in Fig. 2. With a constant heat input via the resistance wire, the rise in temperature as measured by the Beckmann thermometer is exactly the same as that measured by the corrected slope of the recorded temperature.

The hydrothermic equivalent of the apparatus was calculated as follows: With a constant heat input at a given calorimeter temperature under fixed room temperature conditions, the calories engendered in distilled water were equated with those engendered in potassium carbonate solution of known specific heat. The only unknown, the hydrothermic equivalent of the apparatus can be calculated(6). It was 5 ± 1 cc for the digital, and 14 ± 2 cc for the hallucal calorimeter.

*Summary.* A recording digital calorimeter is described.

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## An Improved Technic for Liquid Emulsion Autoradiography.* (20802)

JAMES S. ARNOLD.† (Introduced by Austin M. Brues.)

*From the Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill. and the Radiobiology Laboratory, University of Utah College of Medicine, Salt Lake City, Utah.*

The increasing application of radioactive elements as tracers in biological and medical problems and the necessity for more precise information regarding the effects of radioactive elements on tissues have resulted in continuous improvement in autoradiographic procedures for the detailed study of the distribution of these elements. It is generally recognized that autoradiographic resolution on a cytologic scale can be improved by the elimination of all space separating the emulsion from the tissue, the use of thin layers of emulsion, and the use of thin tissue sections (1-4). In the course of a study of the deposition of long-lived radioactive elements at the cellular level, it has been necessary to improve and modify existing technics for the preparation of liquid nuclear track emulsions for autoradiography. As a result, an autoradiographic procedure has been developed which eliminates all emulsion-tissue separation and allows the preparation of emulsions less than one-half micron in thickness.

*Materials and methods.* All tissues are fixed in acetone at room temperature to minimize migration of bone seeking radioisotopes and fogging of emulsions by chemical constituents. Tissue sections, 2 to 5 microns thick, are cut in an embedding medium such as celloidin, paraffin, or plasticized nitrocellulose(5), and mounted on glass slides with egg albumin or other fixatives. (For very hard tissues that tend to curl, *e.g.*, undecalcified bone, it is advisable to roll the damp section on a slide that has been previously coated with 1.5% gelatin, drained, and air-dried.) The embedding medium is then dissolved in a suitable solvent, *e.g.*, xylene for paraffin, ether alcohol for celloidin, and ether alcohol or acetone for the plasticized nitrocellulose, and the tissue section is dried. NTA and NTB liquid nuclear track emulsions in the solid state in air- and light-tight, Dewar flasks, and maintained at 5°C, were kindly supplied for

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† Present address: Radiobiology Laboratory, University of Utah, College of Medicine, Salt Lake City.

test purposes by the Eastman Kodak Co. NTB emulsion has a high sensitivity, producing short tracks for low energy beta particles or electrons. NTA emulsion has a low sensitivity, responding quite slowly to beta irradiation. Both emulsions are capable of forming alpha tracks.

A small quantity of the gelatinous emulsion is transferred on a clean glass or stainless steel spatula from the Dewar storage flask to a beaker that has been placed in a constant-temperature water bath at 38°C. Melting occurs in 15 to 25 minutes, depending on the amount of emulsion to be used. When the emulsion has reached the liquid state, it is applied to the mounted tissues by means of a soft, clean No. 6 or No. 7 camel's hair brush. The thickness of the emulsion layer is easily controlled by varying the amount of emulsion on the brush. The sweep of the brush should neither start nor stop over the tissue section. With experience, as many as 50 slides may be satisfactorily covered in 5 minutes.

The emulsion coating will gelatinize at room temperature in about 5 minutes. If the emulsion tends to clump on the tissue, the slides should be cooled on a metal plate above an ice bath before coating. After the emulsion coating has gelatinized, the slides are dried in a gentle current of dust-filtered cool air (that may be obtained from an electric fan with a cheese cloth filter). The slides are then stored for exposure in a plastic slide box containing an open vessel of saturated calcium chloride solution. The slide box is sealed so that it it both light- and air-tight and placed in a refrigerator at 0° to 5°C for the desired exposure period which, as in any autoradiographic procedure, depends on the amount of radioactivity present, the distribution of the radioactive isotope in the tissue, the degree of image intensity desired, and the half-life of the radioisotope(1,2,4). All solutions used in the developing process are kept at 18°C to prevent softening and liquefaction of the thin emulsion. The slides are processed in a horizontal position to further minimize distortion. To prevent the accumulation of bubbles beneath the thin emulsion, all solutions are degassed by reducing pressure to the boiling point at room temperature (using a simple water aspirator and vacuum flasks) and all washing steps are eliminated. The slides are held for one minute in D-19 developer (Eastman Kodak) and transferred to acid fixer with hardener for 2 minutes. The slides are then placed in 4% formaldehyde solution with occasional gentle agitation for 5 minutes and then transferred to a second 4% formaldehyde solution for 4 hours.

