A THEORETICAL AND TECHNICAL STUDY OF AUTOGRAPHY AS A HISTOLOGICAL METHOD FOR LOCALIZATION OF RADIOACTIVE ELEMENTS

By

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A Thesis

Submitted in partial fulfillment

of the requirements for the degree of

Master of Science

McGill University

Montreal, Quebec

Canada

May 1950.

A C K N O W L E D G E M E N T S

I wish to thank Professor C. P. Martin for the privelege of working in the Department of Anatomy. No words of mine could ever begin to express the debt I owe to Professor C. P. Leblond for direction, encouragement, stimulation and patience during the course of this work.

To Mr. Hans Torunski, without whose technical assistance none of this work could have been done, I wish to express my sincere appreciation.

The opportunity of working with Dr. J. Gross and Mr. N. J. Nadler has been a privelege. I wish especially to thank them for their explanations of the theoretical considerations involved in autography.

For advisory and technical assistance in the preparation of this manuscript, my sincerest thanks to Miss Winnifred Storey, Miss Patricia Hart, and Dr. Phoebe L. Cox. To my coworkers, Miss J. Cambron, Mrs. Joyce Millette, Miss Catherine Stevens, Miss Louise Cimon, Mrs. Joan Dougherty, Miss Diana Baillie and Mr. Gilles La Roche, my sincere appreciation for the many kindnesses they have extended to me is gratefully acknowledged.

And to my father, Mr. Mitchell Bogoroch, whose forebearance has made all this possible, my appreciation cannot be expressed in words.

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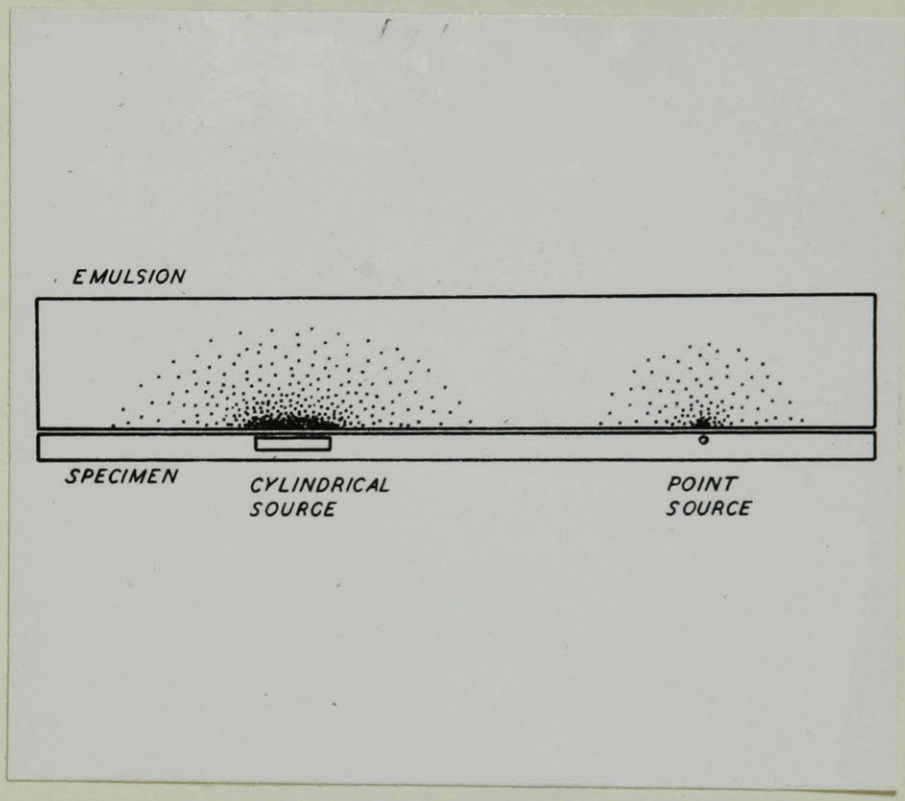
INTRODUCTION

An "autograph", also known as a "radioautograph", "autoradiograph", or "historadiograph", is the "signature" left by a radioactive particle in a photographic emulsion, and is, therefore, the visual evidence of the presence of radioactivity in the structure in contact with the photographic emulsion.

Radioisotopes present in biological material may be detected in two ways: 1) chemically, using the Geiger counter method and 2) histologically, using the above photographic technique - autography. It is the latter technique, however, that not only reveals the presence of the radioisotope within the specimen but also allows the isotope to be traced to its precise site in the tissue structure. This is accomplished by placing a tissue section containing the radioactive material in close contact with silver bromide grains in a photographic emulsion, exposing for a sufficient length of time, and developing the preparation as in ordinary photography. The radioactive emanations cause black silver granules to be deposited in the emulsion over the areas in the tissue section from which the radiations originated. These are rendered visible by the development process (Figs. 1 and 2).

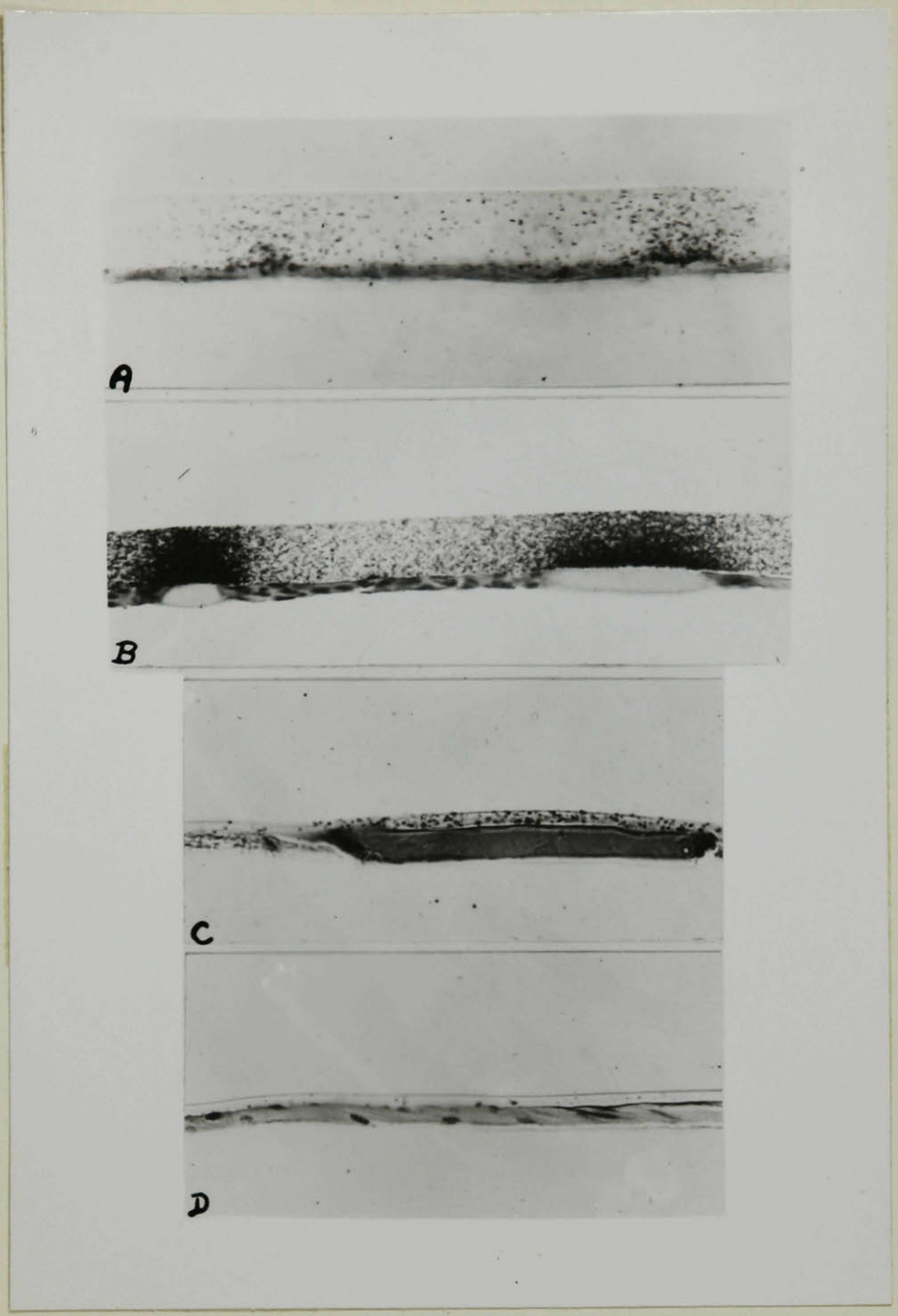
The use of silver bromide grains as a detector of radioactivity has four advantages over other techniques: 1) the effect of the

FIG. 1



Diagrammatic representation of the effect on a photographic emulsion (above) of a point source of radioactivity (right hand side of specimen), and a cylindrical source of radioactivity (left hand side of specimen).

FIG. 2



Profile sections of "coated" autographs. Sections of rat thyroid containing radio-iodine were mounted on strips of acetate film, instead of glass slides, and coated with photographic emulsion, as described in the text (p. 41). After exposure the preparations were developed and fixed to obtain the autographic image, then treated with chromalum to harden the emulsion, embedded in paraffin and sectioned.

The emulsion (upper layer) and section (lower layer) are very close together, i.e., the interspace between them is very small.

A. A cross section of an autograph coated with EK medium lantern slide emulsion and optimally exposed. Two areas of thyroid colloid containing radioactivity are present in the section. Note that the autographic reaction is most dense in the region of the emulsion closely adjacent to the radioactive source. In between the two sources, the emulsion shows a scattering of silver granules which constitutes background fog.

B. A similar preparation to that shown in Fig. A, coated with Ilford Half-Tone Stripping Film. In this case, however, the autograph has been over-exposed. Note the increased spread of the photographic image as well as a marked increase in the background fog.

C. A cross section of an autograph coated with a diluted EK medium lantern slide emulsion (see text p. 42), optimally exposed as in A. An area of radioactive thyroid colloid is visible. Note that closely the autographic image corresponds to the radioactive source and how little interference there is from background fog. The reduction in fog is more apparent in picture D, which is another area from the same preparation in which the tissue is not radioactive. Note that only a few scattered fog granules are visible in the emulsion layer.

radiation is cumulative. The photographic grains allow for minute quantities of radioactivity to be detected if exposed for a sufficient length of time; 2) the image gives a permanent record; 3) the record is two-dimensional, that is, in the plane of the emulsion. Recently, it has been possible to make three-dimensional autographs by following tracks in an emulsion (20); 4) the smallness of the silver bromide granules makes it possible to localize the radioactivity to a comparably small area in the tissue.

It is, therefore, possible by this method to virtually "see" a "labeled" element in an animal tissue. Furthermore, the fate of that element in the animal body may be determined by following the element through from structure to structure.

Although the effect of radioactivity upon photographic emulsions has been known for fifty years, it was not until comparatively renently that autography was adopted as a useful tool in biological research. The method used previous to 1946 - the "contact" method - in which the specimen was put in close contact with a photographic plate or film, was rather crude as compared with what was to follow. Finer methods such as the "coated method" in which liquid emulsion was painted over a radioactive tissue section and the "mounted method" in which a radioactive histological section was floated onto a photographic plate gave results far superior to those of the older method. But these still presented technical difficulties.

With the advent of new emulsions that could be applied to both the coated and the mounted methods, and a better understanding of

the technical considerations involved in the autographic method, it appeared possible to improve the quality of the autograph to an even greater extent. The purpose of this investigation, then, has been to improve the "coated" autographic method so that cellular localization of radio-elements could be visualized. To this end, several types of photographic emulsions were studied in relation to the factors affecting the quality of the autograph, and the autographs obtained were compared. Since some of the newer emulsions were prepared in "strip" form as well as in the older "liquid" form, it was also necessary to devise a method for the preparation of coated autographs with strip emulsions.

The essential result of this investigation has been to demonstrate by a comparison study of the "contact", "mounted", and "coated" methods that the most favorable conditions for autography depend on:

1. the relation between specimen and emulsion, i.e.,
   a) a minimal distance between source and emulsion,
   b) a thin section (5 μ or less),
   c) a thin emulsion coating (10-20 μ),
2. features of the silver bromide grains,
   a) uniformity (for contrast),
   b) small size,
   c) high concentration,

   and
3. characteristics of the β-radiations since particles of low energy give a better resolution.

HISTORICAL RESUME

By a fortunate combination of chance conditions and an inquisitive mind, Becquerel (5) in 1896 made the initial observation that was to provide investigators with a new tool for research - radioautography. One day upon developing a photographic plate which had been stored next to a fragment of uranium ore in a drawer Becquerel found that the plate was fogged. He reasoned that some mysterious rays from the uranium ores might be responsible for the fogging. Thereupon, he placed a layer of crystalline uranium sulphate against a photographic plate wrapped in black paper and upon development obtained a dark image of the crystals on the plate. And so he produced the first known "contact" autograph.

