Alpharadiography: A Simple Method for Determination of Mass Concentration in Cells and Tissues

By LEONARD F. BÉLANGER, M.D.

With the Technical Collaboration of CÉCILE BÉLANGER

(From the Department of Histology and Embryology, School of Medicine, University of Ottawa, Canada)

PLATES 113 AND 114

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ABSTRACT

A simple apparatus for the production of contact microradiographs with the help of a polonium alpha source and nuclear emulsion plates is described.

This apparatus best adapted for soft tissue and low grade mineralization studies offers advantages as to resolution, geometry of specimen as well as ease of operation and cost.

INTRODUCTION

The need for visual appreciation of density differences in microscopic structures is obvious. Various methods such as tissue absorption at specific wave lengths of the spectrum or near spectrum (1), microroentgenography (2), or electron microscopy have already been proved efficient in this respect. The constantly improving definition of autoradiography has prompted the present attempt at utilizing radioactive elements as energy source for microradiography. A pure alpha ray emitter seemed particularly promising on account of the short range and easy absorption of the particles (3). The utilization of charged particles introduces complications of a theoretical nature: the valence state of constituent atoms and the residual charges of the molecules which comprise the absorber may influence the rate of particle absorption by the electron shell. In practice, however, it seemed that if the operational conditions were standardized, differences of alpha absorption as recorded by a fine grained photographic emulsion, would be indicative of mass variations in different parts of the sample.

Materials and Technique

Polonium^{210} (Radium F) was our first choice. It has a rich historical background in autoradiography (4); it is also a practically pure alpha emitter, giving off only 7 or 8 quanta of 8 to 9 $\times$ 10^5 ev. gamma rays per million alpha particles (4) with a half-life of 140 days. A 2 mc. plated source was obtained commercially.[1] The radioactive element was provided in the form of an evenly distributed electroplated deposit on a platinum foil disc 1 cm. in diameter, protected by a thin mica cover, and held to a metal support (Text-fig. 1 *A*) by a sealing ring.

For the present purpose, the polonium source was affixed by its rod support to a rubber stopper held in turn by a pierced rectangular aluminum plate (3 inches x 1 inch) made to fit a standard plastic slide box which was to serve as camera obscura (Text-fig. 1 *A*).

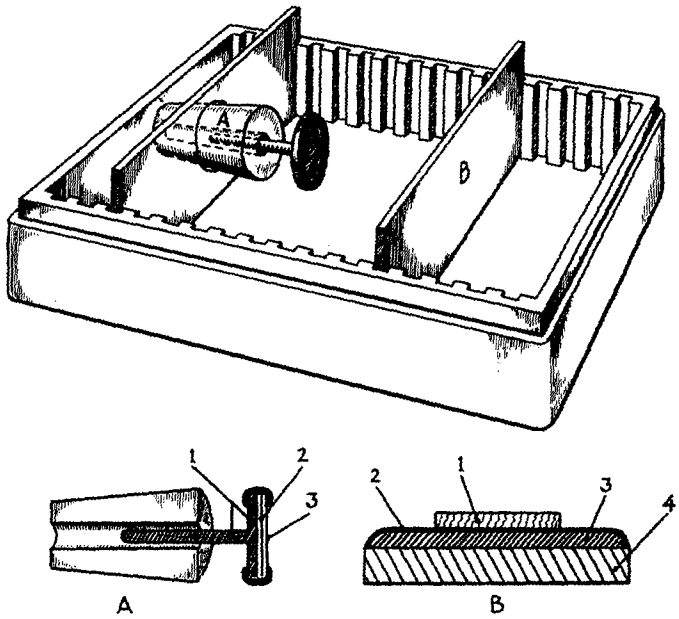
Glass slides coated with NTA nuclear emulsion (E.K.) and others sold as maximum resolution spectroscopic plates (Eastman Kodak No. 649) were used as recorders. These were coated with a thin coat of celloidin by dipping once into a 1 per cent solution in ethanol-ether, and dried for at least 24 hours. The tissues were all fixed in aqueous formaldehyde or in formaldehyde-ethanol.