*Staining.* After the emulsion has been hardened in formalin, the tissues may be stained at room temperature with hematoxylin and eosin without fear of softening the emulsion. The best results are obtained by overstaining the slides in Mayer's hematoxylin and then differentiating in 5% ammonium alum solution until the gelatin of the film is clear. (When preparing undecalcified bone autoradiographs, the sections should be decalcified prior to staining in 5% ammonium alum, which does not attack the silver grains.) The slides are then placed in ammonia water at a pH of 8.5 for 30 minutes. (A higher pH tends to soften and distort emulsions and a lower pH fails to protect against residual thiosulfate attacking the silver image.) The tissues are rinsed for a short time in distilled water and are then stained in aqueous eosin (½% for 30 to 60 seconds). The gelatin is cleared of eosin by vigorous differentiation in 50-90% alcohol for 10 to 15 minutes, and the sections are mounted in clarite or permamount in the usual manner. If a few bubbles of gas should persist beneath a section, they may be removed by soaking for 12 hours in a 1:1 dilution of the mounting medium(6). The autoradiographs are examined under ordinary transmitted light or with a dark field microscope.

*Discussion.* The production of thin emulsion layers and the elimination of tissue emulsion interspace are theoretical as well as practical advantages in the improvements of autoradiographic resolution(1-4). Fig. 1 is a microphotograph of an autoradiograph and 4 microns thick section of a trabecular bone from a rat sacrificed one week following $Ca^{45}$ administration. The line of increased grain density running down the center of the structure overlays $Ca^{45}$ incorporated in the under-

