2×10^{-3} as described above, these numbers indicate that the detection efficiency is probably between 0.25 and 0.5.

Background. Tracks which cannot be associated with stained macromolecules are occasionally observed. Control grids with no uranium were irradiated and typical tracks were observed in them; the number of tracks seen, however, was too few to estimate their probable frequency of occurrence. These tracks probably arise from trace impurities of fissionable atoms in the grids or sample holder.

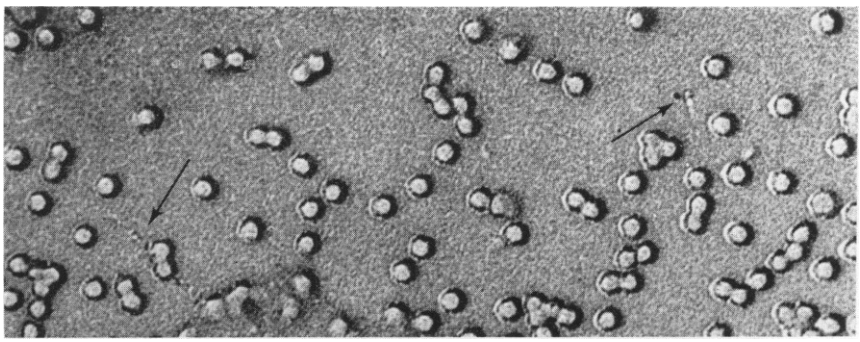

FIGURE 4 Fission fragment tracks from uranium-stained SBMV irradiated to 3×10^{18} nvt. × 80,000.

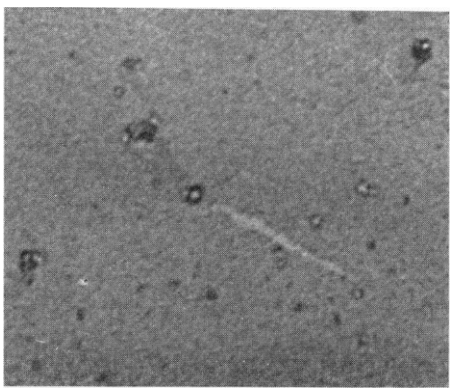

FIGURE 5 Fission fragment track from uranium-stained Coxsackie A9 virus irradiated to 5×10^{17} nvt. × 80,000.

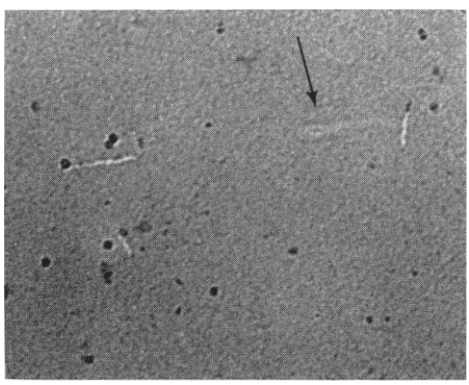

FIGURE 6 Fission fragment track from uranium-stained Coxsackie A9 irradiated to 5×10^{17} nvt. × 30,000.

Background tracks, although not a significant problem with the exposures discussed here, might become a distracting factor under conditions of very low labeling ratios and very high neutron doses.

Resolution. Having shown that fission fragment tracks are closely associated with the uranium-stained macromolecules which are their source, it remains to show to what extent the track itself may be used to determine the loca-