In 1904 London (72) and in 1922 Kotzareff (55) using similar methods, produced crude autographs of the organs of radium-treated animals. By placing a histological section containing polonium against a photographic plate Lacassagne (57, 58, 59) (1924) was able to obtain autographs of much finer definition than had heretofore been possible. Comparison of the tissue section and the autograph made it evident that discrete areas of accumulations of black silver granules in the emulsion could be attributed to definite structures in the tissue section. This technique was used by Lomholt (48) in 1930 with Radium D, a radioactive isotope of lead, and three years later Behrens and Bauman (6, 7) similarly prepared autographs of thorium B, another isotope of lead. During the ten to twelve years that followed this method continued to be used to

detect radium (102), radio-phosphorus (29,36,81); strontium (61,89,103); zinc (77,78,79,80); and iodine (24,25,43,44,45,50,65) in normal and pathological animal tissues.

The contact method has its limitations, however. It was true that radioactivity could be detected grossly in an organ or tissue structure by this method but it became obvious that better methods were needed if autography was to be used widely and successfully as a method of microdetection of radioactivity.

To meet this need, Bélanger and Leblond (1946) (9) developed the "coated" method. Shortly after, in 1947, Evans (35) and others (34) described a "mounted" method. In the coated method liquid emulsion was painted over a stained radioactive histological section on a slide; in the mounted method, an unstained radioactive histological section was floated onto a photographic plate. Both these methods offered a far more intimate contact between section and emulsion as well as allowing section and developed emulsion to be viewed simultaneously. The more intimate contact meant that the action of the radiations from the tissues on the emulsion would result in a less diffuse image than previously (See Theory). The advantage of viewing section and emulsion simultaneously is obvious.

Although it is only 3 years since these techniques have been developed, much literature has appeared in which these methods were greatly instrumental in elucidating the fate of different compounds in the animal body. The mounted method has been used (39,40,41) to study the sites of I^{131} pickup in cancers of the thyroid gland. Van Sallman, Evans, and

Dillon (91,92) used Na^{24} on studies of the eye while Warren and Dixon (105) studied anaphylactic shock with I^{131} labelled antigen. Boyd (21) has modified the mounted method somewhat and has used it in several instances.

The "coated" method has been used most extensively by Leblond, Bélanger, Gross, and co-workers. Their more important work included an elaboration of a theory on the mode of action of the thyroid gland(46,64,66); the destruction of thyroid by large doses of radioiodine (37); a further development of the theory on bone and tooth formation (10,64,70,86); and a study of the turnover of DRNA in tissues of the rat(69).

Modifications of the various basic techniques have appeared in the literature especially with the use of stripping emulsions. Thus McDonald, Gobb, and Solomon were able to trace C^{14} in glycine to the liver using a modification of the mounted method coupled with an inversion method(74,75), while Bélanger (8) using a somewhat similar approach with the coated-inverted method studied the metabolism of P^{32} in bones and teeth.

Notwithstanding the recent advances in autographic techniques, the older contact method is still very much used in many laboratories especially where hard specimens such as bones and teeth do not lend themselves favorably to microsectioning. The method has been used in this laboratory to study P^{32} in bones and teeth (70), and also in California (1,3,22,49,94,95,104) and Chicago (12,13,14, 38) for the study of the metabolism of fission products in the animal body.

Other elements, detected in a similar manner, have been I^{131} in teeth (4), in thyroid carcinomas (73) , and in a lingual thyroid (81); potassium in the brain (23); phosphorus in a urinary calculus(26) as well as in the eye (83,84); and copper in early chick embryos (99) ; and gallium in skeletal tissues (32). Several reviews and analyses of the results obtained using an autographic method have appeared in the literature (2,42,47,48,51,62,98,106).

THEORY OF THE AUTOGRAPHIC IMAGE

When ordinary light meets a photographic emulsion, that emulsion will register the effect of the meeting, and upon development with "photographic developers" will reveal the effect by a deposition of black silver granules. Similarly, when an ionizing particle, such as a β-particle comes in contact with a photographic emulsion, upon development, it, too, causes a deposition of metallic silver at the site of the contact. The mechanism involved is as follows:

Photographic emulsion consists of silver halide crystals or "grains", usually silver bromide, in a gelatin matrix. The concentration of the bromine and the mean diameter of the grain varies with different types of emulsion. The grain is composed of a cubic lattice of silver and bromide atoms arranged alternately and more or less equidistant from each other. The emulsion also contains impurities, the most important of which is sulfide. This plays an important role in the photographic process.

When the emulsion is exposed to radiant energy, there is an excitation and release of electrons from some of the bromine ions. These ions become trapped in the crystal lattice at specific irregularities such as provided by the specks of silver sulfide. Subsequently, silver ions in interstitial positions are attracted to these "development centers" and are electrically neutralized. This results in the deposition of metallic silver, the so-called "photolytic silver" which forms the "latent image". If enough energy is absorbed, so that enough photolytic silver is deposited, the grain so affected can be developed, that is,

rendered visible by precipitation around the development centers of silver so far present as bromide in the grain. Furthermore, these grains will remain unaffected by the fixation process whereas undeveloped grains will be dissolved out (54).

The photographic mechanism in the case of ionizing particles is similar to that for radiant energy except for the first stage. The electrons are brought into the conduction band by direct ionization due to collision of the atomic electrons within the crystals.

Factors Influencing the Autographic Image

In an autograph, an increased density of silver granules occurs in the emulsion over the sites of radioactivity. Localization implies that these areas of increased densities can be associated with tissue structures. The closer two areas of increased densities are and can still be distinguished from one another, the finer is said to be the localization, that is, the better is the "resolution" of the method used.

Resolution and density are both dependent on

a) the geometrical relation of the radioactive source and the overlying emulsion,

b) the energy and intensity of radiation and,

c) the characteristics of the photographic emulsion.

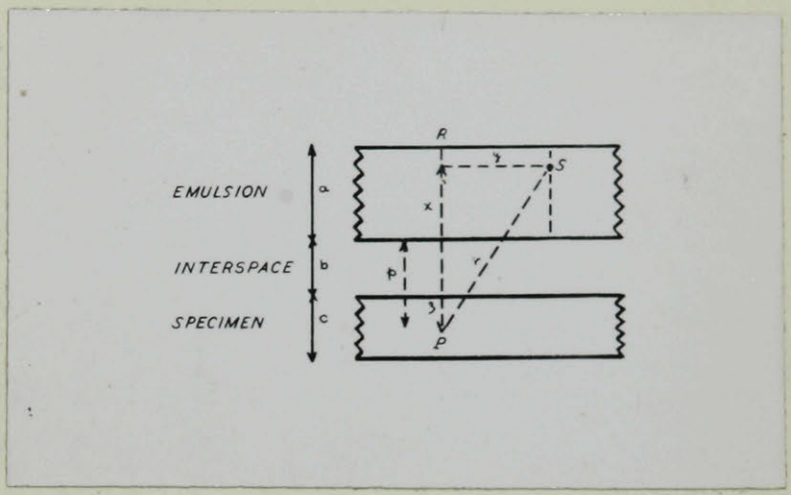
The theoretical considerations involved in the above factors will help to explain the quality of autographs obtained in practice.

a) The Geometrical Relation of the Radioactive Source and the Overlying Emulsion

The autographic preparation consists of the section, the overlying emulsion, and the space between the two, i.e., the interspace (Fig. 3). Any variation in their geometric arrangement such as changes in their respective thicknesses will affect the sharpness and density of the autographic image obtained. To analyze the effect of these factors, Pelc (88) assumed that "P" is a point source of radioactivity in a specimen of thickness "c". The total amount of radiation emitted from the source during a given interval of time, referred to as exposure time, can be designated as "Q". The specimen is separated from the emulsion of thickness "a" by the interspace "b".

Now since the intensity of the radiation at any point "S" in the emulsion is inversely proportional to the square of the distance from P to S (i.e., "r"), then the amount of radiation received at this point can be expressed as $\frac{Q}{r^2}$. This relationship is true for moderate densities only (76 ; see P.). In this case the blackening effect or density of the emulsion at S due to the radiation may be expressed as $\frac{QA}{r^2}$ where A is the sensitivity of the emulsion. This may be calculated for any element x along a line S perpendicular to the plane of the emulsion. The sum of these densities will represent the density P_D, visible through the microscope at a distance "y" from the perpendicular through the point source P. This summation may be obtained by integrating the densities along the perpendicular through S to obtain ^{P}D.

FIG. 3



Schematic representation of the action of a radioactive point source, P, on a photographic emulsion.

P is a point source of radioactivity in the specimen.

S is any point in the emulsion.

a = thickness of the emulsion.

b = interspace between specimen and emulsion.

c = thickness of specimen.

PR is a perpendicular through P, assuming that edges of specimen and emulsion are parallel to one another.

x, y, z - as indicated by dotted lines.

r = distance PS

p = z+b, i.e., distance of point source P from edge of the emulsion. (adopted from Pelc (88)).

$$P_D = \frac{PQ}{y} \tan^{-1} \frac{ay}{y^2 + P(p+a)} = AQP_D, \text{ when } y = D$$

and the maximum density $P_{D\ max}$ may be obtained

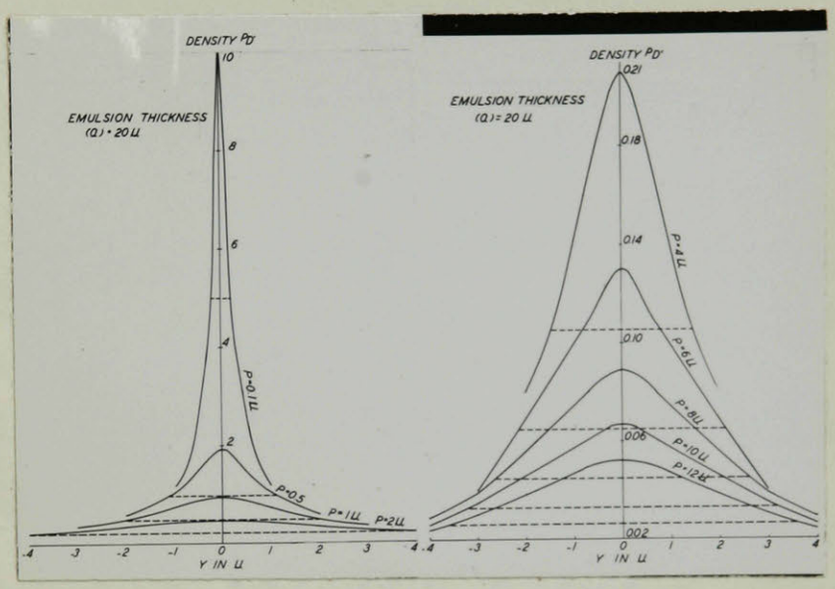
$$P_{D\ max} = AQ \frac{a}{P(p+a)}$$

P_D, and $P_{D\ max}$ being used when AQ is kept constant.

The maximum density ($P_{D\ max}$) will occur along the line PR passing through the point source P. By calculating the values of P_D, in arbitrary units for various distances (y) from the line PR, Nadler, working in this Department, has been able to construct a curve showing a profile of the densities produced by the point source at various points in the photographic emulsion (See Figs. 4 and 5). These curves have been constructed for conditions in which the point source is at various distances from the edge of the emulsion (i.e., distance p is varied). It is evident that the maximum density, ($P_{D\ max}$) is greatest when the source is closest to the emulsion.

If two point sources of the same intensity at the same distance from the edge of the emulsion are separated by a distance equal to the resolution measured, their corresponding photographic densities will be readily distinguishable. Therefore, using as an arbitrary measure of resolution (theoretical) the dispersion of the curve at the point where the density is half the maximum density (as suggested by Pelc), Nadler(Fig. 6) as well as Pelc prepared curves which showed that the best resolution is obtained when the source is closest to the edge of the emulsion. They were also able to show mathematically that within limits the variation in the emulsion thickness has relatively little effect on the resolution of the

FIGS. 4 AND 5

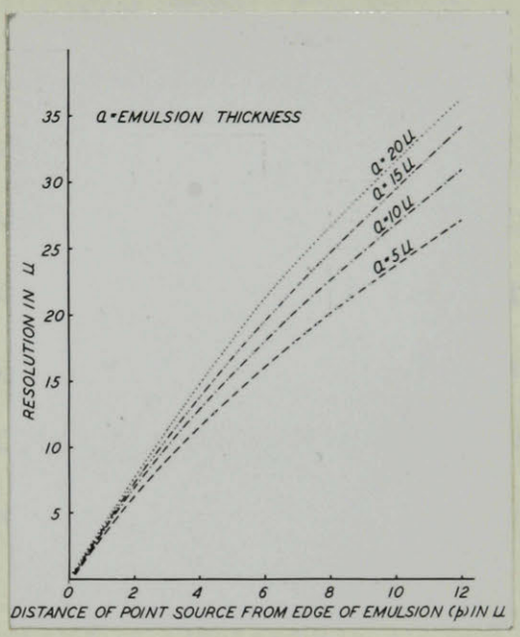


Influence of interspace on resolution and photographic density (P_D') of the autographic image resulting from a point source of radioactivity.

