A New Approach To High Resolution Radioautography[1]

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THE OFT-REPEATED STATEMENT that a radioactive tissue slice will "take its own picture" when placed against a photographic emulsion is true only to a rather limited extent. When there are large differences in radioactivity among various gross structural or functional portions of a tissue, a radioautograph may indeed bear a close similarity to a macrophotographs of the stained tissue section, and such radioautographs have provided information concerning distribution of radioisotopes in very much greater detail and quantity than it would have been feasible to obtain, for example, by microdissection and measurements with a Geiger counter.

If ordinary radioautographs are magnified beyond 5 or 10 diameters in an attempt to examine the radioisotope distribution on a microscopic scale, however, it is found that the magnified radioautographs no longer resemble "pictures" in the usual sense of the word, but appear more like groups of hazy smudges or irregularly distributed silver grains, which reproduce little, if any, of the microscopic structure of the tissue source, and frequently yield little more information than that provided by gross inspection of the original radioautographs. The primary difficulty, of course, lies in the fact that the particles emitted by radioactive atoms show a random distribution as regards their direction of travel. Since, in order to affect a photographic emulsion, the particles must be able to penetrate tissue and emulsion to a significant extent, a radioautograph of a single point source of radioactivity resting on the surface of a photographic emulsion would consist of a group of silver grains distributed within a circular area around (or with a thick emulsion, in a hemisphere under) the point source. The apparent diameter of the circle would depend principally upon the penetrating power of the emitted particles and the intensity of the exposure. The amount of exposure is involved because a minimum concentration of silver grains (or a minimum percentage increase in concentration over that due to background "fog") is required before the emulsion shows a detectable darkening, and the geometry of the system is such that this concentration of grains should be reached first near the source (the geometry being somewhat similar to that in wheels, where the "concentration" of spokes decreases from the hub to the periphery). In the case of most biologically important radioisotopes, which emit β-particles with a spectral distribution of energies, the effect of exposure intensity is made still more important by the fact that particles of all energies emitted can react in the small area immediately around the source, whereas the production of a significant concentration of grains in the much larger area near the maximum range of the particles is especially slow because it depends on the relatively small proportion of highly energetic particles emitted. The diameter of the significantly darkened circle about a point source of radioactivity is usually the limiting factor in the resolving power of the radioautograph, since if a second point source were placed less than this distance from the first, the two "circles of confusion" would overlap and fail to show clearly the double nature of the source.

A variety of approaches to the problem of increasing the resolving power of radioautographs has been reported or suggested. About the only type of approach that has been both moderately effective and generally applicable to biological studies (*1, 2*) involves placing a thin tissue section in intimate contact with a thin emulsion and leaving the tissue and emulsion fastened together for microscopic examination. Use of the thin source and emulsion increases the relative importance of the less penetrating particles and reduces the probability that the more energetic particles will be in the emulsion as they near the end of their paths. Leaving the tissue attached to the emulsion eliminates the need for cross-comparison or realignment of tissue and radioautograph and thus facilitates the use of low exposures by making it unnecessary for the radioautograph to "picture" any histological structures. By examining the combination under high magnification, with only the tissue and the immediately adjacent portion of the emulsion in the plane of focus, one may obtain the effect of an exceedingly thin emulsion. The availability of special β-particle emulsions that have small grain size, high stopping power, and very low background fog (*3*) has still further increased the amount of information that may be extracted from lightly exposed radioautographs with the tissue section still attached. This

[1] Reviewed in the Veterans Administration and published with the approval of the chief medical director. The statements and conclusions published by the author are the result of his own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

simultaneous observation of tissue and radioautograph makes interpretation so convenient that the technique will probably continue to be used for routine studies, even though techniques giving much higher resolution but lacking the simultaneous observation feature become generally available.

With intensely radioactive specimens, it might conceivably be possible to collimate the emitted particles by absorbing the unwanted ones before they reach the emulsion. If a sheet of solid material thick enough to absorb all but a small fraction of the incident particles were placed between an emulsion and a tissue containing an α-particle emitter, a fairly high resolution might be achieved, since the absorber would pass only those α-particles approaching the surface of the emulsion at an approximately perpendicular angle. For β-particles, which are easily deflected and show a wide range of penetrating power, however, the solid absorber would have to be replaced with one resembling a microscopic or submicroscopic honeycomb, the openings of which could pass "well-aimed" β-particles into a very thin emulsion while most of the "poorly aimed" particles were being deflected and absorbed by the walls. It is at least conceivable that such a microhoneycomb exists or could be prepared, but there is some question as to how advantageous its use would be.