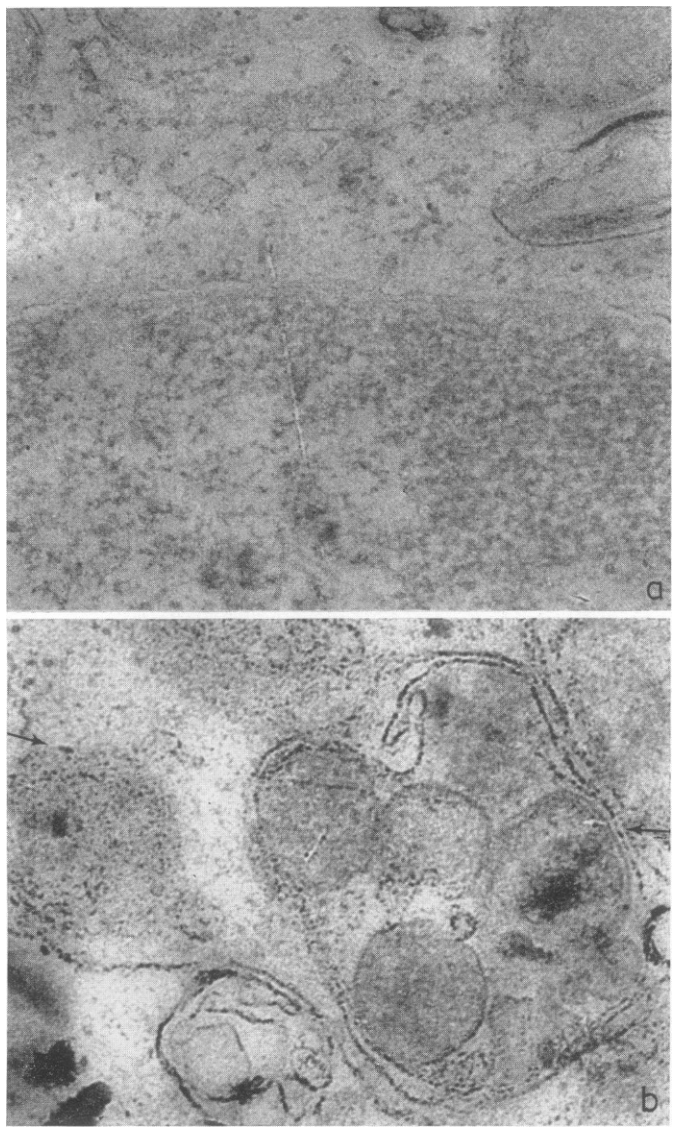

FIGURE 7 Fission fragment track in uranium-stained sections of monkey kidney cells irradiated to 3×10^{16} nvt. $\times$ 60,000.

tion of the fissioned atom. The case for which a segment of a fibrous object, such as DNA, is labeled with uranium is particularly simple since only a linear measurement is sought and the track need only be extrapolated a short distance to the image of the fiber. The linear resolution of this procedure may be estimated from the uncertainty in determining the center of the track. This uncertainty appears to be about ¼ of the track width. This would be the minimum error, or maximum resolution, in determining the linear position of a uranium atom in a thin fiber from its fission fragment track, and this corresponds to a track oriented at 90° to the direction of the fiber; for smaller angles, the resolution would deteriorate. If tracks with angles to the fiber axis of less than 10° are ignored, the average linear resolution is about ½ of the track width, or 50 A for the tracks observed here.

In cases where two-dimensional localization is sought, and no information is available in the electron microscope image other than the track, the situation is more complicated. If it is known that all of the uranium atoms used in labeling are concentrated in a small area, one may look for two or more closely associated tracks and identify their source as the conjunction of their extrapolations with an error on the order of 100 A.

In the more common case, however, when the uranium atoms are scattered separately in the sample so that multiple tracks from one site are improbable, one will usually be able to say only that the track indicates the position of the uranium to an accuracy which is a function of the specimen thickness. For instance, in the case of SBMV, tracks were observed as far as ½ micron from a virus particle; this might be taken as the resolution of a specimen which was 250 A thick.

Because the tissue sections were stained indiscriminately, no estimate of resolution may be inferred from them; however, the resolution in such sections can be expected to be of the same order of magnitude as that for the virus specimens.

CONCLUSIONS

The ease with which fission fragment tracks may be produced and observed in biological specimens demonstrates the feasibility of their use as uranium tracers on a macromolecular or subcellular level. Although uranium is commonly used as an "electron stain" in cell microscopy and groups of as few as six have been observed directly in DNA preparations (4), fission fragment tracks offer a unique method of locating singly dispersed uranium atoms in a variety of biological objects with good resolution.

For the 2 week irradiations described here, about 0.1 per cent of the fissionable atoms will yield a detectable track. Because there is virtually no degradation of the specimen after 2 weeks, one might expect that irradiation in the same facility for extended periods is feasible; for instance, a 20 week irradiation would increase the track yield to about 1 per cent. Even higher yields might be possible in practical irradiation times by using reactors with a higher thermal neutron flux providing

the specimen could survive the irradiation satisfactorily. This, however, cannot be assumed on the basis of the work discussed here because of possible differences in gamma flux and ambient temperature.

Hope for increased resolution in sections as well as macromolecular preparations lies in the additional information to be gained from fission fragment effects in layers other than the chromium; specifically, the faint tracks observed in the lightly shadowed Coxsackie specimens and the dark tracks seen in the tissue sections. Because they occur at different depths in the film, a three-dimensional plot of the course of the fission fragment becomes available and may contribute further information about its source.

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Note Added in Proof. More recent work (MALMON, A. G., *J. Appl. Physics,* 1963, **34,** 3634) describing the appearance of fission fragment tracks in thin plastic films offers the possibility of extending the results described here to unshadowed specimens. Furthermore, it demonstrates the feasibility of obtaining a three-dimensional plot of the path of a fission fragment, suggesting that resolution of a few hundred Angstroms is possible in tissue sections.