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α-autoradiography of $^{10}$B Compound Distribution in tissue by use of Superimposition Technique

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Key Words: Autoradiography of α particles, Superimposition technique, Boron, Neutron capture therapy.

二重撮影法を用いた$^{10}$B化合物の
生体内分布のα-autoradiography

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Since Locher and Kruger suggested the possibility of neutron capture therapy, a number of investigators have been making efforts to establish clinical application of this treatment. Selective accumulation and high concentration of boron in the tumor is definitely needed.

α particles produced by the reaction $^7\text{B}+\gamma = ^7\text{Li}+\alpha$— 2.59 MeV travel only 7—14 microns in tissue and kill the tumor cells. For the success of the therapy, development of a proper boron compound is mandatory. $\text{Na_2B_{12}H_{13}}\text{SH}$ (sodium mercaptopoethalylcypodecaborate) was synthesized originally by the Dupont and is showing favorable results in basic studies and clinical use.

Determination of boron uptake by tumor is performed by chemical analysis. Histological study of boron uptake is inevitable to know fine distribution in tissue for fulfillment of clinical therapy. Autoradiography is required for this study. So far neutron induced α-autoradiography with nuclear track emulsion has been done by previous investigators.594 Also tritiated boric compounds were used for conventional

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Autoradiography. An entirely new technique which is completely different from the aforementioned autoradiographical technique has been introduced for α-autoradiography by the authors.

**α-Autoradiography**

In nuclear physics, geophysics, health physics and other fields, dielectric organic polymer has been calling attention for its ability to register heavy particles. When heavy particles traverse in polymer, they give the damage to the chains of polymer along with the paths (Fig. 1). Etching procedure by NaOH follows it, then a pit is formed and becomes visible under a microscope or even by naked eyes as fogging of the film. Several investigators have been trying to apply this idea for autoradiography.

α-autoradiography has merit that the pathways of α-particles produced by the reaction \( ^{10} \text{Be} (n, \alpha) \), \(^7\text{Li}\) are easily studied in relation to the cells. How α-particles travel in the tissues is clearly visualized.

The use of plastic film without nuclear emulsion has three major advantages: 1) The procedures are simple, requiring only three days to complete the preparation of the plastic film for examination in contrast with several weeks for photographic methods. 2) No darkroom is required; the necessity for total darkness for the critical maneuvers of placing the histologic sections on the photographic emulsions leads to errors which are easily avoidable when one can work with good illumination. 3) A lower background is obtainable.

For evaluation of boron uptake by tumor, α-autoradiography by plastic film has been applied.

**Technique**

1) Cellulose nitrate film.

A sensitive plastic film was made according to the recipe recommended by E.V. Bentor. Recipe: Cellulose nitrate 17%, ethyl acetate 62%, cellosolve acetate 3%, isopropyl alcohol 5%, butyl alcohol 4% and dioctyl phthalate (added as a plasticizer) 4%.

The solution is stirred thoroughly to dissolve completely. A few days aging at room temperature is required for equilibration of the solution. Just before making the film, one dilutes the solution by addition of ethyl acetate of the same amount by volume.

To make the thin film, a glass slide is dipped into the solution for ten seconds and removed vertically. After another ten seconds, the one side of the glass is wiped off and it is placed on the table horizontally overnight: covered with a glass dish to avoid contamination with dust and to dry slowly. A film about 30 microns thick is obtained. Films thus made are placed in the oven for annealing at 60°C for two hours.

2) Activation by thermal neutrons.
A histologic section of tissue containing the concerned beren compound is prepared by a freeze-drying method which avoids translocation of beron molecules from their in vivo sites during the preparative maneuvers. This section is mounted on the film and irradiated by thermal neutrons at a nuclear reactor (Fig. 2.B).

3) Chemical etching.

After three days for radioactive decay of the $^{24}$Na, the film is etched by soaking in 6.5 N NaOH (Fig. 2.C,D). The pits are formed in the plastic film at the sites of damage by heavy particles (Fig. 2.E). The pits at the sites of liberation of $\alpha$ and Li particles upon $^{10}$B disintegration are readily seen (Fig. 3). As an etchant KOH can be used. The extent and phase of the etched track are dependent on polymerization, nitration, plasticizer, annealing, concentration and temperature of etchant, and etching time. With the recipe described above, two hours annealing at 60°C and 6.5 N NaOH, a favorable etching time is 35 minutes. With these conditions, the diameter of a track is less than 1 micron and the length is less than 10 microns.

