SOME OBSERVATIONS ON α-TRACK AUTORADIOGRAPHS OF BIOLOGICAL SPECIMENS

by

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In recent years, our attention has been focussed on the possible harmful effect of thorium and its decay products when deposited in the organism. About 20 years ago, an opaque substance for use in X-ray visualization "Thorotrast" was brought on the market and has been applied quite extensively. More recently, the question has been discussed in the literature whether thorotrast which contains thorium dioxide causes radiation damage*[4,8,9,10,14].

Since thorium and most of its decay products are alpha emitters it seems promising to apply α autoradiography as a means of studying the distribution of these substances in the organism and of estimating the radiation dose to which the tissue is subjected.

The present work deals with the experience gained in an attempt at an accurate localization and a quantitative determination of thorium and its decay products in biological specimens. In a later stage of the work, these investigations aim at a correlation between the intensity of internal irradiation and the damage caused by this irradiation in the tissue. Obviously, such study requires a reliable autoradiographic technique and a possibility of unambiguous interpretation of the autographs.

EXPERIMENTAL

Mammalian tissue containing thorium and its decay products and probably contaminated with radium and its decay products was studied. We have reason to assume that the material is approaching equilibrium with the mesothorium presumably present from the start.

The plates found best suited for the present purpose are manufactured by the Eastman Kodak Company in U.S.A. They are nuclear emulsions of the denomination NTA which are sensitive to particles of high ionization power. The plates, 25 or 50 microns thick, show a very low background of random grains or tracks from natural contamination. After proper processing, they are perfectly clear and free from spots or surface impurities. These plates can be stored for several months.

The tissue containing the radioactive substance had previously been carried through a routine histological fixation and paraffin embedding procedure, and sections 5–8 microns thick were cut on a microtome. When placed on photographic emulsions the sections are unstained. Intimate contact between the emulsion and the tissue is obtained by applying a technique proposed by EVANS[2] and ENDICOTT AND YAGODA[1]. The sections are stretched on warm water, then floated on cold distilled water, and finally lifted from the water surface by means of the photographic plate to be used. The plate carrying the section is dried in air and subsequently stored for exposure in a light tight box containing a drying agent which is considered of importance for the suppression of latent image fading (cf. also [11] and [13]).

* Only a few references of this literature are given; for detailed studies of this problem, the reader is referred to the medical journals.

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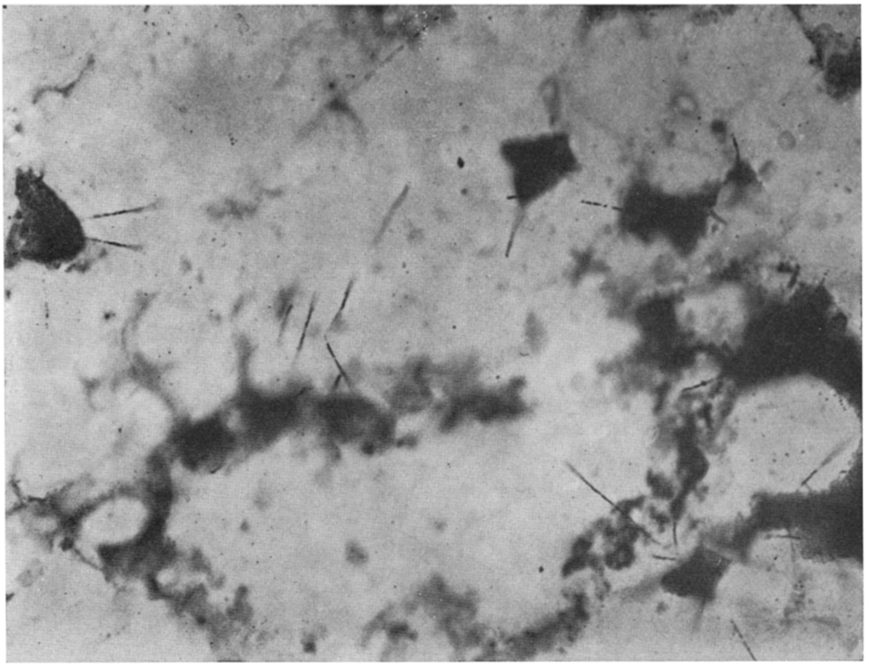