A series of characteristic curves showing the density in arbitrary units at various distances y in the emulsion from the perpendicular through point source (see Fig. 3), for various point sources at distances, p, from the edge of the emulsion. The dotted lines measure the resolution.

These curves illustrate how the increase in the distance p, decreases the maximum density and resolution obtainable with a point source, when the emulsion thickness is 20 μ.

FIG. 6



Influence of emulsion thickness on the resolution of a point source autograph.

Curves illustrating the relation between resolution and distance, p, of a point source from edge of emulsion for various emulsion thicknesses.

It is concluded that the emulsion thickness is of relatively minor significance compared with the distances p.

images produced by a point source as compared to the effect of the distance between the source and the edge of the emulsion. This work suggested theoretically that the reduction of the section thickness and the interspace to a minimum will give the finest resolution. The emulsion thickness plays a lesser role. The calculations involving a line source, a plane source and to a lesser extent a cylindrical source all substantiate this theory. It will be shown in the experimental section below that this theory is in fact applicable in practice. On the basis of these findings, it was advisable to adopt an autographic preparation which consists of a 5 μ section and a 10-20 μ emulsion and which is prepared by methods giving a negligible interspace (mounted, coated, and strip techniques to be described).

b) <u>The Energy and Intensity of Radiation</u>

The effect of each β-particle on the emulsion is not uniform since the photographic action of these particles is a function of their energy which decreases as they travel farther from their source.

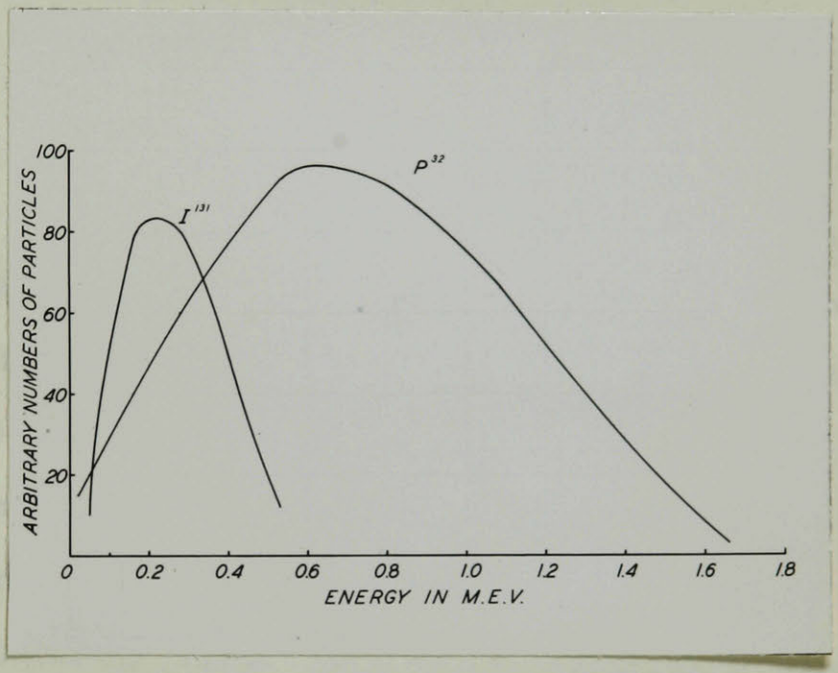
Throughout its path a β-particle loses energy by ionization and radiation (loss of photons) but the rate of these energy losses varies with the initial energy of the particle, as well as with the medium through which it moves. Bethe, Block and others (47) have derived a formula showing the relation between the rate of energy loss per unit length of path (space rate of energy loss) for a single β-particle in a medium of silver bromide. If the assumption is made that this energy loss is contributed to the formation of the latent

image, it follows that the photographic efficiency is increased with decrease in energy of the β-particle.

As the β-particle progresses and loses energy, up to some limit, its photographic efficiency must therefore increase, a fact which has been indicated experimentally by the increasing granule densities toward the ends of C^{14} β-particle tracks. Accordingly, the distribution of blackening, as shown in Figs. 4 and 5 should be modified so that the density is greater at the lateral margins of the theoretical curves. This factor will have an adverse effect on the resolution, especially with higher energy rays, for which the rate of decrease of energy and the corresponding increase in photographic activity is initially less. Thus the photographic action of P^{32} will be increased farther from the source than in the case of I^{131} (most of the β-particles of I^{131} having a lower energy than those of P^{32} (Nadler) and, therefore, a P^{32} autograph will be more diffuse than one due to I^{131} (Fig. 7) (96).

The greater spread of the images resulting from the higher energy rays is also due to their greater range. Thus, the maximum emulsion range for C^{14} particles is about 20 μ; for I^{131}, 200 μ; and for P^{32}, 1400 μ; and therefore, the possibilities of diffusion of the image are smallest for the lowest energy isotopes. The effective range, however, is less than the theoretical values cited, since the β-particles undergo some scattering and, in practice, the spread is considerably reduced by limiting the exposure.

FIG. 7

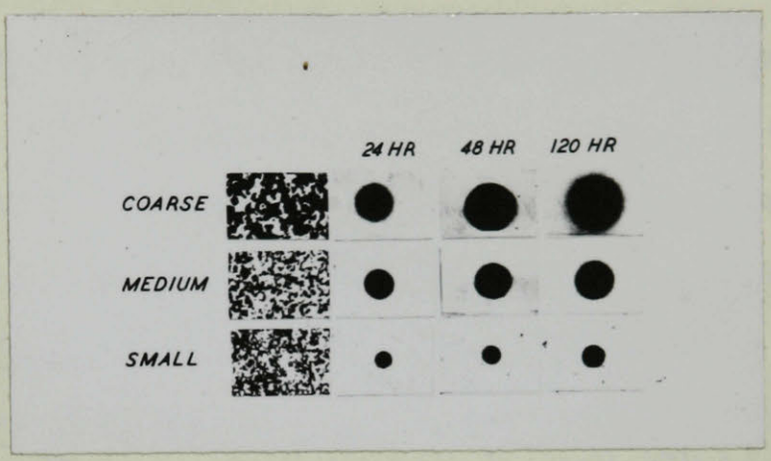


β-ray spectra for I^{131} and P^{32}. The curves illustrate the relative number of β-particles with given energy.

The Amount of Radiation ("Exposure")

The quantity as well as the quality can be shown to have a marked effect on the resolution of the autographic image. The quantity of radiation will depend on the length of time the autograph is exposed (i.e., exposure time). The density is proportional to the amount of radiation up to moderate densities only, since as fewer grains become available for development, there is a lowered statistical probability of β-particles striking unexposed grains. Accordingly, with further increase in exposure there is less increase in density. In the autographic image, this effect will be greatest in the region of maximum density and will result in a flattening of the characteristic curves (Figs. 4 and 5). The resolution should then be considerably poorer than that indicated in the curves. This indicates that low exposure of the autographic preparation is required for optimal resolution of the photographic image. This will be shown experimentally in profile sections of autographs exposed for various lengths of time (Fig. 2). Increased exposure will also result in increased fogging of the emulsion over non-radioactive areas of tissue. This same phenomenon has been demonstrated macroscopically by Boyd (15) whose results are shown in Fig. 8, from which it may be seen that the diameter of the image increases with increasing length of exposure. This increase in image size, or spread in density, will result in poorer resolution.

FIG. 8



Influence of the size of the grain and length of exposure on the size of the autographic image (composite figure made from Boyd, 15). The source consisted of radio-rubidium which acted on three kinds of emulsions identified by the size of their grams (left hand side) for 24, 48 or 120 hours.

The size of the image increased with the size of the grain, as shown by comparisons of the pictures along vertical lines, and length of exposure, as shown by comparing pictures along horizontal lines. Therefore, resolution is improved by decreasing the size of the grain or decreasing the exposure.

c) The Characteristics of the Emulsion

Grain size, grain uniformity, grain concentration, conditions of development and fixation, and fog will all affect the photographic image.

1. Grain size is obviously important. The larger the grain size, the higher is the statistical probability of its being traversed by a β-particle and the greater is the energy absorbed. Accordingly, the larger the grain the more silver atoms are reduced for the same amount of incident radiation. Thus, an increase in the grain size of the emulsion results in an increased sensitivity of the emulsion. This increase is, however, accompanied by a decrease in resolving power, as demonstrated experimentally in Fig. 8 from which it may be seen that the fine grained emulsion gave the most circumscribed image. Thus, from the point of view of localization, the finer grained emulsions are preferable.

2. Grain concentration: The greater the concentration of grains for a given volume of emulsion the greater is the maximum density obtainable. Thus, both Ilford and E.K. Nuclear Emulsions have a high concentration of silver bromide. In addition, the larger number of grains tends to increase the sensitivity of the emulsion, i.e., Ansco.

3. Grain uniformity: Broadly speaking, grains of a given size have a tendency to respond in a similar manner. Therefore, emulsions with a uniform grain size will have a high contrast. On the other hand, emulsions with a wide range in grain size will have a low contrast. In order to obtain a good resolution for a given small area, a high contrast is desirable, although it requires an optimum exposure time in order to avoid over-exposure and loss in resolution. However, a low contrast may be desirable in routine preparations with many sites of different intensities.

Conclusions:

From these considerations it may be concluded that the emulsions giving the best localization will contain a high concentration of small uniform grains, with low background fog, and will have been sensitized to the photographic action of β-particles. These emulsions must be handled and processed so as to obtain the maximum contrast and to prevent an increase in the background fog.

TECHNICAL STUDY OF THE COATED AUTOGRAPH

Materials and Experimental Animals

The tissues for histological preparations were obtained from normal or iodine-deficient albino rats of various weights.

The photographic emulsions used for radioautography were of three general types: 1) on a glass backing; 2) as a strip film; and 3) as a liquid emulsion in bulk. The first type included Eastman Medium Lantern Slide Plates, Eastman Kodak Contrast Lantern Slide Plates, available in Montreal, and Eastman Kodak NTB Plates from Rochester, New York. The second type included Ilford Half-tone Stripping Film obtained from Ilford Company, England; Eastman Kodak NTB Stripping Film distributed by Heineke Corporation of Rochester, New York; and Eastman Kodak NTB 2 prepared and supplied by Eastman Kodak of Rochester for which we are indebted to Dr. Spence and Mr. Swann. The final type, Radioautographic Ansco A, B, and C emulsion, was obtained from Ansco Corporation in Bloomingdale, New Jersey and NTB 2 pellicles from Eastman Kodak, Rochester, New York.

Eastman Kodak D-72, D-42, and D-19 developers, were the photographic developers used while Kodak Acid Fixer served as the fixing agent.

We are indebted to Mr. Engler of the Goodrich Rubber Company of New York for the supply of Geon Latex 652 and Resin Geon 20x200.

The radioactive isotopes I^{131}, P^{32}, and C^{14} were obtained from the National Research Council Atomic Energy Project at Chalk River, Ontario.

Histological Techniques

The histological procedure to be described was that used for all tissues. However, since the thyroid gland was the organ most used in this study, the detail description will deal only with that gland.

Albino rats were injected subcutaneously, intramuscularly, or intravenously with large doses of tracer NaI* or $Na_3(P^*O_4)$ in solution and were sacrificed under ether at definite time intervals after the injection. When radioiodine was used, the thyroid gland was removed by freeing the gland from the surrounding muscle and cutting the trachea below and the thyroid cartilage above the limits of the gland. The organ was then fixed for four hours in Bouin's solution(containing picric acid, formalin, and acetic acid). 10% formalin was the fixative routinely used for other tissues. It was important to use solutions that did not remove the compound being tested or did not in any way affect photographic emulsion.