Celloidin sections of *ca.* 12 μ, cut in 80 per cent ethanol, were brought through 95 per cent and absolute ethanol into ethanol-ether which dissolved most of the celloidin support. They were then mounted directly on the celloidin-coated photographic plate under safelight, by pressing gently with folded fine grade filter paper (Text-fig. 1 *B*). *Paraffin* sections were floated onto warm water for unfolding, then onto cold water and also mounted on the photographic plate. The paraffin was then removed according to the method of Engstrom *et al.* (2).

For a time, celloidin sections were also mounted on a thin saran foil which was in turn folded over the photographic plate, section inwards.

The tissue-celloidin-photographic plate complex (Text-fig. 1 *B*) was then placed into the camera obscura at a distance of 20 to 30 mm. from the source and exposed for periods of 4 hours to 4 days.

[1] Atomic Energy of Canada Ltd., Ottawa.



TEXT-FIG. 1. The apparatus for alpharadiography: *A*, rubber stopper mounted on aluminum plate and bearing alpha source: *1*, platinum plated metal support; *2*, polonium coat; *3*, mica cover. *B*, target: *1*, tissue section; *2*, celloidin coat; *3*, nuclear emulsion; *4*, glass slide.

The tissue section was then removed and at the same time, the protective coat of celloidin was dissolved in a bath of acetone followed by a series of graded ethanol solutions down to water (2). Paraffin sections were previously coated with a strip of cellulose adhesive (Scotch tape) which helped in removing the tissue after the acetone bath (5).

The photographic plate was then developed for 5 minutes in D19 (15°C.), briefly rinsed in 1 per cent acetic acid (stop bath), and fixed for 15 minutes in Kodak acid fixer with hardener. It was then washed for 30 minutes in cold running water, dried in an air stream, and finally covered with a synthetic mounting resin (permount, Fisher) and a glass coverslip.

Sections of mineralized tissue containing radioactive calcium were also mounted on NTA plates and submitted to alpha bombardment in an attempt to obtain combined auto and microradiographs.

RESULTS

Technical.—The *distance* between the source (*A* in Text-fig. 1) and the emulsion-tissue mount (*B* in Text-fig. 1) was found to be *critical* within narrow limits. Alpha particles of Po^{210} do not penetrate more than 3.84 cm. of air at 15°C. (3). At 3.0 cm., tissue absorption was so great that only the crudest features could be recognized. However, at 2.4 cm., only the mineralized portions of the sections, along with dense collagen bands and keratin concentrations, were recorded by the emulsion; everything else had been fully penetrated by the alpha particles.

The ideal distance appeared to be 2.6 to 2.7 cm. Minute differences of density (Figs. 1 to 12) were then recorded in the "alpharadiographs." In actual practice, it soon became apparent that records produced within this critical section-to-source distance were comparable only when objects were not further than 10 mm. on each side of the midline, although the radiation cone itself had a diameter of approximately 35 mm. at that distance, as recorded by the emulsion. All the objects reproduced in the accompanying photomicrographs (Figs. 1 to 12) were well within the optimal target range.

With the present 2 mc. source, *exposure* times of 24 hours with NTA emulsion and 4 days with spectroscopic plates, produced the best images.

The saran-mounted sections required about twice as long and the pictures were less sharp than those obtained by *direct mounting*.

All the pictures shown here, apart from Fig. 6, were recorded on NTA *emulsion*. This emulsion, recommended for alpha records by the manufacturers, has proved at least 4 times faster than the emulsion on No. 649 spectroscopic plates.

The background was definitely darker. The large halide concentration in NTA is probably responsible for its good performance; Yagoda (3) has already discussed the effects of emulsion loading, and Blackett (6) has reported last year having obtained better resolution by using a silver-enriched emulsion.