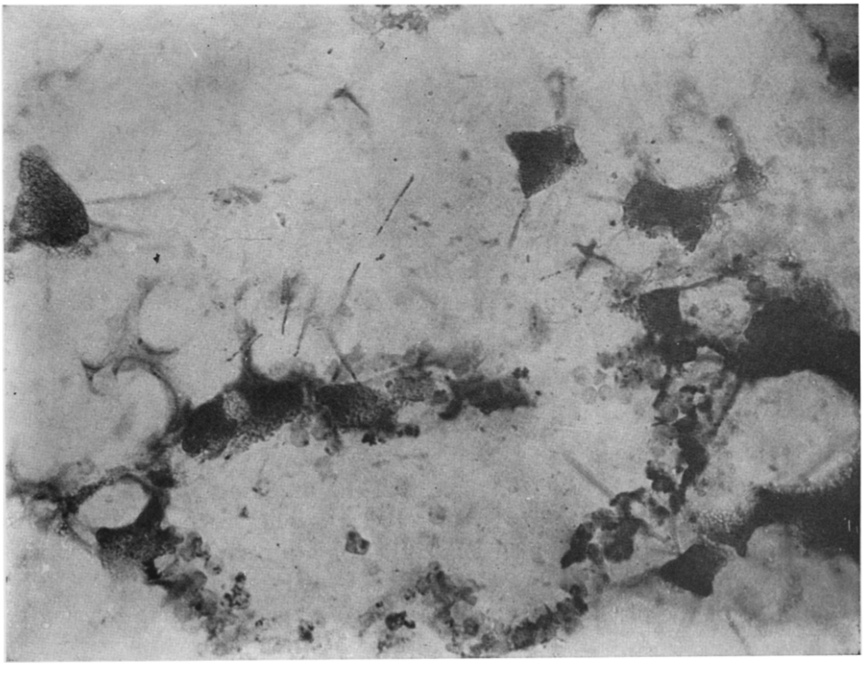

Prior to processing of the plates, the sections are deparaffined in xylol, the plates are carried from 99% to 75% alcohol and then soaked in water. They are developed for 4 or 5 minutes in Kodak D 19 developer, fixed in 30% hypo, washed and dried. The sections are then stained in the routine manner with hematoxylin and eosin, and mounted with balsam and a cover slip. Although the emulsion does take up the stain, this does not interfer with the microscopic examination of the autograph under high power. α tracks are easily and clearly visible in the emulsion when viewed through the tissue.

THEORETICAL

The decay scheme of thorium leads us to expect tracks of various lengths representing different alpha particle energies. However, the NTA plate registers neither beta particles nor gamma rays as tracks. The first 4 decay products of thorium are solid and have long half lives (for example Th = 10^{10} years, MsTh = 6.7 years), their α energies are of the order of 4–5 MeV. Single α tracks from thorium and these decay products should be rather short, the range in emulsion of the longest amounting to about 17 microns. On the basis of the half lives it is improbable that one nucleus undergoes several successive decays within the period of time used for exposure of the plates (days). Therefore, stars of α particle tracks from the first part of the decay scheme can be expected to be rare.

The fifth step in the decay leads to thoron, a gas, half life 54.5 sec. All later decay products are solids of relatively short half lives.

This fact allows to look for two different and characteristic features of the autographs. In the first place, thoron being a gas means that there is a possibility for diffusion away from the point at which thoron is formed. This means that tracks may be found in locations (underneath or even outside the tissue) some distance away from the location of the mother substance. Moreover, since all decay products following thoron have relatively short half lives, short also as compared with the exposure time of the plates, we can expect to observe α track stars representing the decay products of thoron. Also, it seems natural that the autograph should reveal α stars in sites which do not reveal visible deposites of thorium. The range in emulsion of each particle appearing as a track in such a star is determined by the well known energies of the particle. The longest track is that of the α particle from ThC′ with an energy of 8.62 MeV corresponding to a track length of 42 microns in emulsion.

Finally, it can be assumed that some of the thoron has a chance during the exposure of the plates to diffuse to regions outside the tissue section before decaying. It cannot surprise us then to find a considerable number of stars in parts of the emulsion which are not covered by the tissue section. The number of stars should greatly exceed the number of single tracks outside the tissue, and amount to many times the background.

FINDINGS

Examination of the autographs with the tissue superimposed leads to observations which can be explained on the basis of the physics of the thorium decay.