The fixed thyroid was trimmed with a razor blade so that the histological section might contain cross sections of the trachea bounded on each side by one thyroid lobe and capped by the isthmus. This was then bagged in a cheesecloth square, tied with thread and suspended in dioxane solution for one hour. Immersion in six changes of dioxane for comparable lengths of time followed. The preparation was subsequently transferred for one hour to a solution of dioxane-paraffin (1:1) kept in a 70°C. oven. Four changes of melted paraffin under similar conditions were utilized for satisfactory impregnation of the tissue with paraffin. The embedding was accomplished by removing the trimmed thyroid from its

bag and placing it in a prepared dish of melted paraffin. The paraffin was then allowed to harden. This procedure was accelerated by placing the dish of slightly hardened paraffin in cold water.

Trimmed blocks of paraffin that were ready to be sectioned with the microtome were kept in ice water. This prevented the melting of paraffin and resultant distortion of the preparation during sectioning. Five and ten micron sections were used routinely.

Histological slides were prepared by coating 3 x 1 glass slides with a very thin layer of egg albumin; placing 5 to 10 drops of distilled water on the slide which was now on a heated (40°C.) levelling table; and placing the tissue section in the resultant pool. The water eventually evaporated leaving the wrinkle-free section very tightly adherent to the glass slide.

The stains most often used were Hematoxylin-and Eosin and Masson Trichrome for soft tissues whereas Safranin was favored for bones and teeth.

Chemical Techniques

In order to correlate the amount of radioactivity in the thyroid with the autograph it produced, one lobe of the gland of some of the animals was taken for Geiger counter studies. The lobe was dissolved in 10 cc. of 2N sodium hydroxide and a 1 cc. aliquot was plated with a pipette, allowed to evaporate in a 70°C. oven, and counted with a Geiger-Muller counter.

Histological slides containing radioactive sections were also placed under a Geiger-Muller counter to determine the relative amount of activity present.

Two different Geiger counters were used in these experiments. The one used was a matter of convenience at the moment. The first was a Berkeley Decimal Scaler, Model 1000, used in conjunction with a self-quenching, Tracerlab, GM tube which was housed in a closed lead castle. The second was a 64-scaler used in conjunction with a similar type GM tube housed in a Tracerlab castle with manual sample changer. The window thickness of the tube was 2.59 mg per square centimeter. The manner in which the counts per minute were calculated for each sample differed, however, owing to the difference in nature of the two scalers.

The procedure was as follows:-

The constant voltage transformer was plugged in and the power switch turned on. After two minutes the high voltage switch was turned on. After at least ten minutes to warm up the counter was ready to use. The operating voltage, characteristic of the tube in use, was determined by ascertaining its plateau curve. For this determination a standard uranium sample plate is used. The plateau region is one in which the voltage has little effect on the count obtained for any constant sample. In this laboratory all GM tubes are operated in the lower half of their plateau, since the plateau shortens from the higher voltage downwards as the tube breaks down. The plateau need only be checked about every ten days to two weeks for a tube in good working condition.

Following this, all counts were made in the same way. In the decimal counter each plate was counted for a whole number of minutes as shown by a stop watch; the time was determined by the speed of the count. At least 1000 counts were counted and the counts per minute calculated directly by dividing the registered total count by the total time of counting. For the 64-scaler, however, since each registered count

represents 64 true counts, all counts were multiplied by 64 and divided by the total number of minutes of counting in order to obtain the counts per minute of the sample in question.

A background count was made at the start and at the finish of each series of counts made. This count is due to cosmic radiation and spontaneous discharges in the GM tube. Rise in background is due either to contamination or a breakdown of the tube. The count was made by placing a clean glass plate under the tube window and obtaining the count registered. The plate used was identical with those on which the radioactive samples were plated. As the background is relatively low (about 15 to 20 counts/min. for these counters) it is customary in this laboratory to count the background for at least ten minutes. The counts/min. so obtained were then subtracted from the total counts per minute for each sample. This gave the net or true counts/minute.

Owing to high coincidence losses at and above a counting rate of 10,000 counts/minute (losses are 5% and greater), no plates were used that registered a count greater than about 6000/minute. Where a faster rate was found a smaller aliquot was replated and recounted.

A standard uranium sample was counted before and after each series of counts made in order to check the steady operation of the counter.

Duplicate standard I^{131} samples were plated from a known suitable dilution of a known amount of the injected solution. From the count obtained on these plates, the original injected dose per animal was calculated. The use of these standard plates provides a convenient method by which to compare all counts with the injected dose. The amount of decay that has occured at the particular time of counting can be then

disregarded.

The reason for stipulating 1000 as the minimum number of total counts to be made on each sample, arises from the variation in the probable error of counting with the total number of counts made. The probable error can be shown to be equal to $\pm \frac{k}{\sqrt{m}}$ where K is a value proportional to the desired probability and m is the total number of counts made on the sample. In this laboratory 95% probability is used for which it has been calculated that k has a value of 1.960. Now if we allow an error 6.2% it can be calculated from the above formulae that m equals 1000 counts. It can be seen that as the total number of counts made increases then the probable % error falls and vice versa. The actual error in the net counting rate can then be calculated as $\sqrt{(\text{background error})^2 + (\text{sample error})^2}$.

I. Experiments to Perfect the "liquid" Coated Technique

A. The "coated technique" as described by Bélanger and Leblond (9), Percival (85) and Leblond et al. (68) suggested the use of liquid emulsion. The only source of liquid emulsion that could be applied at that time was obtained from Eastman Kodak Medium Contrast Lantern Slide plates (henceforth referred to as Medium emulsion plates). It was deemed feasible in this investigation to try new experimental emulsions as they appeared on the market. It was necessary to examine the method of preparation for coating of the individual emulsions, to determine the relative speeds of each emulsion, and to compare the quality of the autographs obtained. Several of the steps described in the method were reinvestigated in an effort to improve the existing techniques.

The studies were investigated under the following headings:

1. Preparation of Liquid Emulsion for Coating.
2. Amount of Emulsion required.
3. Spreading of Emulsion.
4. Drying and Storage of Slides during Exposure.
5. Development and Fixation of Exposed Slides.
6. Dehydrating and Mounting of Autographs.
7. Comparison of Autographs made with Different Emulsions.

1. Preparation of Liquid Emulsion for Coating

The emulsions used most frequently for routine studies in this Laboratory were medium emulsion, Radioautographic Ansco A, and Ilford Half-Tone Stripping Film. This section will, therefore, be described in the order of the amount of experience with each type. The emulsions studied were as follows:

(a) Medium Lantern Slide Plate Emulsion.

(b) Thinned Emulsion of Medium Plate Emulsion.

(c) Liquified Ilford Half-Tone Stripping Film.

(d) Radioautographic Ansco Emulsions.

(e) NTB Plates.

(f) NTB_1 Stripping Film Liquified.

(g) NTB_2 Pellicles.

(a) Medium Lantern Slide Plate Emulsion was prepared for coating by the method of Leblond, Percival, and Gross, i.e., Medium Emulsion Plates (3 1/4 x 4") were allowed to remain in distilled water at 20°C. for 10 minutes after which time the emulsion was sufficiently softened (it had absorbed water) so that it could be scraped from the plate into a 50 cc. beaker with the aid of a glass slide. The beaker was then placed in a 37°C. water bath and after 15 minutes the emulsion was sufficiently liquid to be applied to the histological slide. Each emulsion plate supplied enough to coat six slides.

(b) To test the effect of Thin Emulsion (See Fig.2) as postulated by theory (Pelc,88 and Nadler,47),Medium emulsion was prepared as above. When completely liquified, 7 cc. of distilled water and 1 cc. of 1% dupanol solution were added for every 2 cc. of emulsion. In this way the emulsion was diluted five times. The solution was then allowed to remain for another 10 minutes in the water bath before it was used. During this time a homogeneous solution was obtained. One emulsion plate could then supply enough emulsion to coat 30 slides.

By varying the ratio of emulsion to water, it was possible to obtain emulsions of various dilutions. When the coating dried, however, the concentration of silver bromide in the gelatin was the same as that of undiluted emulsion.

(c) It has also been possible to liquify Ilford Half-Tone Stripping Film Emulsion. The stripped strip-emulsion (See Strip Autograph) was placed in distilled water as in a) and was found to liquify homogeneously after 15 minutes in a warm water bath.

Several attempts were made to liquify the stripping film without presoaking. The film was cut into pieces small enough to fit into a 50 cc. beaker and was warmed as above. The melting was not found to be uniform. Furthermore, the cutting of the emulsion resulted in a slight increase in the amount of background fog. This method was therefore abandoned.

By adding 1% dupanol as in b), a sample of thinned Ilford emulsion was easily prepared.

(d) The only type of emulsion which did not require soaking prior to liquefaction in a water bath was Ansco X-ray emulsions. The three types of emulsion, A, B, and C, are supplied in the gelled state. Therefore, by using a glass slide to scoop a small quantity of emulsion from the container into a 50 cc. beaker placed in a warm water bath, after 15 minutes liquid emulsion was available for coating. This emulsion is understandably more concentrated and therefore more viscous than the ones prepared by presoaking. However, it is still found to flow freely.

(e) With the development of Nuclear Track Beta Emulsions, which were made specifically for detecting β-radiations, NTB Plates became available in 1948. These plates consisted of 3" x 1" glass slides coated with nuclear type emulsions of approximately 100 μ thickness. It was possible to liquify this emulsion in the same manner as in a) above. One plate supplied emulsion to coat one slide.

(f) NTB emulsions have also been prepared as Strip Emulsions on a celluloid backing (NTB_1) and on a glass plate backing (NTB_2). (See section on Strip Autographs for details of stripping of emulsions). Different lengths of time were used for soaking the NTB_1 emulsion (25 μ thick, 4" x 5") for the softening process. In order to obtain sufficient quantities of

liquified emulsion in the beaker placed in the water bath, it was found necessary to presoak the emulsion strip for at least 30 minutes. This type of strip emulsion was supplied with a celluloid backing and to which it was intimately attached. Since the celluloid did not dissolve in the warming process, much of the melted emulsion still adhered to it resulting in a considerable loss of emulsion. By using 1% dupanol solution in the distilled water, more of the emulsion was made available. The celluloid was removed from the beaker prior to coating, with as little agitation to the dissolved emulsion as possible. It was found that one strip (4" x 5") yielded sufficient emulsion to coat approximately two slides.

(g) NTB2 Emulsion has also been prepared as pellicles, that is, concentrated emulsion (250 μ) with no backing. The pellicle (4" x 5") is fairly brittle and since it is very concentrated (loaded with silver bromide) and thick, 10 minutes in 20°C. water was found to be insufficient. After 10 minutes the emulsion was limp but showed little signs of liquifying completely even after 30 minutes in the 37°C. water bath. When water was added to the beaker, the emulsion slowly began to liquify but strands of thickened undissolved emulsion tended to clog the medicine dropper even after one hour.

The soaking process was repeated using 1% dupanol solution instead of water for one hour. This procedure, however, did not improve the complete liquefaction to any extent.

Summary

1. All except Ansco emulsions required presoaking for for complete and homogeneous liquefaction.

2. The optimum temperature for soaking in distilled water was found to be 20°C.

3. All emulsions were liquified at 37°C. Eastman Kodak Contrast Process Plates and NTB 2 pellicles gave a stringy emulsion when liquified and could, therefore, not be used.

4. (a) Ansco radioautographic emulsion has been the simplest to prepare for coating while

(b) medium plate emulsion and Ilford stripping emulsion, although requiring more skill and time, gave very satisfactory results.

(c) The most cumbersome and wasteful preparations were with the strip emulsions on a cellulose acetate backing which did not dissolve with heat.

5. Thinned emulsion was easily prepared by diluting the melted emulsion with 1 cc 1% dupanol (a drying agent) and water.

6. A wratten One (Red) safelight at a distance of at least three feet was found to give sufficient light by which to work and yet have no noticeable effect on any of the emulsions with the possible exception of Ansco emulsions. It was, therefore, decided when using Ansco and working fairly close to the light

source to use a Wratten Three (Dark Green) safelight, or to use a red light suspended from the ceiling at a distance of at least six feet. If the emulsion is allowed to remain closer to the safelight, fogging will result. This has also been observed by Percival.

2. Amount of Emulsion Required

Percival (85) and others (68) suggested the use of five drops of emulsion to cover all but one-half inch of the surface of the histological slide. The uncoated portion allowed the experimental markings on the slide to remain visible. During the three years in which this work was carried out it was realized that this was a somewhat wasteful procedure since it was only rarely that the histological section occupied more than one square inch of slide. Furthermore, in order to standardize the thickness of the emulsion coat applied, it was considered advisable to define an exact area (usually one square inch) on the slide with a diamond pencil. This served two purposes. First, it was now possible to deliver an exact amount of emulsion to a definite standard area. Secondly, since a subsequent step in the technique required the emulsion to flow from side to side, the diamond pencil markings not only kept the emulsion within the bounded limits but also made for a more even flow.