Probably the highest degree of radioautographic resolution possible has been obtained with α-particle emitters (*4, 5*). Using special α-track emulsions under high magnification, the path of an α-particle may be traced back to the point at which it entered the emulsion. Under ideal conditions, with an exceedingly thin tissue section in perfect contact with the emulsion, the source of the particle might be estimated with an error of less than a micron. Although it has recently become possible, under suitable conditions, to produce visible β-particle tracks in an emulsion, the tortuous paths of these light particles would make their tracing much more difficult and their extrapolation into the tissue section very much less accurate than in the case of α-particles (*6*).

Any procedure that overcomes the problem posed by the random direction of the emitted particles to such an extent that it is capable of showing the source of nuclear particles on a truly microscopic scale is likely to become involved with a secondary limitation on the useful resolution achievable with a radioautograph: the number of radioactive atoms that can be packed into any given microscopic or submicroscopic structure is severely limited (especially under physiological conditions), and their emission of nuclear particles is random with respect to time. For this reason it will probably never be possible to obtain highly enlarged radioautographs showing the clarity and structural detail of pictures taken through light or electron microscopes, with their practically unlimited source of neatly collimated photons or electrons. If this secondary limitation (in the number of particles available) becomes of major importance, it may be found that the information locked in the radioautograph can be extracted only by an exhausting statistical approach (correlating the data from a large number of similar structures in order to estimate the relative concentration of radioisotope in that type of structure). It can be seen, for example, that if a radioautograph showed only three particles issuing from a given cell, and indicated that all three particles came from one vacuole, the finding might be interesting but would, by itself, have little statistical or biological significance, for a second radioautograph (if sufficient activity remained) might very well show several particles from other parts of the same cell and none from that particular portion. If the primary problem of the random direction of the particles is minimized by wasting a large proportion of the energy emitted from the tissue, the limited supply of particles may become a serious factor even at rather low degrees of resolution and require a statistical approach or result in a radioautograph too light to be detected in the background "fog."

The collimation of particles by absorption of unwanted ones would certainly involve tremendous wastage of the available energy, and the use of limited exposures of thin, fine-grain emulsions in order to minimize the effect of the high-energy particles also fails, in a sense, to make full use of the radioactivity available. Even with the elegant α-track procedure one must limit exposure to a level which avoids a confusion of tracks over any given microscopic structure, and the technique for tracing α-tracks one at a time would usually necessitate a statistical approach for mapping the fine-scale distribution pattern of the α-emitters.

A uniform electrostatic field could pull the nuclear particles toward paths normal to the plane of the emulsion and thus might improve resolving power without entailing further wastage of energy, but the potential gradient that could be maintained without arcing would probably show little effect on the circles of confusion from most of the biologically important radioisotopes. A uniform magnetic field could cause the particles to follow spiral paths between collisions and thus might improve resolution to some extent by decreasing the average straight-line penetrating power of the particles.

The ideal solution would be a focusing arrangement that could cause all the particles emitted in any direction from a given point in the specimen to impinge on a corresponding point of an enlarged image on a photographic plate. Then the exposure could be as intense as the specimen could make it, and, because of the enlarged image, exceedingly fast large-grain emulsions could be used to lay down a maximum amount of silver for each unit of energy available. Marton and Abelson (*7*) reported the achievement of a 30-μ resolution using a very thin but highly radioactive layer of a monergic (internal conversion) β-particle emitter and a low-aperture magnetic lens at a magnification of 1.6 diameters, but a generally applicable procedure along these lines seems quite remote unless fundamental advances in electron optics produce a high-aperture electron lens corrected for

spherical and "chromatic" (variable energy) aberrations.