Most of the tissue examined shows regions where smaller or larger crystal-like foreign bodies can easily be distinguished. Other regions are tree from such particles. After a few days' exposure of the emulsions carrying tissue sections, these "foreign bodies" are found to be the place of origin of a great number of α tracks entering the emulsion at all angles. The majority of these tracks is short and stars are very rare in

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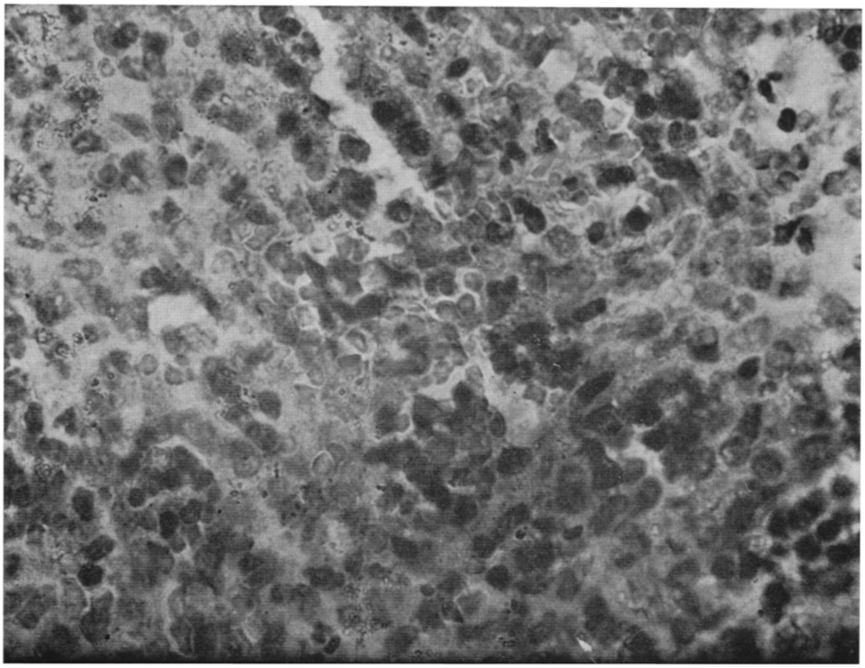