Biological.—Human and animal tissues fixed in formaldehyde or formaldehyde-ethanol (in order to avoid artefects due to uptake of salts from the fixative) could be divided into 4 categories relative to the "alpharadiographs" produced under the presently described conditions:

1. Showing *high absorption* were: all mineralized tissues (Figs. 6, 12); also demineralized bones (Fig. 1) and teeth, ligaments, tendons, tendon insertions inside cartilage (Fig. 1), perichondrium (Fig. 1), periosteum, connective tissue trabeculae in glands (Fig. 4), muscle (Fig. 1), red blood cells (Figs. 10, 12), hair, the stratum corneum of skin, the cornified portion of the papillae of the tongue (Fig. 3), the surface epithelium of the stomach, cells rich in RNA such as the ganglionic cells of the cerebellum (Fig. 9), and the acinar cells of the pancreas (Fig. 11); also certain cells (yet unidentified) in the pars distalis of the hypophysis (Fig. 10); the white matter of the central nervous system.

At the cytological level, the nucleoli (Fig. 9) and the striated border of the intestinal villi (Fig. 8) were remarkably absorptive; also intercellular cement (Fig. 4).

2. Showing *low absorption* of alpha particles were: mammalian cartilage (Fig. 1), mucus cells (Fig. 4) apart from the surface epithelium of the stomach (Fig. 7); also areolar connective tissue (Figs. 3, 7, 8), the molecular layers of the cerebrum and the cerebellum (Fig. 9), pancreatic islets (Fig. 11), some cells of the hypophysis (yet unidentified, Fig. 10).

3. Showing *moderate absorption* were the younger layers of the skin, tongue (Fig. 3), and other keratinized structures; also the eosin bodies of the cerebellum (Fig. 9), young bone at the periosteum, and neoplastic trabeculae in an osteogenic sarcoma (Fig. 5).

4. Showing *variable absorption* were the cells of connective tissue (Fig. 7), the bone marrow, the pituitary (Fig. 10). Highly variable were the *nuclei* of cells from diversified tissues (Figs. 2 to 4, 6 to 11).

Some *tissue features* became strikingly apparent due to the differences in density of adjacent constituents. Such were the distribution of *collagen* as thick bundles at the sites of cartilage insertions (Fig. 1) or as thin fibers between individual cartilage cells (Fig. 2) or between the acini of mucus glands (Fig. 4); also the *globular units of salt* in mineralized cartilage and their extracellular location (Fig. 6). The distal arrangement of the high density material in the *pancreatic acini* (Fig. 11) corresponds to the localization of the ergastoplasm. In the area of the *isthmus of the stomach glands*, the concentration of dense material is apical and minimal (Fig. 7) as compared with that of the more mature cells above, where the mucus becomes highly concentrated and occupies most of the cell (Fig. 7). On the other hand, the dense material in *chondrocytes* (Fig. 2) and also in *osteoblasts* is distributed evenly all over the cell. The *osteoclasts* were also generally dense; some of these cells, however, have shown several ovoid areas of low density, probably the nuclei.

The polymorphic response of the nuclei of different cells (Figs. 2 to 4, 6 to 11) seems to be parallel to haematoxylin affinity.

Longitudinal sections of *cardiac and skeletal muscle*, which showed portions tapering down to less than 10 μ in thickness, revealed the alternate arrangement of dense A bands and alpha-transparent I bands.

Certain features are presently unexplained. Among these is the nature of the dust-like material surrounding the nucleus of the intestinal epithelium (Fig. 8); also, further comparative observations will be required to identify the opaque and transparent cells of the hypophysis (Fig. 10).

The sections of mineralized bone containing Ca^{45}, have produced an easily recognized *autoradiograph* (Fig. 12) after an exposure of 1 week prior to alpha bombardment. Since the radiocalcium had been introduced 4 days prior to sacrifice, the autoradiograph was at some distance from the formative border of the trabecula (Fig. 2). Against the white dense background of the mineralized bone "shadow," the uneven intensity of the autoradiograph was very apparent.

DISCUSSION

This is, we believe, a first attempt at recording alpha absorption patterns with biological material. The definition obtained is encouraging.

Under ideal conditions, the photographic records could be examined at 1,000 diameters. Magnifications of 50 to 500 were common practice (Figs. 1 to 12). The accompanying photographs were not enlarged from the original photomicrographic negatives; they are all contact prints and thus demonstrate pure microscopic definition.