There remains an approach which theoretically would show many of the advantages of an excellent focusing arrangement and some additional ones: if the *tissue itself* could be *mechanically enlarged* before the radioautograph is made, as a picture on a balloon is enlarged when the balloon is inflated, the maximum diameter of the circle of confusion about each radioactive point source would remain the same as before, but each point in the tissue would be farther away from every other point; ergo, increased resolving power. This principle has occasionally been involved to some extent in radioautography under special conditions. For example, in one unpublished instance in Hamilton's laboratory a partial microdissection performed by the microtome during preparation of a bone section resulted in an unexpectedly clear demonstration of the presence of plutonium in the periosteal tissue.[2] Similarly, dilute blood smears on β-particle emulsions provided Boyd *et al.* (*8*) with beautiful radioautographs showing the presence of high concentrations of radiocarbon in some of the widely separated blood cells and its relative absence in others.

An early thought in connection with mechanical enlargement of tissue sections was that it might be possible to perform a microincineration of a tissue section containing a nonvolatile radioisotope, fuse the ash into the glass mount (or between two layers of glass), and then blow the glass into a very thin bubble. A microincinerated specimen, with its lack of cohesion between various portions of the ash, may yet prove to be the best type of preparation for tissue magnification, but the only radioactive tissues immediately available for testing were rat thyroids containing easily volatilized radioiodine. Mostly out of curiosity as to what might happen, a thin thyroid section was placed between two slightly flattened lead balls and the two balls were then squeezed in a small hydraulic press, on the theory that each part of the tissue would be tightly held by the pressure from the lead atoms above and below and that as the lead atoms began to flow outward the tissue might expand with the lead. When the two flattened disks of lead were peeled apart, the inner surface of each showed an area with a visibly different texture from the rest. The shape of this odd-textured area corresponded roughly to the shape of the thyroid section but was about twice as large as the original section. When a radioautograph of the inside surface of each disk showed a magnified outline of the gross histological structures of the thyroid section, with a clarity at least as good as that of the original radioautograph, a series of experiments was begun to test some of the possibilities of enlarging intact tissue sections. These experiments have not progressed to a point at which it is possible to outline a comprehensive set of optimum conditions for mechanical magnification of tissue sections or to predict the ultimate resolution achievable by the procedure, but the results obtained indicate that this type of technique may be of real value in biological radioactive tracer experiments. The work was greatly facilitated by the discovery that the tissue did not need to be between two equally thick pieces of lead in order to expand evenly. This meant that the tissue could be mounted on a relatively thick piece of lead, covered with lead foil or with a piece of lead that would be quite thin after expansion, and the radioautographic film finally exposed right through the thin lead covering.

An example of the improved resolution obtained in these preliminary studies is shown in Figs. 1 and 2. The tissues used were 7-μ sections of a nodular colloid goiter surgically removed from a patient six days after he had received 1.66 mc of I^{131} orally. A paraffin section from the formalin-fixed nodule was floated onto an albumin-coated microscope slide, dried, and tightly pressed against "No-screen" x-ray film for 4 hours to give an ordinary natural-size radioautograph, which was photographically enlarged 48 diameters (Fig. 1*b*). The section was then deparaffined, stained with hematoxylin and eosin, mounted in balsam, and a photomicrograph prepared (Fig. 1*a*). The next serial section was mounted, stained, and photographed in the same manner,[3] and is reproduced as Fig. 2*a*. The cover slip and balsam of the second slide were removed with xylene, and the slide was then placed for a few minutes each in amyl acetate, amyl acetate containing 1% pyroxylin (Parlodion, Mallinckrodt), and amyl acetate containing 5% pyroxylin. The thick pyroxylin film was allowed to harden in air, scratched in a long rectangle about the section, and then gradually floated or teased off in warm water, taking care to proceed slowly enough to allow time for the water to soften the albumin between the section and the slide.

The pyroxylin-embedded section was placed on the center of a 40-mm lead disk, 13 mm thick, and a drop of amyl acetate was used to flatten it and attach it to the disk. The lead disk was then placed in amyl acetate for 2 hours, after which it was gently removed and the excess amyl acetate and pyroxylin wiped from around the tissue section. As soon as the tissue appeared on the point of drying out, a lead foil about 0.1 mm thick and 4 mm square was carefully placed over it, and the disk, tissue, and covering foil were immediately subjected to 8 tons of pressure between

[2] Shown informally at a Manhattan Project Conference in 1945. (The author used, without specific acknowledgment, this and other bits of then-classified information concerning plutonium metabolism in calculations for his National Nuclear Energy Series volume, and is happy to be able now belatedly to acknowledge J. G. Hamilton, at Berkeley, and W. Langham, at Los Alamos, as the principal sources of that information.)