Fig. 2a

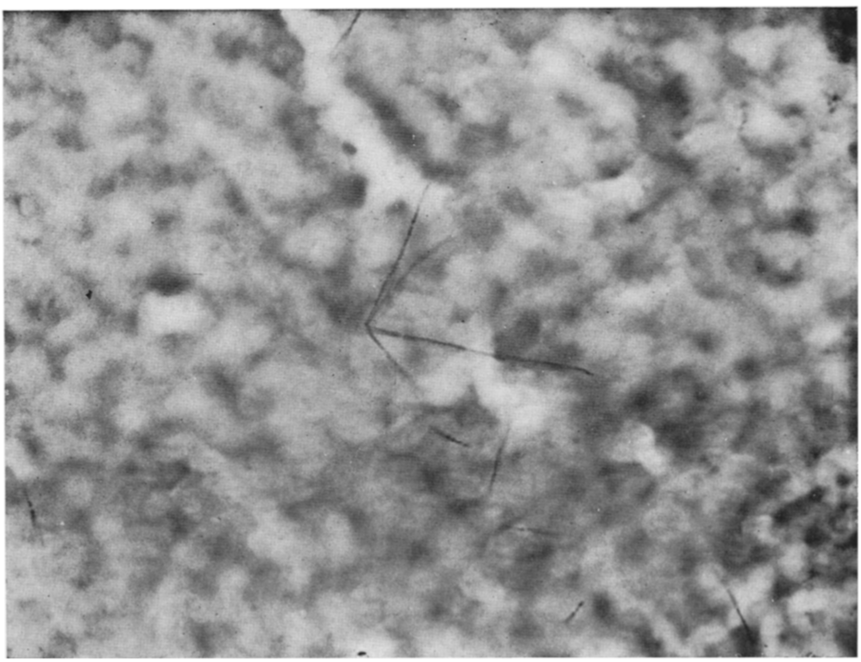

Fig. 2b

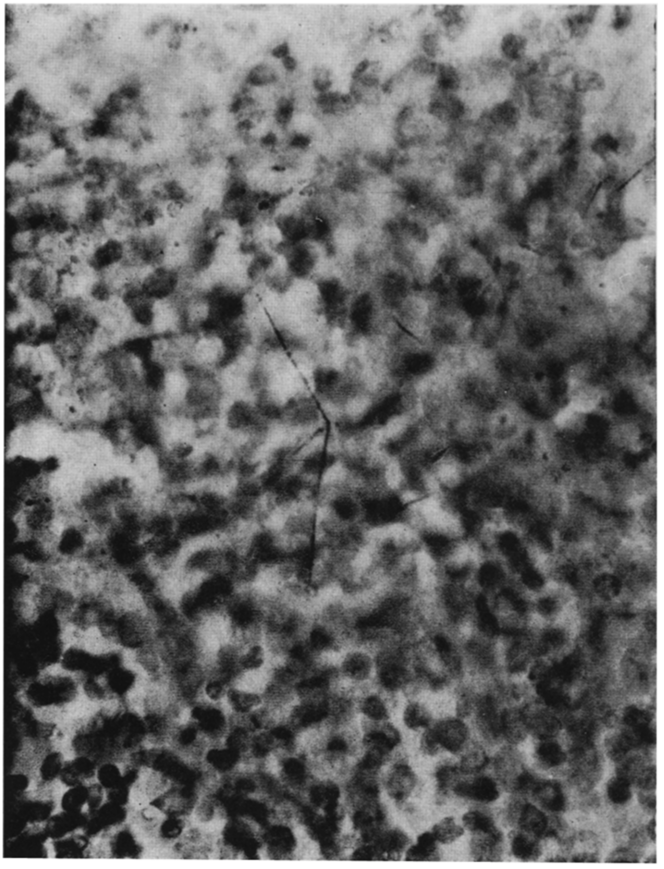

Fig. 2c

Fig. 2. Liver section. (*a*) tissue in focus; (*b*) and (*c*) emulsion in focus. Plate Kodak NTA, exposure 56 days. Average 10 tracks from 150 sq. micron clumps. Typical 4 prong ThX-star (*b*) and 3 prong Th star (*c*) in centre.

the immediate proximity of the crystal-like foreign bodies. In regions of the tissue where foreign bodies could not be detected under the microscope (800 ×) the number of tracks and their lengths vary considerably. In some cases, single tracks are numerous and stars are rare. In other cases, stars are found in great number and they dominate the picture. It has not yet been possible to find a definite correlation between the track picture and the type of tissue examined.

Moreover, the plates show a great number of tracks in regions of the emulsion outside the tissue sections. Both single tracks and stars occur. Plates carrying tissue which reveals a great number of stars under the tissue display many stars at some distance from the tissue proper, while on other plates single tracks are dominant both under the tissue and outside. Most stars have either 3 or 4 prongs.

The background of tracks due to natural contamination was determined on plates which were processed without having been in touch with tissue sections.

