Technical Papers

Carbon 14 Beta Track Autoradiography[1, 2]

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Autoradiographs produced by beta particles from histological specimens have until now consisted of randomly distributed silver grains. As a beta particle passes through an emulsion of low sensitivity, only a small number of grains are made developable. The random distribution of these grains (in some cases only one grain per beta particle is produced) does not give a characteristic signature of the path sufficient to identify a single disintegrating atom.

Such a signature, or track, of alpha particles has been used to detect alpha-emitting atoms in tissues (4). When the tissue is placed directly on the emulsion, location of a distintegrated atom can be approximated in the cell by changing the focal plane of the microscope, thereby following the track to its origin at the surface of the emulsion just beneath the cell.

Alpha particles, whose energies and ionizing potentials are much higher than those of beta particles, give well-defined, straight tracks of developed silver grains in nuclear track emulsions. Until recently none of the nuclear track emulsions would show beta tracks. Photographic manufacturers in England and in this country have now developed emulsions which show tracks of beta particles approaching an energy of one Mev. These emulsions have been so sensitive that long exposures of histological specimens having a low concentration of, for example, C^{14}, lead to unsatisfactory results. This is owing to an accumulation of a large background of tracks from cosmic events. The Eastman Kodak Research Laboratory recently made an emulsion of intermediate sensitivity with which we have registered C^{14} beta tracks beneath a tissue section in considerable excess of the background tracks. These emulsions also enable one to follow the track of the beta particle to its point of entry.

Two experimental NTB emulsions on glass plates, No. 416,297, 100μ and No. 417,104, 25μ thick, were prepared to have a sensitivity such that they would register as tracks only beta particles up to approximately 0.4 Mev.

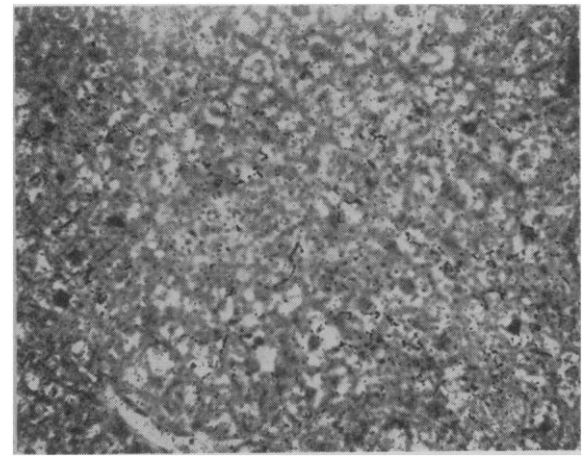

FIG. 1. Photomicrograph of an autoradiograph of a liver section from a rat injected with C^{14}-labeled glycine. Magnification × 360. The plane of focus of the microscope is immediately beneath the section, making the cells appear unsharp while a number of beta tracks are sharp.

These emulsions are now designated as NTB2. It is this feature that makes possible the registration of C^{14} beta particles with a maximum energy of 0.165 Mev without registering much random ionization.

The liver section of a rat injected with glycine labeled with C^{14} in its alpha carbon atom was studied. (Autoradiographs of blood cells from this same rat were previously reported (*2*). Three μc was given intraperitoneally and the rat was sacrificed after 25 hr. The tissue was fixed in Bouin's solution, embedded in paraffin, and cut at approximately 7μ. A tissue section was floated on water and picked up by slipping the photographic plate beneath it in the water and lifting the tissue out on the emulsion (*4, 5*). After partial drying, the plate was placed in a black plastic slide-box and set aside in a cold room at approximately 7° C for exposure. Several grams of a desiccant ($CaCl_2$) was placed in the box to keep the humidity low, in order to delay decay of latent images. After exposure and removal of the paraffin (*4, 5*), the plates were developed in Eastman D19 for 20 min and fixed in 30% hypo at approximately 20° C. The tissue was stained in hematoxylin and eosin and mounted in the conventional manner.

The emulsion underneath the tissue sections showed about the same number of random grains as parts of the emulsion outside the area of the sections. However, we have found a large number of beta tracks, the majority of them starting very close underneath the tissue and ending further down in the emulsion (cf. Fig. 1). It is typical of these beta tracks, in contrast to straight alpha tracks, that they are curved, frequently showing sharp bends in any direction. The grain density increases markedly as the particles lose energy by collisions, and the tracks often end in a hook or loop of high grain density. Although most of the tracks we have observed