[3] The author is indebted to a number of members of the Long Beach Veterans Administration Hospital staff for their cooperation in this study, and in particular to M. E. Morton and the Radioisotope Unit for making available some radioactive human tissues obtained in the course of their diagnostic and therapeutic work, to B. E. Konwaler and the Laboratory Service for assistance in the preparation of special sections and slides, and to T. Masterson and the Medical Illustrations Laboratory for the photographic reproductions. The radioiodine was obtained from the Oak Ridge National Laboratory on allocation from the U. S. Atomic Energy Commission.

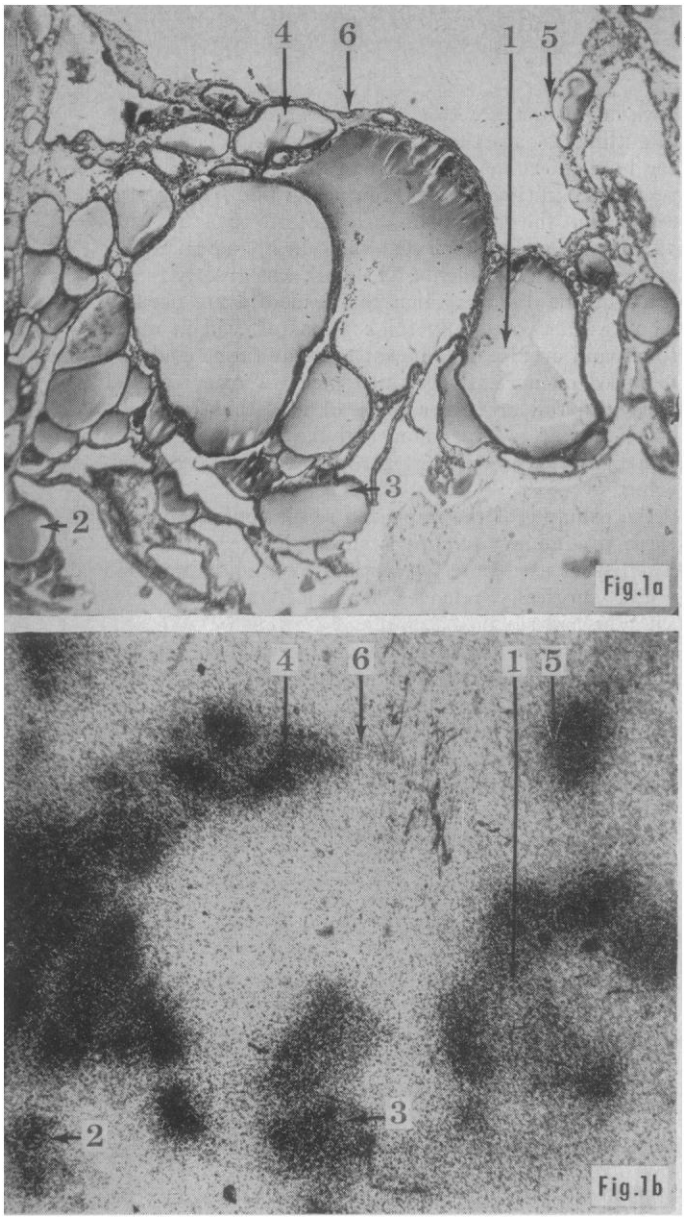


FIG. 1. *a*, Photomicrograph of 7-μ section from nodular colloid goiter surgically removed from patient six days after oral administration of 1.66 mc I^{131}. Formalin fixation, hematoxylin-eosin stain. × 48. *b*, Radioautograph prepared from above section before it was processed for photomicrography. "No-screen" x-ray film was pressed against paraffin section on albumin-coated slide for 4 hr, tank-developed 7 min at 68° C in Eastman x-ray developer. × 48.