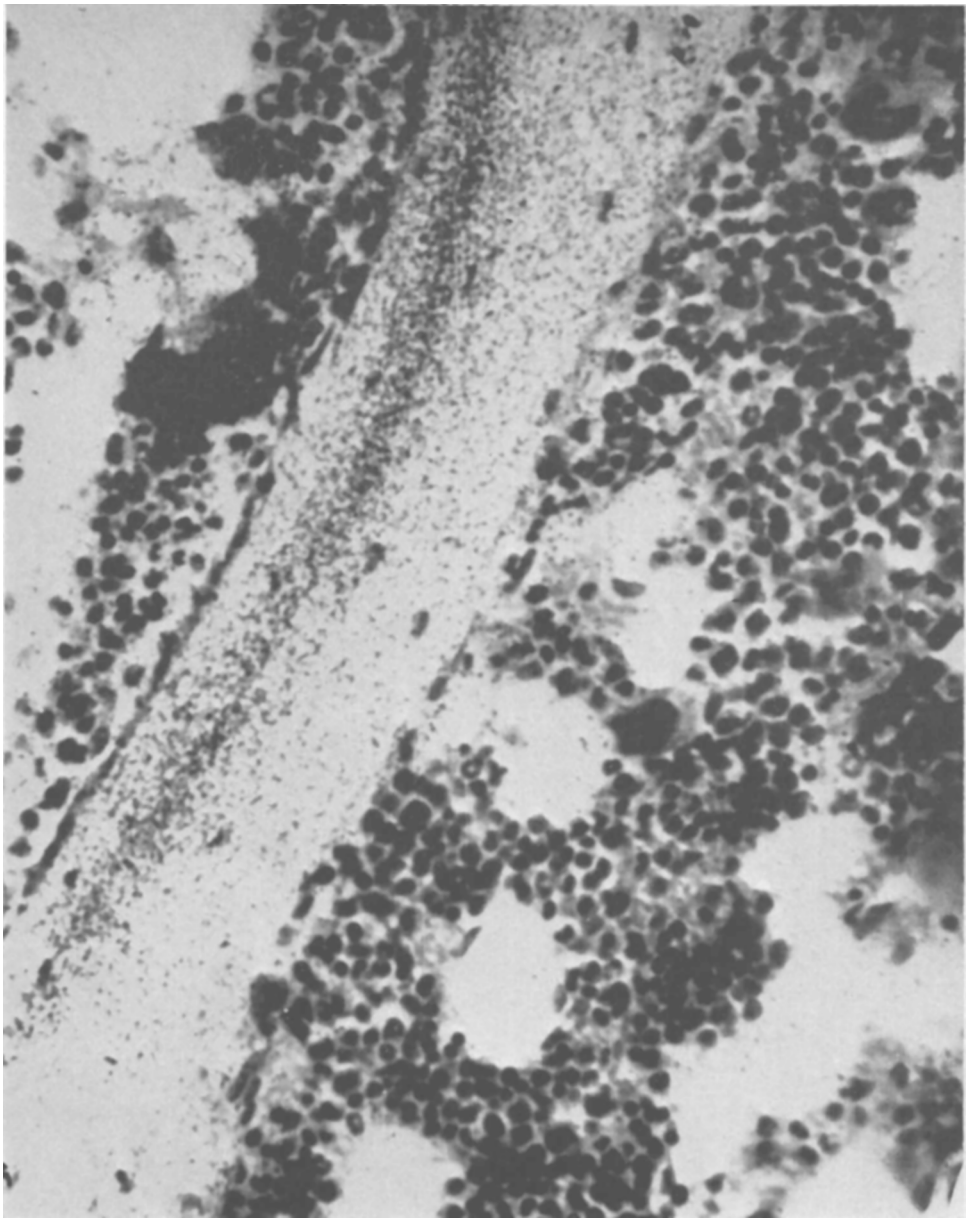

FIG. 1. Microphotograph of an autoradiograph and 4 $\mu$ thick section of a trabecular bone from a rat sacrificed 1 wk following $Ca^{45}$ administration.

lying bone, which was deposited one week prior to sacrifice. The osteocytes and various cells of the marrow may be compared to the width of the line of increased grain density. It is apparent that this line is no wider than 14 microns in the center. However, no accurate estimation of the absolute resolution of the autogram is possible because the width of the strip of bone containing the increased $Ca^{45}$ concentration is not determinable.

In addition to considerations of resolution, the thin emulsion layer has a distinct advantage over thick ones in high magnification microscopic study and photomicroscopic reproduction of detailed autoradiographs. The depth of focus of most microscope

objectives with a 20× or greater magnification is less than 3 microns. When a histological section and intimately superimposed autoradiographs are viewed simultaneously one or the other tends to be out of focus. Because of this limitation of the objective depth of focus, any diminution in emulsion thickness, section thickness or emulsion tissue interspace will facilitate study of the high resolution autoradiogram.

Several problems arose during the course of the development of the described technic which are peculiar to extremely thin emulsion layers of liquid-coated autoradiographs. The first of these is the adherence of the emulsion to tissue in an even layer. Addition of wetting agents, precooling of slides, and dilution of emulsion(2,6) all tend to minimize the tendency of the emulsion to clump; however, occasionally all of these procedures fail on certain preparations for undetermined reasons.

Thin layers of emulsion are subject to cracking when stored at the low humidity produced by most chemical desiccants. When actual cracking of emulsion does not occur, sufficient tension may develop to produce characteristic fine, irregular lines of closely packed silver grains. Emulsion cracking and tension artifacts are virtually eliminated by maintaining a constant relative humidity of 40% during the exposure period. This is simply done by placing an open vessel of saturated calcium chloride solution into the exposure slide box and exposing autograms at 0-5° C.‡

The tendency of bubbles to form between emulsion and tissue during development and fixation procedures was initially a problem. This phenomenon probably occurs in thick as well as thin emulsion preparations; however, the distortion produced by it is much greater in the case of thin emulsion layers. Bubbles began to appear in the developing solution and continued to accumulate throughout all processing steps. The tendency of bubble formation was finally controlled by 1) eliminating all washing procedures, 2) decreasing development and fixation period to a minimum, 3) degassing of all processing solutions prior to use, and finally 4) hardening emulsion in 4% formaldehyde solution. If thin emulsion layers are not hardened in formaldehyde they soften and disintegrate in most aqueous solutions above 20°C.

Chemical fogging of the emulsion has rarely been encountered when processing acetone-fixed tissues. The reason for this is as obscure as the actual mechanism of chemical fogging.

*Summary.* A method for the preparation of autoradiographs with thin liquid coated emulsion layers has been described.

‡ Suggested by William P. Norris of Argonne National Laboratory.

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