In order to be sure that all the preparations were being coated with equivalent amounts of emulsion, the same medicine dropper was used for all the coatings. It was argued that if all the air were expelled from the dropper and the dropper then filled to its capacity with emulsion, the first two drops used per slide in every case should yield the same amount of emulsion for each specific type of emulsion. This procedure was therefore adopted.

It has been found possible to use one drop of emulsion per square inch of slide. However, this relatively small amount did not always give a perfectly even film especially with such thick emulsions as Ansco. This, too, was due to the fact that the emulsion dried easily at room temperature, and, therefore, required extremely rapid manipulation. This amount of emulsion was used only when very thin emulsion films were desired and is not suggested as a routine.

3. Spreading of Emulsion

The evenly coated slides were put on the warm side of a leveling table* (See Fig. 9). This heat allowed the emulsion to remain liquid and the leveling table insured a final even spread (warm side) and gelling (cool side). The effect of time during which the slides were left on the warm side of the leveling table was investigated to determine the optimum warming time. Twelve slides were coated with medium plate emulsion according to the method of Leblond et al. (68) and allowed to remain on the 37°C. side of the leveling table for different intervals of time (0-5 minutes). The developed autographs were examined under the microscope for fog granules. The amount of fog granules present in the autographs left for 0 minutes on the warm table was taken as the control and given three plus signs. If the number of black silver granules per field increased to twice the number of these in the controls, nine plus signs were given. An intermediary number of plus signs were assigned to the autographs containing more background fog granules than the controls but less than twice of these in the controls. The results as seen in Table I indicated that there was no apparent increase in the amount of fogging with the length of stay on the warm plate for these time intervals.

It was noted, however, that when the emulsion was allowed to dry on the cool side of the table following the warming

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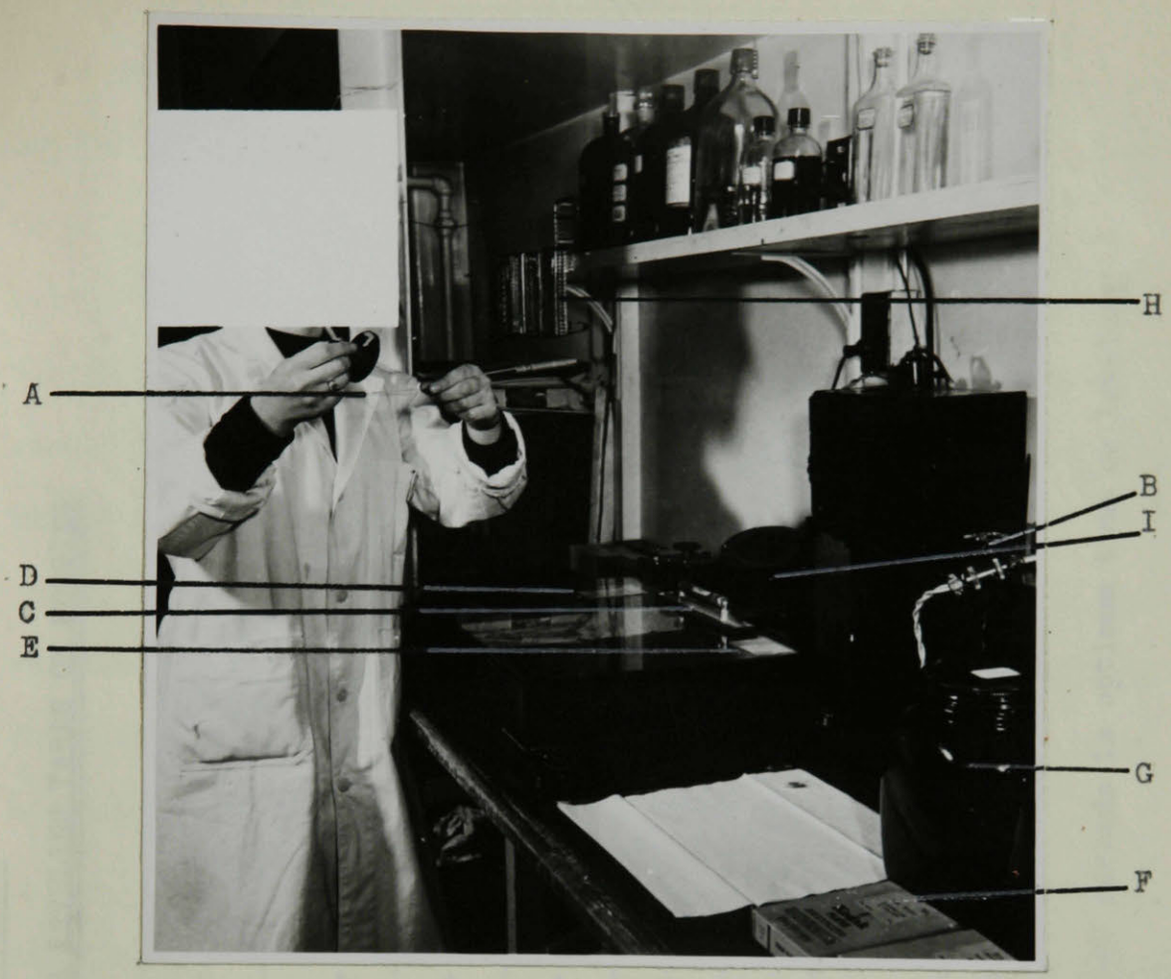
*The leveling table consists of a glass top, with a heating element under one end, adjusted to give a temperature of 37°C. The whole is set on leveling screws and made horizontal with the aid of a spirt level.

FIG. 9.



Author in the process of preparing a liquid coated autograph in a photographic darkroom. Note

A. Two drops of emulsion from a medicine dropper are being placed on a histological preparation
B. Beaker containing melted emulsion, in a constant temperature water bath (37°C)
C. Leveling spirit
D. Preparations left on the warm side of the leveling table for 30 seconds after coating to insure a uniform spread
E. Liquid coated autographs drying on the cool side of the leveling table
F. Labeled box in which to store autographs
G. Labeled Jar in which to store boxes
H. Slide carrier in which autograph preparations are placed during the development process
I. Wratten Series 1 Safelight

TABLE I

THE EFFECT OF THE WARM LEVELING TABLE ON FOGGING

Table at 37°C.	Fogging at 0 seconds	Fogging at 15 seconds	Fogging at 1 minute	Fogging at 5 minutes
Slides 1-4	+ + +	+ + +	+ + + +	+ + + +
Slides 5-8	+ + +	+ + +	+ + + + +	+ +
Slides 9-12	+ + +	+ + + + +	+ + +	+ + +
	0 seconds	15 seconds	1 minute	3 minutes
Appearance of emulsion coat	Uneven sometimes Occasional bubbles	Smooth even coat	Smooth even coat	Thick blob of emulsion in middle of slides Uneven drying

Conclusion: (1) No significant increase in the amount of fog with the length of stay of autographs on warm leveling table

(2) For a smooth even coating, 15-30 seconds is optimum time on leveling table

procedure, the emulsion dried leaving a smooth even film only in those slides which had remained for 15 seconds or one minute on the warmed table. When the coated slides were unheated prior to drying the emulsion film was not always even and sometimes contained bubbles; whereas when the slides were allowed to remain for three minutes or longer on the warm table, the emulsion dried rapidly from the periphery leaving a thick blob of emulsion in the middle of the slide.

The most convenient warming time for technical reasons was taken as being 30 to 60 seconds, since another preparation could be coated during that interval, thereby avoiding any loss of time.

To test the evenness of the coated film, profile sections of autographs of 5 and 10 μ thyroid sections coated with undiluted and diluted medium plate emulsion were made. Celluloid, obtained by an X-ray film soaked free of its emulsion, was used as a base on which histological tissue sections were mounted. It was necessary to make the base adhere to a glass slide so that the preparation could subsequently be coated evenly. Both household cement and celluloid dissolved in acetone to form a paste were used. Unfortunately, neither of these were completely satisfactory. In many cases little ridges which were obviously undesirable were formed in the celluloid. The developed autographs, mounted on the base, were separated from the glass slide and the whole preparation was again embedded. Cross-sections of the autographs were then prepared.

This whole procedure was repeated using dialysing paper instead of celluloid as a base. A clean glass slide was eased into a dialysing sac envelope which was then smoothed out. The section was mounted on this, coated, exposed, and developed. The whole preparation was then separated from the glass slide, embedded and cut as above.

The cross-sections of autographs were examined and the relative thicknesses of emulsion, celloidin base, and tissue sections were measured. Fig. 2 C is a photograph of a profile section of a 5 μ thyroid tissue coated with thin emulsion, while Fig. 2 A is one of a section coated with undiluted medium plate emulsion.

Because of the uneven surface caused by the celluloid and dialysing sac, the coat of emulsion obtained was not as uniform as was normally obtained in practice. Nevertheless, in general, the emulsion coat per slide was fairly uniform with an approximate thickness of 20 μ for two drops of emulsion and 30 μ for three drops; the celloidin coat was less than 1 μ thick; and the tissue section 4.5 and 9.5 μ thick. Table II lists the measurements taken of some of the sections thus obtained. An ocular micrometer was used for all the measurements. The slight increase in the emulsion thickness over reactive centers was accounted for by the fact that unreduced silver bromide was dissolved out from the emulsion by fixation whereas the reduced metallic silver remained over the reactive areas.

TABLE II

MEASUREMENTS OF THICKNESSES OF EMULSION AND SECTIONS OF PROFILE SECTIONS OF COATED AUTOGRAPHS

	Section	Emulsion	Reaction area
Autograph No. 1	3.0 μ	21.1 μ	
	4.8 μ	22.1 μ	
(2 drops emulsion)	4.3 μ	20.4 μ	x
	4.8 μ	17.5 μ	
	5.0 μ	18.0 μ	
	4.5 μ	18.7 μ	
	5.0 μ	18.7 μ	x
	6.8 μ	18.7 μ	x
	3.0 μ	17.5 μ	
	4.8 μ	20.4 μ	
	Av. 4.6 μ	Av. 19.5 μ	
Autograph No. 2	5.0 μ	18.7 μ	
	4.5 μ	20.4 μ	
(2 drops emulsion)	5.0 μ	20.4 μ	
	3.5 μ	22.1 μ	x
	3.5 μ	20.4 μ	x
	6.8 μ	18.7 μ	x
	Av. 4.4 μ	Av. 20.1 μ	
Autograph No. 3	3.4 μ	34.0 μ	
	3.4 μ	32.3 μ	x
(3 drops emulsion)	5.0 μ	28.9 μ	
	5.0 μ	29.8 μ	
	5.0 μ	30.0 μ	x
	5.0 μ	31.7 μ	x
	4.5 μ	27.2 μ	
	4.8 μ	27.2 μ	
	Av. 4.5 μ	Av. 30.1 μ	
Autograph No. 4	3.4 μ	34.0 μ	
	5.0 μ	34.0 μ	
(3 drops emulsion)	3.4 μ	34.0 μ	
	3.4 μ	34.0 μ	
	5.0 μ	34.0 μ	x
	5.0 μ	32.0 μ	
	4.0 μ	32.0 μ	
	Av. 4.1 μ	Av. 33.4 μ	
Autograph No. 5	8.5 μ	32.0 μ	x
	8.5 μ	35.0 μ	x
(3 drops emulsion)	9.5 μ	30.0 μ	x
	Av. 8.8 μ	Av. 32.3 μ	
Autograph No. 6	9.0 μ	37.0 μ	x
	11.5 μ	30.0 μ	x
(3 drops emulsion)	8.5 μ	35.0 μ	
	11.0 μ	37.0 μ	x
	Av. 10.0 μ	Av. 34.5 μ	

4. Drying and Storage of the Slides during Exposure

It had previously been suggested that after 15 minutes on the cool side of the leveling table the emulsion was sufficiently gelled so that the slides could be stored for exposure. It was found, however, that in preparations containing a very large amount of radioactivity, an autograph could be obtained as early as one hour after coating. This meant that at 15 minutes after coating the emulsion already recorded the effect of a considerable amount of the radiations emitted. Any slight movement of partly dried emulsion during the transfer of the slides to the site of storage would necessarily result in a displacement of emulsion and an obvious distortion in the results. Furthermore, this slight movement could also make for an uneven film when the emulsion finally dried.

Fifteen minutes was found to be sufficient drying time for thinned emulsion only. Medium plate emulsion and melted Ilford emulsion required up to one hour whereas Ansco and NTB pellicles required longer times.

Temperature and humidity obviously played an important role in the drying process. In order to keep these factors constant, it was decided to install an air-conditioning unit in the darkroom used for autography. The room temperature was kept at 68°F. and the air fairly dry (about 60% humidity). In addition to being the best condition for the preparation of autographs, it was also the most comfortable conditions under which to operate.