An important factor in obtaining good resolution has been the direct mounting technique of Engstrom *et al.* (2), itself borrowed from autoradiography (9), thus reducing to a minimum the tissue-emulsion distance.

It seems also that the heavily loaded fine grain NTA emulsion has been a happy choice when sharp contrast was desirable. Spectroscopic plates (E.K. 649) have produced a better record of multiple gradients.

The actual long exposure times may be considered objectionable. However with sources of up to 80 mc. per cm.^{2}, available commercially, exposure times of ½ hour with NTA and 2 hours with maximum resolution spectroscopic plates (E.K. 649) are theoretically possible. These periods would be of the same order as those presently recommended for x-ray microradiography (2). Furthermore, the relatively low cost of the apparatus and its small size and mobility permits the use of several instruments at the same time. On the other hand, the simplicity of the equipment and operations described here puts this technique within the capabilities of any experienced histology technician.

It was very satisfying to recognize that some of the biological observations resulting from "alpharadiography" were quite in accord with facts already in the literature, particularly in regards to microroentgenography of soft tissues (pancreas, keratinized epithelia, nuclei, and nucleoli) as reported by Fitzgerald *et al.* in a chapter in reference 2. This observation places the two methods on common grounds in regards to mass determination.

It may be argued that exposing biological material to alpha bombardment may cause an actual activation of certain atoms of the tissue (10). However experience tells that the small amount of radioactive material produced would itself not significantly influence the emulsion in such a short time. Activation of secondary x-ray fluorescence by alpha bombardment has proved negligible in this type of work as well as in autoradiography (11, 12).

An apparatus presently under construction will permit minute variations (0.01 mm.) of the source-plate distance; the polonium plate will be attached to a micrometric screw with locking device and will be moved from outside the box. On the other hand, the shape of the plated surface will be modified to match the geometry of the photographic plate. With these mechanical improvements, there should be no lateral decrease of penetration and *no limit to the surface of the object* being submitted to bombardment. This feature seems to obviate a definite limitation of the "fine focal spot" method of microroentgenography (2, 7, 8).

There are definite limitations of "alpharadiography" in regards to penetration. Mineralized sections of bones and teeth, apart from those showing the very onset of calcification (Fig. 6), were hopelessly opaque except in the areas corresponding to the marrow cavities (Fig. 12). It is possible that other radioactive sources could be utilized for these denser tissues; this problem is currently under study.

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Explanation of Plates

Plate 113

Fig. 1. Upper extremity of humerus of rat, demineralized. Alpharadiograph on NTA. × 32.