polished and slightly oily steel platens. This pressure gave about a 30 per cent enlargement of disk, tissue, and foil, and firmly fused the three together. The disk was then trimmed to a smaller circle, keeping the still-visible foil near the center, and pressed again, giving a further expansion. The trimming and pressing were continued until the foil had been expanded about threefold; the disk was then trimmed to give a square slightly larger than the foil. This square of lead was gradually expanded, first in one direction and then in the other, in a small rolling mill (two polished steel rollers, 77 mm in diameter by 150 mm long, geared together, and turned by a hand crank in heavy brass bearings, which were set in an iron frame arranged to allow adjustment of the space between the rollers). As the lead square became too thin for convenient handling it was backed by a lead sheet cut to the same size, and the expansion and backing with lead sheets continued until the square was as wide as the rolling mill. A sheet of "No-screen" x-ray film was taped to the foil side of the square, and the film and lead were scratched to facilitate subsequent realignment, wrapped in black paper, and covered with a thick sheet of sponge rubber, a board, and a 100-mm layer of lead bricks. The film was developed after two weeks and showed that the tissue section had been mechanically enlarged approximately 20 diameters. This radioautograph of the expanded tissue was further enlarged photographically (Fig. 2*b*) to give a total enlargement roughly comparable to that of the ordinary radioautograph (Fig. 1*b*) and the photomicrographs (Figs. 1*a* and 2*a*). A casual inspection shows that the expanded tissue suffered some distortion (portion to right of arrow 1 expanded too much in horizontal direction, and portion around arrow 2 expanded too much toward lower left quadrant), but the major "landmarks" are shown with a clarity that permits fairly detailed comparison with the corresponding photomicrograph. Distortion is apparently most likely to arise from nonparallel surfaces of the platens in the hydraulic press, and from flexibility of the rolling-mill frame, which causes the edges of the lead square to expand more than the center. In addition to reproducing the major landmarks, Fig. 2*b* gives a fairly faithful rendering of many microscopic details that are more or less completely lost in Fig. 1*b* (e.g., breaks in the colloid indicated by arrows 3 and 4, a line of cells, possibly with shrunken colloid attached, arrow 5, and what appears to be a closely spaced group of microfollicles, arrow 6).

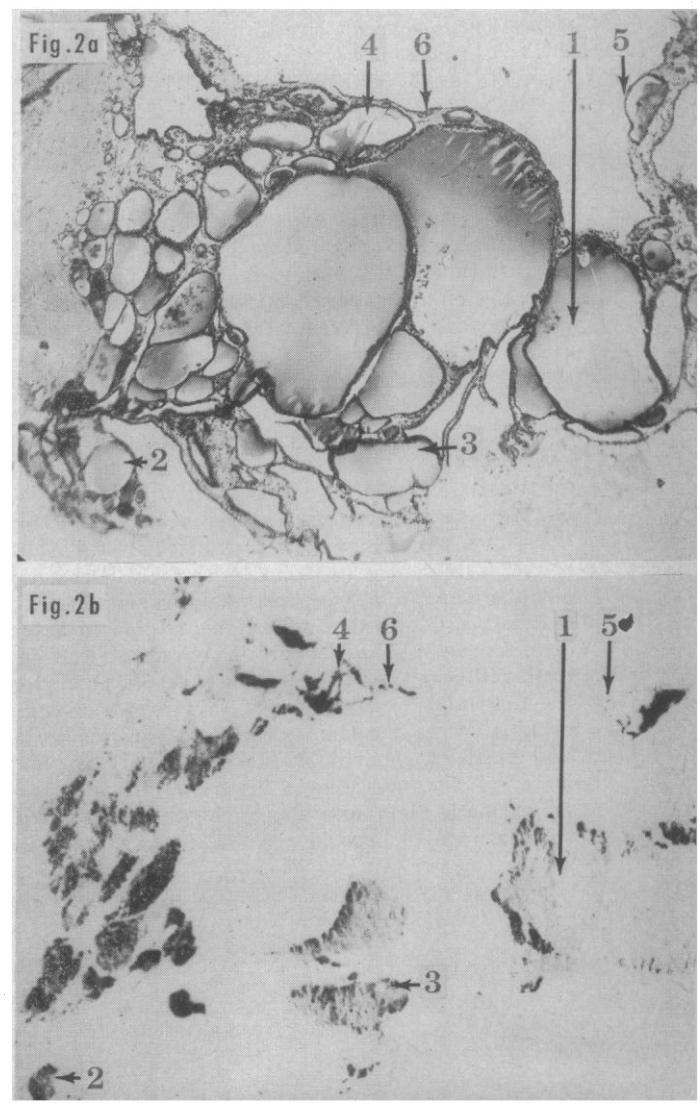
Some of the fine structure apparent in Fig. 2*b*, however, is probably an artifact. The radioactive colloid, for example, shows a markedly granular radioautograph. It seems probable that radioiodine is not distributed with exact uniformity throughout the colloid of any one follicle, for higher magnifications show the colloid to be filled with vacuoles of various sizes, and carefully prepared thin, tissue-thin-emulsion thyroid radioautographs occasionally appear to show a non-uniform distribution of radioiodine within a single follicle. Examination of radioautographs of tissues at various stages of enlargement and tests of expansion in lead of materials other than tissues (e.g., gold leaf) strongly indicate, however, that most of the granularity of the expanded colloid arises because the expanding lead fails to "stretch" the tissue out smoothly molecule by molecule, but rather tears it into microscopic fragments and then carries these fragments apart while maintaining approximately their original orientation relative to their neighboring fragments. The resolution obtainable with any given type of tissue section and technique of mechanical enlargement will probably be limited by the size of these separated fragments. Measuring the distance between centers of