To test the effect of temperature on autographs made with different dilutions of radioactivity, six pieces of filter paper one square centimeter were moistened with one drop each of P^{32} solution.

a) Two pieces each contained 3,000 counts

b) Two pieces contained 300 counts

c) Two pieces each contained 30 counts

One piece of a, b, and c were placed on a medium plate lantern slide and covered with a glass plate in a pressure cassette. The cassette was allowed to remain for 24 hours at 25°C. The remaining three pieces were treated as above but kept at 6°C. in the icebox.

When the two plates were developed both showed equal intensities of blackening. There was no apparent difference due to temperature, during this short length of time.

It was found by experience, however, that when autographs are stored at room temperature for any length of time the amount of background fog increased progressively with time. When the autographs were transferred immediately after preparation to a coldroom, the amount of background fog did not increase considerably during exposure. This strongly advised the storage of slides at reduced temperatures, especially for long exposure periods.

The effect of <u>moisture</u> on the intensity of the photographic image was examined with both Medium Lantern Slide Plates and Contrast Process Plates. One Medium Plate and one Process Plate were soaked

in water for several minutes. Each of the two wet plates and two dry plates were then exposed for three-quarter seconds to white light from a photographic enlarger equipped with a wedge. After development and fixation, it could be seen that the dry image was 50% more intense than the one obtained with the soaked plate.

This phenomenon was similarly tested using twelve coated histological slides of which six were stored in a phosphorus pentoxide atmosphere while the remaining six were stored without a drying agent. All the slides were kept at 0°C. for two days after which time they were developed. The autographs stored with phosphorus pentoxide all displayed sharper images than the controls. It was also established that P_2O_5 as well as calcium chloride did not inhibit the sensitivity of emulsion. It was, therefore, decided to store autographs in a dry atmosphere.

Previous to the time when a drying agent was used, slides were stored in slide boxes wrapped in three layers of heavy black paper and sealed with cellulose tape. The individual boxes were then stored in a coldroom. It was necessary to devise a system whereby slides could be kept in a dry atmosphere and yet be easily accessible for development. This was accomplished by painting ordinary candy jars inside and outside with several coats of white and black paint. These jars were then tested for light leaks and were found to be light-proof. To insure the fact that the slides would not become exposed to light of any sort in case

any of the paint from the jars chipped, Eastman Kodak M Plate boxes were lined with corrugated paper to be used as slots for the autographs. These boxes, known to be light-proof, were labeled as seen in Fig. 9, giving detail as to contents, relative count, data of coating, and signature of the operator. The boxes were then arranged so that the slides would sit horizontally in the light-proof jars which were numbered. Each jar contained calcium chloride confined in a small cosmetic jar with a perforated lid. The black jars were then stored in the coldroom at 0°C. Later it was found advisable (106) to store the preparations at even colder temperatures which prompted the use of a deep freeze (-10°C.) for storage.

A sample of the records kept of autographs is seen in Table III. Using this system of recording, the exact whereabouts of every autograph made was known.

TABLE III

AUTOGRAPH RECORD SHEET

Box Number	Experimental Number	Particulars	Run by	Number of slides	Number of Jar	Date of Coating	Development	Completed
1	1001	I^{131} stained thyroids; 200 cts.; Ansco	J. Doe	10	1	Jan. 1	Jan. 10	
2	1001	Unstained thyroids; 200 cts.; Ansco	J. Doe	10	1	Jan. 1	5-Jan. 4; 5-Jan. 10	
1	1002	P^{32} stained bones; 1000 cts.; medium emulsion	D. Jones	15	2	Mar.15		
2	1002	Unstained bones; 1000 cts.; medium emulsion	D. Jones	15	2	Mar.15	5-Apr. 15;	

5. Development and Fixation of Exposed Slides

The development of exposed slides is of the same importance in autography as in routine photographic procedures and similar techniques are used in ascertaining the proper development time. Two minutes in D-72 developer was recommended as giving the most satisfactory results. In regard to development time, as development progresses, the resolving power rises to a maximum, drops slightly, then remains approximately constant (76). However, overdevelopment will cause a progressive darkening of the whole emulsion.

Practically, the most satisfactory development time is the time sufficient for the developer to reach the deeper layer of the emulsion, thereby developing all silver grains due to radiation. Such a minimal development keeps the background fog down to a minimum. For autographs of average emulsion thickness, one and a half minutes has been adopted for development time. This time may be shortened to one minute when thin emulsions are used.

A rigid temperature control was found to be essential since the speed of the development action follows a curve which rises as the temperature becomes higher. The temperatures of all the solutions, then, were kept below 20°C. Higher temperatures caused the emulsion to melt or develop bubbles. Any melting could result in a displacement of the emulsion with the areas of reaction moving away from the radioactivity sites.

After rinsing in clear water for a few seconds, the autographs were fixed. Ten minutes were usually allowed for fixation but it was found that since the quantity of silver bromide was relatively small due to the thin coats of emulsion, fixation for six minutes was found to be sufficient to remove all the unactivated silver.

Following fixation the slides were washed in cold running tap water for at least 15 minutes. This removed all the fixative, thereby preventing precipitation of hyposulfite crystals on the autograph.

The displacement phenomenon was a serious factor to consider. It could arise through the melting of the emulsion as already mentioned, or could occur during processing because of the gravity pull when the slides were held vertical. To control this as much as possible, the slides, while being developed, fixed, and washed, were always kept horizontal so that any swelling in the emulsion would not shift it in a downward direction.

If mass production was to be the rule, some carrier arrangement which would allow the slides to remain horizontal was indicated. This was easily arrived at by using a hand-made slide carrier which could accomodate 16 slides. The slides were fixed in their position by two elastic bands embracing the carrier which was then allowed to journey through the various developing and rinsing solutions always remaining on its haunches, so to speak. (Fig. 9).

6. Dehydrating and Mounting of Autographs

At the time when the coated method was first described, the autographs were allowed to dry at room temperature after they had finished being washed. When they were completely dry they were preserved by mounting with a drop of Canada Balsam and a glass cover slip. Upon examination, the preparations were often seen to contain artefacts. The drying apparently also dried the tissue section which would then appear to contain numerous dark irregularities. This type of artefact was sometimes mistaken for an autographic reaction and had to be avoided.

To remedy this, autographs removed from wash water were immediately dehydrated in 95% alcohol for three minutes, in absolute alcohol for three minutes, in absolute alcohol-xylol (1:1) for three minutes, followed by three three-minute changes of xylol. The treatment with xylol reduced the incidence of artefacts. These were further reduced by allowing the autographs to remain for 24 hours in a balsam solution diluted with xylol. Depending on the condition of the tissue section, the preparation was removed from this solution and the excess balsam was allowed to drain off. Two drops of the balsam solution used as mounting medium were allowed to flow directly over the autograph covering the tissue section. The slide was then lowered to meet the waiting coverslip on the edge of the table. This resulted in an even, bubble-free spreading of the balsam between the two layers of glass. This final bathing in balsam was the last step in the preparation and preservation of the autograph.

7. Comparison of Autographs Made with Different Emulsions (See p. 57)

Since a liquid coated autograph could be prepared from any one of several types of emulsion, it was considered that a comparison study using this technique with each of the various emulsions would provide valuable information for future routine preparations. To obtain histological material with comparable amounts of radioactivity per unit area of tissue, the thyroid gland of a normal adult male albino rat injected with I^{131} and sacrificed 6 1/2 hours after the injection, was fixed in Bouin's solution. Histological sections were stained with hematoxylin and eosin. Twenty-two slides were counted with the Geiger counter and were found to contain comparable amounts of radioactivity.

a) Four of the slides were coated with medium emulsion.

b) Four were coated with melted Ilford Emulsion.

c) Four were coated with thinned melted Ilford Emulsion.

d) Five were coated with melted NTB_1 strip emulsion.

e) Five were coated with Ilford Stripping Emulsion (See section on Stripping Plates).

Considerable numbers of coated autographs have been prepared using Radioautographic Ansco A Emulsion. At the same time that many of these were done, control autographs using medium emulsion were also prepared. It was, therefore, possible

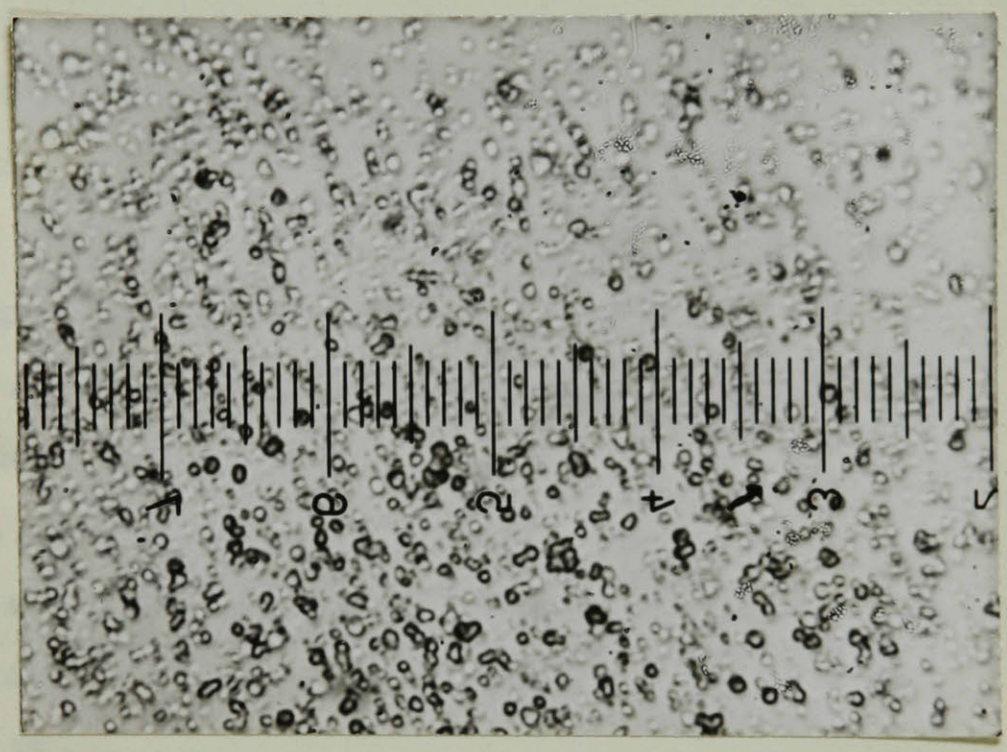
to include the results obtained with Ansco A in the above study. The autographs were examined under the microscope for sensitivity, grain size, resolution of the emulsion, amount of background fog present, retention of stain, and artefacts. These factors were studied at both low and high magnification.

Arbitrary values were given to these factors. For resolution, minute cellular localization that could be distinguished at 900 diameters (i.e., blackening could be attributed to a definite area in the cell) was given a resolution value of A if no background fog was present. B was given if fog granules interfered in some cases while C denoted good cellular localization (i.e., blackening could be attributed to a particular cell at 400 diameters). When autographic reactions could be associated with particular follicles but with no particular cells in that follicle at 400 diameters, the value given was D. Only cellular or "ring" reactions, and not colloid reactions, were used for this criteria. If a magnification of not more than 100 diameters could be used to examine the preparation, the value given was E. In this case, although one colloid reaction could easily be distinguished from a neighbor colloid reaction, at a magnification higher than 100 diameters, no association of autographic reaction and tissue structure was possible due to high amounts of background fog.

The sensitivity of the emulsion as used in these experiments was determined by the length of exposure time that was necessary to give an autographic reaction. If less than one half-life of exposure was necessary, six plus signs were assigned. (Half-life refers to the period of time it takes for an initial amount of radioactivity to have disintegrated so that only half of that initial amount of radioactivity remains. The Half-life of I^{131} is eight days). If two half lives were necessary, three plus signs were used while exposures for the duration of six half-lives were given one plus sign. Although these values are at best very crude, they may still serve as guides for subsequent routine work.

The grain size was determined by examining under the microscope the size of developed and undeveloped silver grains of each emulsion. Nine hundred diameters was the magnification used. The mean diameter of a silver bromide grain (Medium plate emulsion) was determined as 0.4 μ. The experiment consisted of microphotographing (green filter, 2300 x) an emulsion coated slide. This was further enlarged six times. Four random lines were drawn on the print and the average diameter of all grains (24) touched by the lines were measured. (Fig. 10 is a print of the negative before

FIG. 10



Undeveloped silver bromide granules from an autograph coated with medium lantern slide plate emulsion. x 2300

it was enlarged).