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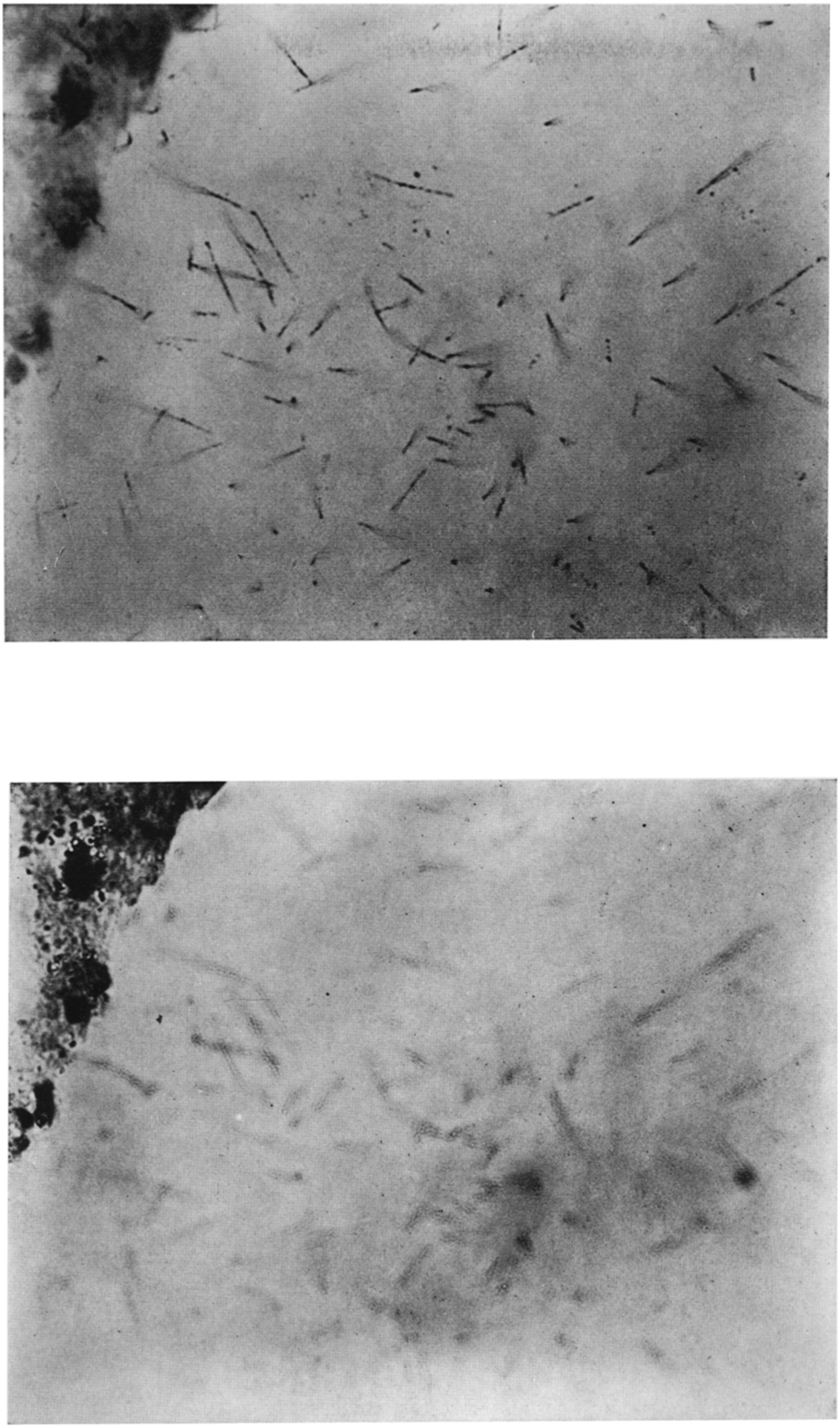

b

a

Fig. 3. Muscle section on Kodak NTB showing outside the tissue an area where an α emitter is leached out. (*a*) tissue in focus; (*b*) emulsion in focus. Exposure 12 days, field 150 × 150 microns, 150 tracks per field.

ARTEFACTS

Before a quantitative or even qualitative interpretation of the present observations can be attempted it seems necessary to discuss a number of artefacts.

1. The photographic emulsion proper is the cause of some surprising phenomena. The physics of elementary particles requires the path of an alpha particle—also in a photographic emulsion—to be a straight line, sometimes showing a sharp bend towards the end of its range. The tissue autographs, however, depict a considerable number of bending tracks of a grain density which makes their classification as α tracks undisputable. U-shaped and C-shaped tracks are frequent. There are several indications of this phenomenon being an artefact caused by distortion of the emulsion. Bending tracks are most frequent along the borderline of the tissue section and in its holes and interspaces. The direction of bending tends to be the same for a great number of tracks within a small region, while very few single tracks are bent in a crowd of straight ones. Occasionally, one branch of a star is curved, and this is, as a rule, a track diving steeply into the emulsion and traversing a large part or even the total depth of the emulsion. This is apparently due to distortion. The literature on cosmic ray particle photographs contains a detailed discussion of similar phenomena, and several remedies are proposed all of which aim at a more careful processing of the plates (OCCHIALINI *et al.*[5,6,7]).

A tissue section in direct contact with the emulsion slows down diffusion of the developer and fix. The uptake of water in the gelatin will be different underneath the tissue and outside, also the degree of swelling, the rate of washing and drying. The tissue and emulsion will probably dry to different water contents. All this can explain the formation of distortion in the emulsion, and also the fact that the phenomenon is most pronounced at the borderline between tissue and emulsion, in holes and interspaces.

2. A phenomenon which makes the quantitative interpretation of the present autographs rather ambiguous is the presence—in many cases—of a great number of tracks in certain regions, preferably along the borderline of the tissue and in holes and interspaces, especially in the proximity of visible thorium deposits. The primary impression is that some of the radioactive material is leached out of the tissue and carried into the paraffin embedding. It is typical of these clouds of tracks alongside the tissue that they consist exclusively of single tracks and that the clouds appear as roundish formations. This points towards residual droplets of the solvent (maybe alcohol during fixation of the tissue) included in the paraffin embedding. Leaching phenomena are also encountered in the autoradiographic study of tissue containing soluble beta emitters, for example, phosphorus or iodine[3].