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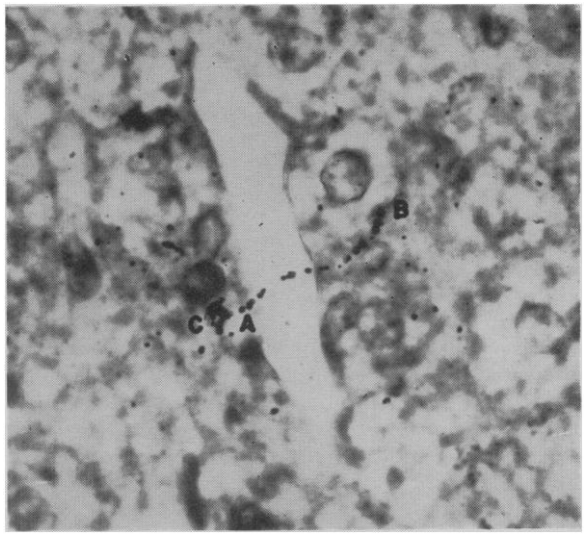

FIG. 2. Photomicrograph of an autoradiograph of a liver section from a rat injected with C^{14}-labeled glycine. Magnification × 900. A composite photomicrograph of a view at tissue level and a mosaic of a beta particle track in the emulsion. The photomicrograph of the tissue was taken at a focal plane just above the tissue-emulsion interface. The composite of the track, starting at the interface and running in three dimensions in the emulsion, was made in the manner presently used for the study of the physics of nuclear tracks. These were superimposed and rephotographed. The increasing grain density along the track shows that the beta particle entered the emulsion near *A* and stopped (at a lower point in the emulsion) at *B*. The clump of grains at *C* may indicate a lower energy beta particle which suffered several deflections, but of this we cannot be certain. The cell nucleus near the origin of the track has been slightly retouched to show its size relative to the silver grains.

started in the proximity of the tissue section, not all of them can actually be retraced to a well-defined locus or a part of a cell. A detailed statistical analysis of tracks is required in order to decide such questions as the cytological location and histological distribution of the C^{14}. We have also observed tracks starting almost in the middle of the emulsion and running in all directions. Such tracks may be due to random cosmic events, to radioactive substances naturally occurring in the tissue and gelatin, and to photoelectrons from x-rays generated by the beta particle.

Fig. 1 is a photomicrograph of beta particle tracks immediately beneath a liver section. This was taken with a 4.3-mm fluorite objective having a depth of focus of several microns in the central portion of the field. Thus a higher proportion of the three-dimensional tracks can be seen. The objective was focused just beneath the tissue-emulsion interface, giving sharply focused tracks in the center and recognizable cell nuclei in the fringing field for comparison.

To prove that the tracks were made by beta particles from the injected C^{14}, and not by background ionizing particles, counts of the number of tracks per field under the tissues were compared with the background of the emulsion which did not support a tissue section. The tracks were countered under oil immersion and while focusing up and down through the emulsion. The following results show that the tracks were produced by C^{14} beta particles:

	Tracks per field
Mean of 40 fields beneath tissue	18
Mean of 20 fields of emulsion unassociated with tissue (background)	2
Corrected mean	16
σ, Standard deviation	5.5

The C^{14} activity of the liver was determined by means of an ionization chamber, as described elsewhere (*1*). On the basis of these measurements, we have calculated that the quantity of tissue delineated by one field (a field volume of $75 \times 75 \times 7$ μ^3) in the microscope can be estimated to have an activity of approximately 20 disintegrations per day, assuming uniform distribution. The autoradiograph exposure time was ten days, which would give approximately 200 disintegrations in the field volume. However, this figure requires several corrections. We have to take into account: 1) a reduction of about 60% for self-absorption of soft beta rays in the tissue section of 7 μ thickness (*6*); 2) a reduction slightly greater than 50% for geometry; 3) an unknown factor for latent image fading; and 4) an unknown amount for failure to count low energy beta particles because of our criterion in defining a track.

A minimum track was defined as four closely spaced grains. Hence, beta particles with an energy insufficient to produce a track of at least four grains would not be counted. Also, it is probable that one or two grains in a row can be lost due to latent image fading. In that case, tracks consisting of 4 or 5 grains would no longer pass the criterion of a track. Since a track of three grains represents a beta particle energy of approximately 20–25 kev, all particles of this energy value and below would not be counted.

Applying the first two corrections, approximately 40 beta particles enter the emulsion. The number of countable tracks is less and by applying Corrections 3 and 4 the value of 16 tracks per field is approached. Thus, we arrived at a reasonable agreement between the results of track counting and ionization chamber measurements for this first attempt at quantitative autoradiography.

We have recently shown that autoradiography of isolated cells offers information about the uptake of a metabolite (*3*). For example, random grain autographs of yeast cells indicate a nonuniform uptake of P^{32}. The technique of counting individual tracks as described above suggests the possibility of a similar study in a histological specimen. In view of this new possibility, we plan to determine the track density under the peripheral and central cells of the liver lobules to investigate the nonuniformity of distribution of labeled compounds.

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