a large number of the visible points of radioactivity in the radioautograph reproduced as Fig. 2*b* (allowing for the degree of mechanical enlargement) indicates that the diameter of most of the fragments is about 10 μ. Some portions of the radioautograph show the presence of considerably larger fragments or may even indicate that large fragments have held together for part of the expansion and then started to break up. Other portions, however (e.g., arrow 1), indicate that the fragments are so small (and so closely spaced) that the resolving power of the radioautograph is insufficient to show them clearly as separate fragments at this relatively low degree of mechanical enlargement. High degrees of mechanical magnification (up to 100 diameters) of intensely radioactive sections[4] generally resulted in very spotty radioautographs because of fragmentation of the tissue, but even these frequently showed some areas in which the size of the tissue fragments was apparently not the limiting factor in the resolving power of the radioautograph. Some of the uniformly exposed areas were obviously due to poor contact between lead and film, but many have shown sharply defined edges of the radioactive area and give a basis for predicting that an improved mechanical magnification technique may eventually yield radioautographs with very high resolving power throughout (though it is hardly to be expected that expansion in lead will ever be made so smooth that cleavage of chemical bonds by "stretching" will become a common procedure). In the meantime, an easily obtainable resolution, such as that illustrated in Fig. 2*b*, may be sufficient to solve some difficult problems of isotope distribution, provided the interpretation is made with the realization that much of the apparent fine structure may be an artifact produced by fragmentation of the tissue.

A wide variety of techniques for expanding tissues in lead gave a fairly good resolution and reasonably low distortion. No procedure was developed whereby the resolution achieved could be determined accurately, but judging roughly from the granularity of the radioautographs of colloid follicles it appeared that some of the best results were obtained when the lead disk employed for the initial expansion in the hydraulic press was fairly thick and had no ratio of thickness to diameter such that application of 8 tons of pressure would increase the diameter by about 20–50 per cent. This apparently resulted in an optimum balance between the grasping and the expanding forces applied by the lead, and although the optimum conditions seemed not to be very critical, they might be expected to vary somewhat with the type of tissue and the physical condition of the section. Several grades of lead were tested without finding any that were distinctly superior, but it seems probable that further experimentation with very pure lead and with lead alloys might determine the most desirable qualities of materials to be used for tissue expansion and indicate whether the size of the tissue fragments is more dependent on the granularity of the lead or on the cohesive forces in the tissue itself.

A few experiments with a 200-ton hydraulic press indicated that the degree of expansion obtainable with a reasonable thickness of lead was quite limited as

FIG. 2. *a*, Photomicrograph of serial section adjoining that used for Fig. 1. × 48. *b*, Radioautograph prepared from same section after it was embedded in lead and mechanically enlarged (along with the lead) by pressing and rolling. Approximate enlargement: mechanical, × 20; photographic, × 2.4; total, × 48. The radioautograph was exposed for two weeks through a very thin (approx 0.2 μ) layer of lead covering the expanded tissue. Film and development as in Fig. 1 *b*. For explanation of arrows, see text.



[4] Figuring the amount of radioactivity required for a satisfactory radioautograph of an enlarged tissue is somewhat complicated. The increase in exposure required after enlargement would be proportional to the increase in the area of film covered by the tissue if the radioisotope were uniformly distributed in the tissue, if the enlargement were smooth (molecule by molecule), and if the original tissue section were large as compared to the penetrating power of the emitted particles. In practice, however, the isotope is generally concentrated in small areas and the enlargement involves fragmentation of the tissue, so the required exposure increases much less rapidly than the area of the tissue section. The advantages of the tissue enlargement technique are most pronounced with highly radioactive sections (for local overexposure does not interfere with the radioautograph of adjacent weakly radioactive areas), but a tissue with a level of radioactivity that is convenient for preparation of natural-size radioautographs on thin, fine-grain emulsions is likely to yield fairly satisfactory radioautographs on fast emulsions after moderate mechanical enlargement.