The amount of background fog was determined by the number of developed silver grains over a non-reactive unit area (one microscope field). The emulsion with the least amount of fog was given one plus sign. Two plus signs were given for twice that amount of fog and so on.

If none of the original stain was removed during the autographic procedure, stain retention was given a value of A or excellent. If the intensity of the stain was reduced but still gave good differentiation, C or good was assigned. If practically all the stain was removed, E or poor was assigned.

A summary of the results of these studies is seen in Table IV .

To compare the various radioautographic Ansco emulsions, six coated autographs were prepared using Ansco A (two slides), B (two slides), and C (two slides). All the slides were exposed to white light from a photographic enlarger equipped with a wedge and then were routinely developed and fixed. It was found that Ansco A was the most sensitive, while B was the least sensitive. The background fog seemed to be more pronounced in Ansco B but this may have been a property of the batch only. The grain size and

TABLE IV

COMPARISON OF AUTOGRAPHS MADE WITH DIFFERENT EMULSIONS

EMULSION	NO. OF SLIDES	RESOLUTION	FOG (x 100)	FOG (x 400)	GRAIN SIZE	SENSITIVITY	STAIN	ARTEFACTS
Medium Emulsion	4	D	+ + +	++ + + + +	Medium(0.4 μ)	+ + +	D	Emulsion stippled in one case
Thinned Liquid Emulsion		B	- -	- -	Medium(0.4 μ)	+ + +		
Liquid Ilford	4	C	+ +	++ +	Small	+	C	Tissue dried in one case only
Thinned Liquid Ilford	4	B	+	+	Small	+	D	Some cells dry; blobs in emulsion
Thinned Liquid NTB_1	5	B	+	+	Small(0.2 μ)	+	D	Tissue dried in all cases; background contained black accumulations
Thinned NTB_2		B	+	+	Small(0.2 μ)	+ +		
Ilford Strip Film	5	C	+ +	+	Small	+ +	D	Colloid appeared black in some
Ansco A		E	+++++	+++++++++ +++	Large	++++ ++++	D	

+++ Noticeable amount of fog
++ Fair amount of fog
+ Very little fog

concentration appeared somewhat comparable for the three types. Since Ansco B and C did not seem to offer any advantage over Ansco A emulsion, the two types were never used in this Department for routine autographic work.

Summary of Results

1) NTB_1 diluted emulsion offered the best resolution under low and high powers. Diluted melted Ilford Strip Emulsion was next best, followed by melted Ilford Emulsion, Ilford Strip Emulsion, and medium plate emulsion. Ansco Radioautographic emulsion offered the poorest resolution of the group.

2) Ilford Stripping Emulsion had the smallest grain. Ansco Emulsion had the largest and coarsest grain. The medium emulsion grain was intermediate. NTB Strip Emulsion grain was only slightly larger than that of Ilford Emulsion.

3) Ansco radioautographic emulsion was the most sensitive. Medium emulsion was next and Ilford Strip Emulsion followed. Melted Ilford Emulsion and NTB_1 melted emulsion were roughly equally sensitive while diluted Ilford Strip Emulsion was slightly less sensitive.

4) Ilford Stripping film and NTB_1 emulsion had the greatest number of grains of silver bromide per unit area while Ansco Radioautographic Emulsion had the least.

5) Background fog was least in diluted melted Ilford, Ilford Stripfilm, and melted NTB_1 emulsion. It was slightly more pronounced in Ilford Emulsion and greatest in Ansco Radioautographic Emulsion. Undiluted medium emulsion contained less than Ansco but more than Ilford.

6) Celloidin was used as a protective coat, however, the amount of stain removed from the sections was approximately the same in all cases.

7) Most artefacts occurred with diluted melted NTB_1 Stripfilm emulsion. The tissue appeared black in areas probably due to drying. In no cases was the emulsion entirely free of dark areas or dirt.

Discussion

It was suggested by theory (106) that emulsions containing the smallest grain size and the greatest number of grains would offer the best resolution. This is in fact true since both Ilford Half-Tone Stripping and NTB_1 Emulsions both contain very small grains (0.1 μ - 0.2 μ) as well as being "loaded" with silver bromide granules and both offered the best resolution of the emulsions tested. Conversely, radioautographic Ansco A Emulsion, with the largest grain size, offered the poorest resolution.

It has also been shown that the sensitivity of a photographic emulsion containing large silver bromide grains is greater than that of an emulsion containing smaller ones, if no special "sensitizer" has been added. This was found to be the case for the emulsions tested. Radioautographic Ansco A with the largest grain was by far the most sensitive while Ilford Half-Tone Stripping Emulsion with the smallest grain size was the least sensitive. Because NTB_1 emulsion contained a "sensitizer" it was slightly more sensitive regardless of the small grain size.

The background fog was found to be least in thin emulsions. As will be explained again in a following section, this was due to the fact that fog is distributed evenly throughout an emulsion. The autographic reaction, however, is most intense in certain areas in the emulsion close to the tissue. The uppermost layer of emulsion which is not present in thin emulsions does not contain any autographic reaction but does contain fog granules. Therefore, a thinner emulsion will give an autographic reaction almost or as intense as a thicker emulsion but the background fog will be much less in the former case. This has been shown to be true in these studies.

Suggested Uses for Each Type of Emulsion

As a result of the conclusions reached by this study, it was possible to suggest the use of some emulsions for specific purposes.

1) Until more work is done with the newer types of emulsion, control slides should always be run with Medium Emulsion. Medium emulsion may be used for slides containing medium activity.

2) Melted Ilford Stripfilm (coated as such or diluted with dupanol) may be used for non-quantitative work with tissues of fairly high radioactivity to obtain good localization.

3) Diluted NTB1 emulsion may be used for very fine localization. If many slides are coated using this emulsion, the unreactive parts (artefacts) will be recognized as such and proper interpretation will be possible. Soaking the film in dupanol instead of in water may prevent this and other types of artefacts.

4) If the amount of radioactivity in a tissue is too low to warrant use of any of the above, Ansco Radioautographic Emulsion A should be used.

8. Description of the "Liquid Coated" Technique

As a result of these studies on the autographic technique, the following steps are those followed in this Laboratory in the preparation of liquid coated autographs. All the steps marked with an asterisk are carried out in the darkroom. Fig. shows one of the steps in the preparation.

*1. A. Medium Emulsion Plates are soaked in distilled water for 10 minutes at 19-1°C. This allows the emulsion to swell sufficiently so that it can be scraped with a histological slide into a 50 cc. beaker.

B. If liquid emulsion is obtained from the manufacturer (e.g., Ansco A Radioautographic Emulsion) the emulsion is scooped into a 50 cc. beaker directly from the manufacturer's container.

*2. The beaker is placed in a water bath kept at 37°C. for 15 minutes after which time the emulsion is sufficiently liquid for application.

*3. During the above procedure the stained slides are warmed on the leveling table.

*4. The warm slide is taken in one hand. Two drops of emulsion for every square inch of slide to be covered are applied to the slide with a medicine dropper to prevent the occurrence of bubbles in the emulsion.

*5. The drops are spread evenly and quickly with a camel's hair brush.

*6. The slide is rotated from side to side along its long axis so that the emulsion flows from edge to edge making an even film. This may be seen in the red light reflected from the safelight on the surface of the emulsion.

*7. The slide is returned to the warm side of the leveling table for 30 to 60 seconds to insure uniformity of emulsion and is then slide gently to the cool side of the plate. After one hour the emulsion has hardened sufficiently so that the slides may be stored in light-tight boxes.

*8. The slides are always stored in a horizontal position to prevent any displacement of emulsion. These boxes are placed in light-tight jars which contain a drying agent. The jar is transferred to a deep-freeze for the duration of exposure.

*9. Development is in Kodak D-72 at 18-20°C. for one and a half minutes, with fixation in acid fixer for six minutes.

The slides are washed for 15 minutes in water below 20°C. to prevent buckling and peeling of the emulsion. They are immediately dehydrated in 95% alcohol, absolute alcohol, alcohol-xylol, and three changes of xylol. Three minutes in each bath is necessary for complete dehydration. To prevent artefacts, the slides are immersed in a 1% solution of balsam diluted with xylol for at least one hour. Longer times are recommended for hard tissues. Slides should at all times be kept horizontal.

10. The section is then mounted in balsam under a coverslip and the preparation is allowed to dry at room temperature.

B. Reduction of Fog

If optimum resolution was to be obtained, the factors which tended to decrease resolution had to be reduced to a minimum. Attention was given above to the most satisfactory geometrical relation of the radioactive source and the overlying emulsion, as well as to the energy and intensity of radiation and the amount of radiation. It is now necessary to consider the characteristics of the emulsion. Since grain size, grain uniformity, grain concentration, development and fixation are discussed elsewhere, the remaining factor left to consider is "Fog". Fog is the photographic density obtained when unexposed portions of emulsion are developed. Gelatin normally protects silver bromide from reduction unless photolytic silver has been produced; but all emulsions contain some grains that are developable spontaneously. These grains probably contain development centers of increased sensitivity. This is termed "inherent" fog.

Fog may be also caused by mechanical pressures, abrasion or shock; or by chemicals such as wooden supports, hypophosphates, arsenites, and stannous salts (106). Fog caused by mechanical or chemical agents could be easily avoided using proper precautions. It was, indeed, a challenge to attempt to remove inherent fog in order to obtain a fog-free emulsion in which every developed grain would be due to the action of a radioactive particle from a tissue source.

1. A method to remove fog from photographic plates was described by Leibermann and Barschall (71). This method was, therefore, applied to medium contrast lantern slide plates. Four medium emulsion plates were carried through D-11 developer (20°C.) for six minutes to develop all the undesirable grains. The slides were then washed thoroughly in six changes of water (18°C.) for thirty minutes. This was followed by draining and drying of the slides for three minutes. They were subsequently immersed in a potassium permanganate sulfuric acid bath ((0.2% $KMnO_4$) two parts, one part 1% H_2SO_4) for 30 minutes (18°C.) and again washed thoroughly in six changes of water (18°C.) for 30 minutes. The potassium permanganate sulfuric acid acted as an oxidizing agent oxidizing all the developed grains. The slides were allowed to dry for two hours. It was found, however, that two hours did not dry the slides appreciably.

To remove the yellow-brown hue taken on by the emulsion and to remove the excess potassium permanganate, the slides were immersed in a 10% solution of sodium bisulfite ($NaHSO_3$) for five minutes after which time they were again washed in six changes of water for 30 minutes.

One slide was developed immediately in D-72 (20°C.) for two minutes and fixed for 10 minutes. Upon examination, the emulsion was found to be practically free from fog. However, considerable amounts of emulsion were lost from the plate.

Since close to 75% of the emulsion was lost with this procedure, several variations in the technique were attempted. Washing time was reduced from 30 to 20 minutes throughout in one experiment, from 20 to 10 minutes in another which was repeated several times with other minor variations in time. However, the loss of emulsion remained considerable.

It was then decided to repeat the procedure as follows:

Nine medium emulsion plates were developed in D-11 (1:1 water) for six minutes (20°C.), washed in running water (15°C.) for 10 minutes, then soaked in the freshly prepared oxidizing solution for 30 minutes. The slides were again washed for 10 minutes (15°C.).

(a) Six slides were dehydrated in 95% alcohol* for three minutes (and for five minutes in a subsequent experiment.

(b) Three slides were left at room temperature for three minutes. All the slides were then soaked in 10% $NaHSO_3$ for five minutes, followed by a 30 minute wash (15°C.) in running water. The slides were again dehydrated in 95% alcohol for five minutes and allowed to dry completely for four hours.

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*Alcohol was shown to have no effect on the sensitivity of the emulsion. One plate was partially immersed in 95% alcohol for two minutes. The plate was then exposed and developed. An even development occurred.

One side of (a) and one of (b) were developed for four minutes in D-11 (20°C.) and fixed for 10 minutes. Again no fog was observed at either low or high power. However, these slight modifications in the technique had no effect on the amount of emulsion that drifted away in the various solutions, most notably, in the sodium bisulfite.

The amount of emulsion remaining on the slides was scraped into a beaker and several radioactive histological preparations were coated with the "fog-free" emulsion (a). At the same time, medium emulsion coated (b), Ansco A coated (c) and Ilford liquid coated (d) autograph preparations, when dry, were taken through the Leibermann-Barschall procedure. All the slides (a), (b), (c), (d) together with an exposed fog-free medium emulsion plate (e) were developed in D-11 for four minutes and fixed. The results were as follows:

(a) The specimen coated with "fog-free" emulsion was fogged.

(b), (c), (d) The specimens coated with medium emulsion, Ansco Emulsion, and Ilford Emulsions retained very little emulsion to speak of, although the remaining emulsion was "fog-free" no autograph was visible. The stain was completely removed from all the sections.