The disadvantage of this procedure is that tissue sections are damaged by strong alkalinity. This drawback is fatal for histological study. To solve this problem, an overlapping histologic technique was
worked out as described in the following chapter.

4) Superimposition technique (Fig. 4).

After neutron irradiation of the plastic film adherent to the freeze-dry section of the biological specimen containing boron, the section on the film is stained with dyes and a microphotograph is taken. The NaOH solution is then applied to the section. After an appropriate time, the NaOH and the damaged section are wiped off the plastic film.

Only etched pits are now visible under the microscope in the field which is again microphotographed. This picture is superimposed precisely on the one previously taken, permitting one to determine the relation of the heavy particle tracks to the intra- and extracellular structures.

Once the film on the glass slide is placed on a microscope stage, it does not move during the remaining procedures. This precludes displacement of the photomicrograph of the tissue vis-a-vis that of the etched film.

**Experiment**

At present time, Na₂B₁₂H₁₄SH is deemed as one of the most promising boron compound with a favorable uptake into tumor and low toxicity. Using α-autoradiography by plastic film, the precise distribution of Na₂B₁₂H₁₄SH in tissue was studied.

150 μg/g body weight of Na₂B₁₂H₁₄SH was administered intravenously through a tail vein in male adult Wistar rats bearing a subcutaneously transplanted glioblastoma-like tumor induced by N-nitrosourea. Injection was performed very slowly taking 5–10 minutes. Tumors and brains were removed from animals and pieces of each organ were obtained immediately, 1–2 mm³ in size.

The tissue blocks were put through a freeze-drying procedure and embedded in epoxy resin. Displacement of highly water soluble boron compound in tissue was avoided through these procedures. The tissue blocks were cut into sections 5 microns thick by an ultramicrotome. The sections were picked up on a wooden stick and placed onto the cellulose nitrate film without use of any liquid. The sections easily adhered to the cellulose nitrate film by pressing lightly with a plastic capsule.

The sections mounted on the cellulose nitrate film were taken to the M. I. T. reactor and exposed.
Fig. 5. Numerous $\alpha$ tracks are seen in association with the cells. Glioblastoma; 3 hours after injection of Na$_3$B$_4$H$_9$,SH.

Fig. 6. Glioblastoma; 22 hours after injection.

Fig. 7. Only a few tracks are seen in the normal brain parenchyma. 22 hours after injection.

to thermal neutrons ($3\times10^{14}$n). After 3-5 days for radioactive decay (mainly $^{24}$Na in the slide glass), a microscopic examination was done. The sections were stained with Methyleneblue+Azure II. Overlapping photographs were taken under the microscope using the superimposition technique and etching procedure with 6.5 N NaOH.

Fig. 5 shows an autoradiogram of $^{10}$B of the tumor of the rat sacrificed 3 hours after injection. Numerous $\alpha$ tracks are observed diffusely over the tumor tissue in association with the cells. Fig. 6 is an autoradiogram of the tumor of the rat sacrificed 22 hours after injection. Tracks are seen abundantly over the cells. These pictures show the evidence of accumulation of boron in the tumor. Considering the direction of tracks of the particles and thickness of the tissue section, there should be a greater number of $\alpha$ particles in a tumor cell than apparently recorded on the film. On the other hand, very few tracks are seen in the normal brain parenchyma of the rat killed 22 hours after injection (Fig. 7).

**Conclusion**

To study distribution of boron in tissue, $\alpha$-autoradiography by use of the cellulose nitrate film instead
of nuclear emulsion was developed. Although high concentration of sodium hydroxide gives damage to the tissue section during the procedure, the superimposition technique gave a solution to this problem.

\[ \text{Na}_3\text{B}_6\text{H}_12\text{SH}, \] a boron compound already in practical use, was re-studied by using this new technique. Autoradiograms showed favorable results with abundant \( \alpha \) tracks over the tumor tissue and with very few tracks over the normal brain tissue.

This technique has several advantages to conventional autoradiography by means of nuclear emulsion. The procedure is simple. No darkroom is required. A lower background is obtainable. This technique is a useful means not only for further development of boron compounds but for other \( \alpha \)-autoradiography for plutonium, radium, thorium etc.

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References