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compared to that easily attained with a rolling mill. The resolution obtained with the large press[5] was not particularly good under the conditions employed, and even with hardened steel platens an inch thick, the edges of a six-inch lead disk were pressed thinner than the center, causing a considerable distortion of the embedded tissue. Some of the difficulties with this press might have been eliminated if highly polished platens had been available.

Some remarkable liberties may be taken with a heavy and accurately machined rolling mill. Using a silversmith's mill (after swearing to remove all bits of lead from the shop and taking an oath of secrecy concerning the one-time presence of such a base metal there), it was found possible to take a lead-embedded tissue section 3 mm in diameter, roll it out into an ellipse 3 mm × 250 mm, cut the long strip into three sections, and then, by subjecting each section of the strip to a second rolling at right angles to the first, to restore the tissue to approximately its original shape. The major internal structures of the tissue were still recognizable in a composite radioautograph, and the resolution appeared to be about as good as that achieved by the gradual expansion of a lead square in the light rolling mill constructed in the laboratory shop.

A few experiments, generally with inadequate techniques and equipment, have failed to turn up a suitable substitute for lead. Silver appears to be too hard (though it could be made to take an impression of a thyroid section), and it requires high-temperature annealing during the expansion process. The sample of presumably pure gold tested also appeared to be insufficiently malleable, and an attempt to use gold-beaters' techniques in expanding tissues, even with easily fusible lead, resulted in the jarring or blowing of fragments of tissue away from their proper positions. Use of a transparent material for the expansion would avoid the problem of transferring the tissue section intact after the photomicrograph was obtained, and would permit simultaneous microscopic observation of the tissue fragments and their radioautographs, at least during the early stages of expansion of the tissue. A number of low softening point thermoplastics tested appeared to show little promise, but the tests were not carried out under ideal conditions, and it is conceivable that among the host of plastic formulations and fabrication techniques available there might be some that would be suitable.

It is possible to expand a tissue section to a few diameters in lead, peel apart the pieces of lead and then, with a coat of collodion, to remove a portion of the tissue fragments sufficient to give a radioautograph showing the main structures. The number of lead particles removed by the collodion is so great, however, that microscopic inspection of the tissue fragments is essentially impossible, and some cleaner substance would probably have to be substituted for the lead as a first step in rendering this sort of technique useful. An attempt to adapt this lead expansion–collodion film procedure for preparation of thin sections for electron microscopy (*9*) also failed to show much promise. Of all the materials tested as possible substitutes for lead, the waxes appeared to show most nearly the properties required for tissue expansion. Placing tissue sections between thin disks of paraffin or beeswax and pressing out between cellophane-covered platens resulted in the formation of nearly transparent wax disks in which the tissue was pressed so thin or fragmented to such an extent that it was rather difficult to find fragments for study under the microscope. Radioautographs prepared from such wax disks, however, indicated very poor resolution, and the tendency to local streaming in the expanding wax was so great that the radioautographs rarely showed any recognizable similarity to the original shape and structure of the tissue section. It seems possible that the advantages of direct microscopic observation of the tissue fragments would be such that a relatively poor expansion technique employing a transparent medium, in which the tissue could be broken into recognizable fragments and the fragments separated a reasonable distance without being squeezed too thin for microscopic visibility, might prove of greater value than techniques achieving much higher resolution with an opaque expanding medium.