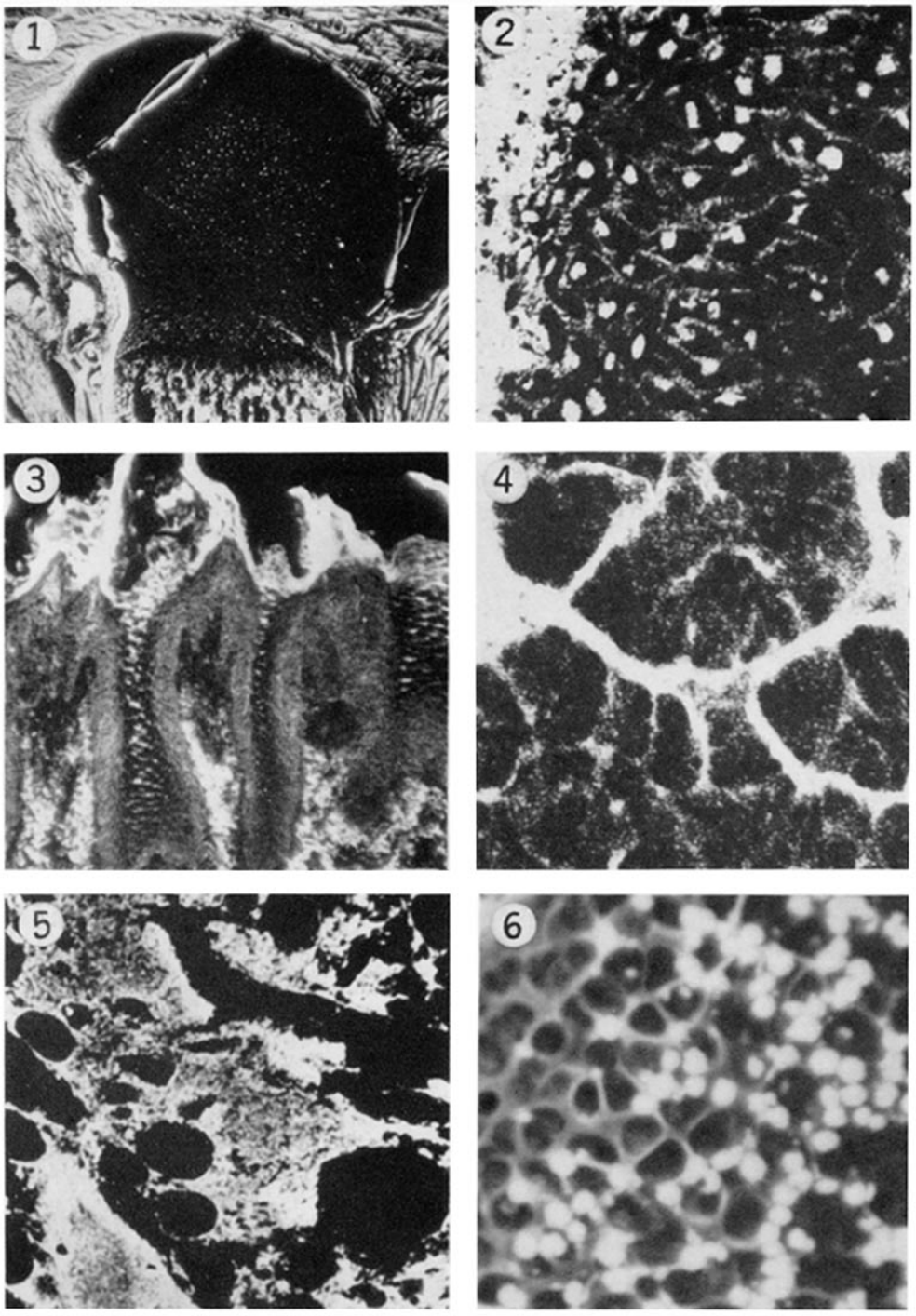
Fig. 2. A portion of epiphyseal cartilage of chick; on the left, a collagen bundle. Alpharadiograph on NTA. × 500.

Fig. 3. Filiform papillae from the human tongue, Alpharadiograph on NTA. × 73.

Fig. 4. A portion of a mucous gland from the human tongue. Alpharadiograph on NTA. × 500.

Fig. 5. A portion of a human osteogenic sarcoma, demineralized. Alpharadiograph on NTA. × 256.

Fig.6. A portion of the cartilage surrounding the cochlea of a young rat; undemineralized celloidin section. Alpharadiograph on spectroscopic plate No. 649. × 300.



(Bélanger: Alpharadiography)

PLATE 114

FIG. 7. A portion of the surface, pit, and isthmus of a fundic gland of the human stomach. Alpharadiograph on NTA. × 300.