Obviously, leaching of radioactive material makes the result of track countings in given regions very uncertain. The number of tracks may be much too high because leached material is re-deposited in a region where it has not been present originally, or, the track count is too low because part of the material originally present has been leached out. For this reason, the present author considers the development of a fixation method which avoids this phenomenon a very important task. Experiments are in progress using freezing-drying of the tissue.

EVALUATION

More than any of the artefacts discussed so far, a necessary correction of the results

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for self-absorption complicates the qualitative and especially the quantitative evaluation of the autographs. If the radioactive substances were evenly distributed in the tissue or if they were aggregated to form bodies which are small as compared with the range of the α particles emitted, the correction for self-absorption would be accessible to a mathematical treatment, provided the thickness of the tissue section and the average size of the active bodies are known. In the present case, however, the radioactive material is deposited in the tissue in aggregates the sizes of which vary from clumps of 1×1 micron to clumps 100×100 microns wide (7 microns thick) (the particle size of the injected colloidal suspension is of the order of 10^{-2} microns). While for small size clumps the number of α particles emitted per time unit is proportional to their volume (third power of the radius) the radiation reaching the surrounding tissue from large size clumps is proportional to r^2 or even to a still smaller power of r. Moreover, the absorption coefficient of the tissue is different from that of the aggregates. These factors introduce considerable uncertainty both concerning the true number of tracks in a given tissue region and the grouping of tracks according to their lengths. The track of a short range α particle may appear relatively long if its point of origin is situated near the interphase tissue/emulsion. The track of a long range α particle may appear relatively short because the particle had to traverse the tissue at such an angle that most of its path falls inside the tissue, or, it originates from the centre of a large clump of thorium deposit and has lost most of its energy on entering the emulsion.

The question remains what information can be obtained from the autoradiographs of tissue containing thorotrast. In the first place, it is possible to obtain detailed information as to the site of the radioactive material in the body, and in different tissues. Moreover, there is no doubt that an overall counting analysis of the tissue autographs is not invalidated by absorption or self-absorption, and an estimate can be obtained of the total number of disintegrations per time unit reaching a given volume of tissue. This value offers a basis for dosage calculations. From the observations made so far it is apparent that the major quantity of the thorotrast is deposited in large clumps and the actual tissue dose therefore becomes much smaller than the dose calculated on the basis of the total number of disintegrations from a given quantity of thorium injected as thorotrast. It need not be stressed that a correlation of this information with the histological findings may open the way for a better understanding of the effect of internal α irradiation on tissue and on various types of cells. Moreover, it may be possible, after further improvement of the histological as well as the photographic technique, to study the distribution of the different decay products of thorium, and other contaminants, as compared with the distribution of the mother substance in the organism. Such information, in turn, will give some indication as to which means would be worth trying in an attempt at removing the harmful components from the respective regions of the body.

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assisted with the processing of the plates and the track counting. My sincere thanks are due Dr GEORGE A. BOYD, Oak Ridge Institute of Nuclear Studies, for valuable criticism and for innumerable discussions of autoradiographic problems.

POSTSCRIPT

After the present manuscript was completed, a paper on similar problems appeared in abstract in the October 30, 1950, issue of *Nuclear Science Abstracts*[12]. A discussion of the results of A. I. WILLIAMS must be postponed until her paper is accessible.

SUMMARY

The application of α autoradiography in the study of biological specimens containing thorium and its decay products is described. The experimental procedure is outlined, various artefacts and necessary corrections are discussed.

RÉSUMÉ

L'application de l'α-autoradiographie à l'étude de spécimens biologiques contenant du thorium et ses produits de dégradation est décrite. La méthode expérimentale est indiquée et divers artéfacts et corrections nécessaires sont discutés.

ZUSAMMENFASSUNG

Die Anwendung der α-Autoradiographie bei der Untersuchung biologischer Proben, welche Thorium und dessen Zerfallsprodukte enthalten, wird beschrieben. Die Methode wird kurz besprochen; verschiedene Artefakte und notwendige Korrektionen werden ausführlich erörtert.

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