No systematic study has as yet been made of the resolution obtainable with tissues other than thyroid, or with various section thicknesses, fixation techniques, or procedures for rendering the tissue sections more frangible. Transferring a section to a lead disk and then heating to a point which browned the section but failed to volatilize the radioiodine or melt the lead resulted in decreased resolution, probably because of oxidation and irregular hardening of the lead surface. Heating after the tissue had been pressed between two pieces of lead resulted in the formation of gas bubbles between the layers of lead and loss of resolution. In one test of nonthyroid tissues a droplet of a pond water culture containing a variety of microscopic animals was placed on a lead disk, and a droplet of radioactive iodine-iodide stain was added. The droplet was removed slowly by a thin strip of filter paper, the former position of the droplet covered with lead foil, and the disk expanded. The radioautograph showed a high background of general radioactivity; because of the lack of a guiding photomicrograph interpretation was uncertain, but there were a number of intensely darkened areas visible to the naked eye, which appeared to correspond to the shape of some of the larger forms of protozoa present in the culture. A rotifer, which was easily picked out in the radioautograph by several observers, appeared to have been expanded in quite small fragments, though there was one noticeable break in the radioautograph of its extended flagellum. If it is possible to develop a suitable procedure for preparing, photographing, and expanding frozen sections, radioautography with short-lived radioisotopes would be facilitated, for it should be

[5] The author is indebted to E. H. King, Engineering Department, University of California at Los Angeles, for operating the big press for these experiments.

possible to expand frozen sections in lead and have them placed against a photographic plate within a few minutes after their removal from the animal. A technique for expanding frozen sections should also be very useful as a means for binding in place the volatile and diffusible radioactive substances ordinarily lost or displaced during tissue processing procedures.

On occasion it has been desirable to cut out a small portion of a lead square and the expanded tissue embedded in it in order that further expansion of the selected portion of tissue might be carried out more easily. With a tracing of the radioautograph as a guide such cutting can be done with a high degree of accuracy, and feats of microdissection which would originally have been exceedingly difficult may be reduced by the tissue expansion to a level of difficulty approximating that involved in the cutting out of paper dolls. Although radioautographic procedures have been used for some time to localize radioactive *elements* on a relatively fine scale, studies of the biochemical transformations which a radioactive element or compound undergoes have for the most part been limited to whole organs or macroscopic tissue structures. With experiments of the type in which the use of high concentrations of radioactivity is allowable, this simplified microdissection technique, combined with very sensitive analytical procedures such as paper chromatography of radioactive compounds (*10*), may aid in extending our knowledge of intermediary metabolism by facilitating a more accurate localization of the sites at which various biochemical processes take place.

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Technical Papers

The Influence of Skin Temperature upon the Pain Threshold as Evoked by Thermal Radiation[1]

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That reduced skin temperature can result in cutaneous analgesia is well known, and skin temperatures near 0° C cause local anesthesia (*1*). Also, Schumacher (*2*) and Graham, Goodell, and Wolff (*3*) have reported that vasodilation of the superficial vessels of the skin lowers the cutaneous pain threshold. However, a systematic investigation into the effect of the level of skin temperature upon the pain threshold for pricking pain evoked by thermal radiation has not been reported. To investigate this matter quantitatively the following experiments were performed.

The pain threshold on the blackened skin of the forehead and the back of the hand of 4 subjects was measured by the Hardy-Wolff-Goodell (*4*) method, in a room at 26° C. Skin temperatures were measured with a radiometer (*5*) prior to each test of pain threshold. The subjects then moved into a room at 8° C, and the skin temperature and pain thresholds on the forehead and back of the hand were measured at 5- to 10-min intervals over a period of 1 hr. The subjects returned to the room at 26° C, and the measurements were continued for 2 additional hr as the skin temperature returned to control levels.

The results of these observations are shown in Fig. 1 by the solid line drawn through the averaged readings plotted for the forehead and the hand. The same relationship of pain threshold to the level of skin temperature was observed for both areas. Cooling of the skin 10° C resulted in an elevation in pain threshold of roughly 200 millicalories/sec/cm².

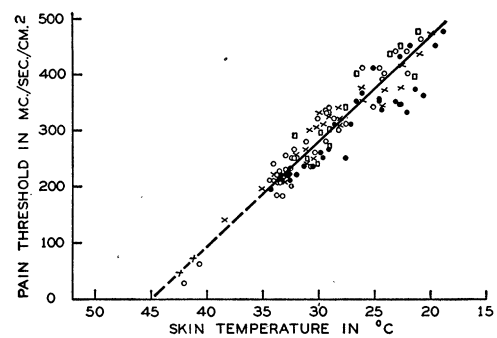

FIG. 1. Relationship between pricking pain threshold and skin temperature.

In a second experiment, the blackened skin of the forehead of 2 subjects was irradiated with intensities of thermal radiation which caused elevation of skin

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