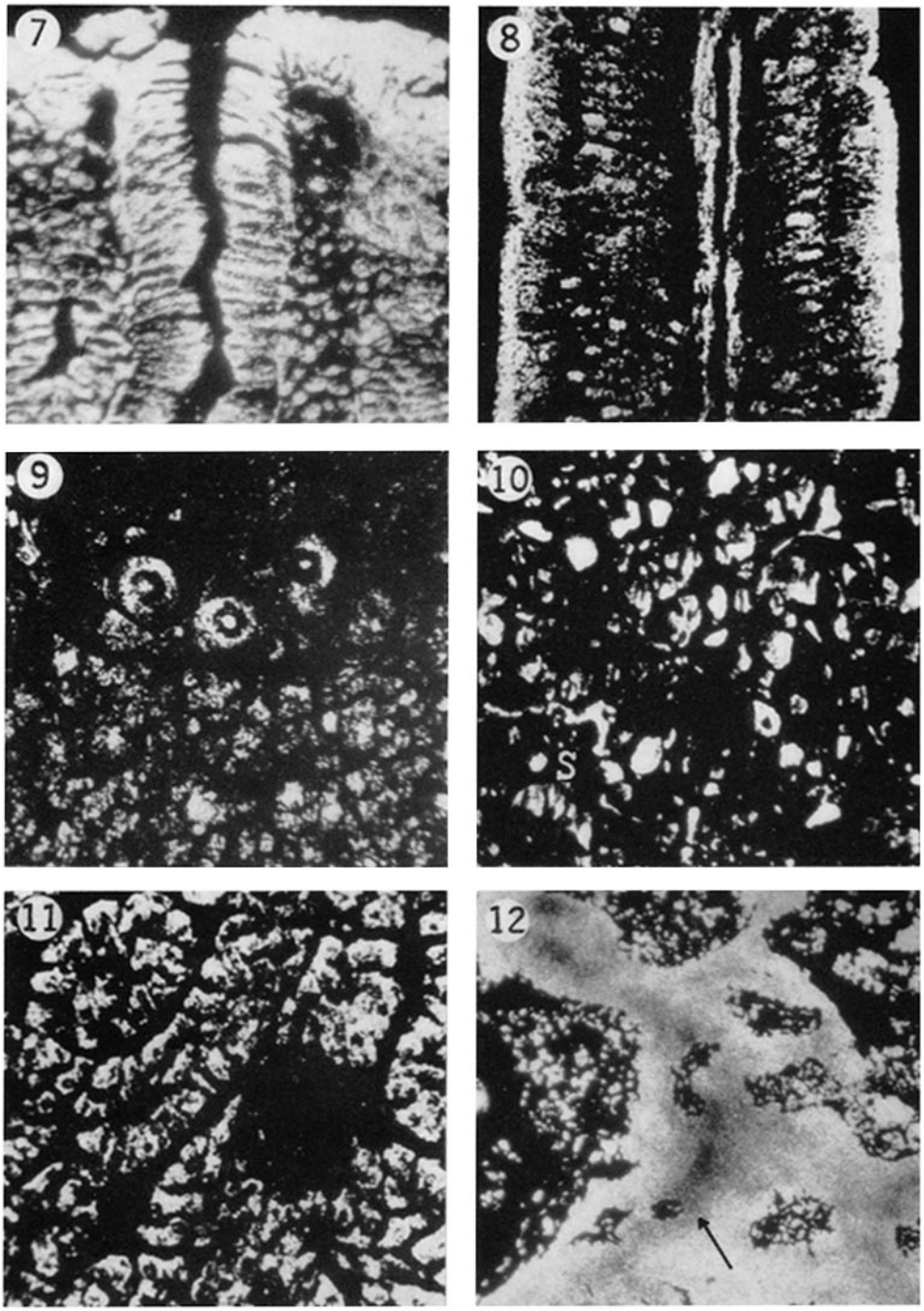
FIG. 8. A portion of an intestinal villus of chick. Alpharadiograph on NTA. × 500.

FIG. 9. A portion of cerebellar lamella of rat; from the top down: the molecular layer, the ganglionic cells, the eosin bodies. Alpharadiograph on NTA. × 500.

FIG. 10. A portion of the pars distalis of a human hypophysis. *S*, sinusoid. Alpharadiograph on NTA. × 500.

FIG. 11. A portion of chick pancreas. The large black spot is an islet. Alpharadiograph on NTA. × 256.

FIG. 12. Combined auto and microradiograph from an undemineralized celloidin section of the head of a young rat sacrificed 4 days after a tracer dose of Ca^{45}. NTA exposed 1 week to Ca^{45} then alpharadiographed. The arrow points to the autoradiograph. × 256.



(Bélanger: Alpharadiography)