(e) The exposed defogged medium emulsion plate when compared with an ordinary emulsion plate exposed and developed for the same length of time revealed

that the sensitivity of the defogged plate was greatly reduced (approximately 75%). This may in part have been due to the loss in concentration due to the dilution phenomenon. The fogging observed in (a) was probably due to an increased sensitivity to heat.

The procedure was again repeated using 30% solution of potassium ferric alum, an emulsion hardening agent, as a five minute bath for plates and autographs immediately before immersion in sodium bisulfite in the defogging method and before immersion in acid fixer in the ordinary development of the slides. Unfortunately, this method, too, did not appreciably improve the above results.

Conclusion

Because of the great loss in sensitivity of the emulsion by the defogging method, it was abandoned as a means to reduce inherent fog from emulsions used for autography.

2. Reduction of Intensity of Autographs

It was at times necessary to reduce the intensity of some of the autographs obtained. The black granules due to spontaneous fog have a great compactness of silver and a greater solubility in certain chemicals than those of the image. To test the effect of different reducers, four medium plate lantern slides were exposed a section at a time, and later developed in D-72 developer for two minutes (20°C.).

The plates were then broken in half so that two pieces were identical. One of each set was used for reduction in either Kodak Reducer or 10% potassium ferricyanide and 10% sodium thiosulfate. The other half of each plate was kept as a control.

1. The first was reduced for one minute with Kodak Reducer.
2. The second was reduced for two minutes with Kodak Reducer.[1]
3. The third was reduced for one minute with 10% $K_3Fe(CN)_6$ and 10% $NaHSO_3$.[2]
4. The fourth was reduced for two minutes with 10% $K_3Fe(CN)_6$ and 10% $NaHSO_3$.

The percentage reduction of blackening was the same for all intensities of black in 1 and 2. However, in 3 and 4, the blackening was reduced inversely proportionally to the intensity, that is, the darker the appearance of the emulsion, the less the blackening was reduced. It was decided, therefore, to use 10% $K_3Fe(CN)_6$ and

---

[1] Subtractive Reduction Formula (33)

$KMnO_4$	0.25 g.	Ingredients were mixed and made up to 1000 cc.
Water	500 cc.	Preparation was taken through this solution for one
H_2SO_4	4 cc.	minute, rinsed, and then allowed to remain in Potassium Metabisulfite for a few minutes to remove the manganese precipitate.

[2] Supra Proportional Reduction formula (33)

Solution A	Potassium ferricyanide	100 g.
	Water to make up to	1000 cc.
Solution B	Sodium thiosulphate	100 g.
	Water to make up to	1000 cc.

The slides or autographs were washed thoroughly to remove all acids from fixing bath. The two solutions were mixed just before use taking 10 parts of A and 90 parts of B to remove fog. For greater activity, the proportion of A was increased.

10% $NaHSO_3$ for Supra proportional reduction of autographs and Kodak Reducer for subtractive reduction where all densities are diminished by the same value.

It must be realized that the autographic results obtained using these reducers are not entirely true. It must be understood, too, that any type of quantitative work is not recommended following any type of reduction. Interpretation of results, therefore, must be made accordingly.

3. The only satisfactory method to reduce the relative amount of fog was found to be the dilution of emulsions which resulted in a thin coating retaining it original concentration of silver halide and presumably its sensitivity. Since with moderate exposure, the photographic effect is close to the tissue surface (Fig. 2 A) while fog granules are distributed uniformly throughout the emulsion, reduction of the emulsion thickness resulted in a greater decrease of the fog density than of the image density. This is illustrated by the profiles of dilute emulsion autographs shown in Fig. 2 . Fog has been reduced to a few scattered granules in the unexposed portions of the emulsion (Fig. 2 D) while the reaction over the radioactive colloid (Fig. 2 C) has almost the same density as the equivalent layer of the emulsion in the ordinary preparations (Fig. 2 A) for the same exposure.

C. Protection of Stain in Prestained Tissue Sections

If sections were to be stained before coating (prestained) it was necessary to have the stain protected by a thin film impermeable to developer and fixer. In addition, this film would have to be as thin as possible as it would make up the interspace (See Theory). Furthermore, it had to be completely transparent and have no effect on the sensitivity of the emulsion.

Extensive studies to find such a protective medium were made by Percival. He found that more than any other medium that he tested, a film consisting of two coats of 1% celloidin dried for more than 12 hours prevented the removal of stain from histological sections by developer and fixer. The procedure of dipping stained sections in a 1% solution of celloidin in alcohol, allowing the slides to dry somewhat, and then dipping the slides once more in this solution was adopted as a routine measure. The slides were put upright and allowed to dry completely for 12 hours before they were coated.

The protection that the celloidin offered the stain was found to be erratic, however. Sections stained with hematoxylin and eosin, although very often appearing less intense after the development process than when first freshly stained, still presented very clear histological detail. The hematoxylin was

removed to a somewhat greater extent than the eosin. The extent to which the stain was removed was greatly increased in sections stained with a Masson Trichrome stain. In this case, each of the components of the stain was removed to a comparable degree.

Since the alkalinity of the developer solution was presumably responsible for the removal of the acid dyes, an attempt to neutralize the developer with acid fixer was made. This reduced the efficiency of the developer, but did not contribute sufficiently to the retention of the stain to warrant the adoption of this procedure.

It was found by experience that fresh fixer tended to remove more stain than did fixer that had previously been left exposed to the air for some time. Since sulfur dioxide was the gas that was given off during this time, the reduction in acidity of the acid fixer seemed to be the factor responsible for the phenomenon.

Another attempt to reduce the amount of stain removed was, therefore, made by diluting the acid fixer with water or developer. Several dilutions varying from 50% to 10% solutions were attempted. The ultimate results of these trials was that longer times were required for complete fixation. The amount of stain removed, however, was not considerably reduced. These methods were, therefore, abandoned.

The search for a new protective base led to studies of new plastics and resins as protective media. The two products with properties most suitable for this purpose were Resin 200X20 and Latex 652, both produced by the Goodrich Rubber Company. Polystyrene, a polymer, was also included in the study.

Resin 200X20 is manufactured in a granular form and may be dissolved in a variety of solvents, the recommended one being 90% toluene and 10% methyl ethyl ketone. The methyl ethyl ketone is added to increase the clarity of the resulting solution. 1%, 5%, and 10% solutions were prepared by dissolving 20 g. of the granular resin in 180 cc. of xylol to which 20 cc. of methyl ethyl ketone were added. This 10% solution was kept as the stock solution from which 1% and 5% solutions were prepared by appropriate dilutions. It was found that the 10% solution was fairly viscous and contained many bubbles while the 1% solution had a low viscosity and contained very few bubbles. All solutions were clear and completely transparent.

Latex 652 is a white liquid with a consistency of homogenized milk while polystyrene is available as white crystals that may be dissolved in xylol.

As a preliminary experiment seven slides that had been stained with Masson Trichrome were coated with two coats of 1% celloidin and were allowed to dry completely for 12 hours.

a) Two slides were dipped into a 5% solution of resin

b) two slides were dipped into a solution of Latex

c) and d) remained untreated except for the celloidin coats.

a), b), and c) were taken through D-72 developer for two minutes, fixed for 10 minutes in acid fixer, and then washed for 30 minutes. These lengths of time were all slightly in excess to those employed routinely for autographs. d) was kept as a control for the intensity of the stain.

The stain was almost completely removed in the slides which had received two coats of celloidin only while the staining intensity of the slides treated with either resin or latex was identical to that of the control. An examination of the resulting film revealed that the coat of latex was comparatively thick while the coat of resin was thicker than the coat of celloidin.

To see whether more dilute solutions could be used and to test their effect on photographic emulsions, 24 slides were prepared of which 12 were stained with hematoxylin and eosin and 12 were stained with Masson Trichrome. The effect of the various protective media on these stained sections can be seen in Table V.

It was immediately obvious that group six could not be used for slides stained with hematoxylin and eosin since when the latex was used directly after the celloidin coat, the hematoxylin was removed as well as some of the eosin and an orange preparation

TABLE V

EFFECT OF VARIOUS PROTECTIVE MEDIA ON STAINED MEDIUM EMULSION AUTOGRAPHS

Protective base	Number of slides stained with H. & E.	Number of slides stained with Masson Trichrome	Effect on Emulsion	Appearance of stain after development
1. 2 coats 1% celloidin	2	2	0	removed
2. 1 coat 10% resin	2	2	0	removed less than in 3 or 4
3. 1 coat 5% resin	2	2	0	removed less than in 4
4. 1 coat 1% resin	2	2	0	removed
5. 1 coat latex	2	2	0	removed
6. 1 coat 1% celloidin and 1 coat latex	2	2	0	orange with H. & E.

Conclusion: 1) Resin or latex alone does not offer a satisfactory protection of the stain.

2) Celloidin, resin, and latex do not affect the sensitivity of medium plate emulsion.

resulted. In the case of the Masson Trichrome stained slides, the stain spread over the entire slide.

All the slides were coated with medium emulsion, developed, fixed, and mounted. An autographic preparation of each group was exposed to light prior to development and each was found to contain an equal density of reduced silver grains. The remaining unexposed slides were all found to contain a normal amount of background fog granules. This indicated that neither of the new protective bases affected the sensitivity of medium emulsion.

The stain in this experiment, however, was removed in all cases, the least amount being removed where two coats of 1% celloidin was the protective base. It was decided, then, that resin or latex alone would not offer a satisfactory protection of the stain.

Since in the preliminary experiment in which a primary layer of 1% celloidin was used, complete protection of the stain was realized, the following experiment was carried out to see whether a thin protective layer could be achieved which would again protect the stain. Twenty slides were prepared of which 10 were stained with hematoxylin and eosin and 10 were stained with Masson Trichrome. Each group then contained one slide with each stain. The slides were coated with thin coats of various solutions as seen in Table VI . All the slides were taken

TABLE VI

PROTECTION OF STAIN USING POLYMERS OF VARIOUS CONCNETRATIONS

Protective base	Number of slides stained with H. & E.	Number of slides stained with Masson Trichrome	Appearance of stain after development process
1. 2 coats 1% celloidin	1	1	removed
2. 1 coat 1% celloidin (dried 1 hour) 1 coat 1% celloidin	1	1	removed
3. 2 coats 1% celloidin (dried 1 hour) 1 coat 10% resin	1	1	persists
4. 2 coats 1% celloidin 1 coat 1% resin	1	1	persists
5. 1 coat 1% celloidin 2 coats 10% resin	1	1	persists
6. 1 coat 1% celloidin 1 coat 10% resin	1	1	persists
7. 1 coat 1% celloidin 1 coat 5% resin	1	1	persists
8. 1 coat 1% celloidin 1 coat 1% resin	1	1	persists
9. 1 coat 1% celloidin 1 coat 1% latex	1	1	H. & E. stain appeared orange before developing
10. 1 coat 1% celloidin 1 coat 1% polystyrene	1	1	removed

Conclusion: 1) The thinnest layer which completely protects the stain consists of 1 coat 1% celloidin and 1 coat 1% resin.

2) Neither 1% celloidin alone nor polystyrene protect the stain.

through D-72 developer solution for two minutes, fixer solution for 10 minutes, running tap water for 30 minutes, dehydrating media, and were then mounted.

A considerable amount of stain in groups 1, 2 (1% celloidin) and 10 (polystyrene) were removed while in group 9 a bleaching of the eosin was noted prior to the development process. The stain was completely protected in all the other groups as could be seen when the slides were compared with the stain from a control slide. Since group 8 which consisted of one coat of celloidin and one coat of 1% resin was the thinnest layer of all those tested which offered satisfactory results, this mode of protection has, therefore, been adopted for all future coated autographs.

II. A. Experiments with Strip Emulsion

The growing interest in autography during the last few years prompted emulsion manufacturers to develop emulsion films of determined thicknesses that could be applied without difficulty to a radioactive histological tissue section. These emulsion films were named stripping emulsions and as the name implied were capable of being stripped from their base and transferred to a new one.

The immediate enthusiasm which greeted this development was greatly due not only to the fact that contact nearly as intimate as afforded by the coated or mounted methods could now be obtained but also to the fact that the absolute thickness of the emulsion used would now be known, would be uniform throughout, and could therefore be used as a means to make autography a quantitative technique. A simple method of preparation of this new type of autograph was necessary. Experiments were, therefore, directed towards the study of the preparation of the emulsion strip for application, the method of application, and each of the subsequent steps followed in the production of a satisfactory autograph was also studied.

1. Preparation of the Emulsion for Application

Since all the emulsion strips are very thin and easily pliable, they were supplied on a glass or celluloid support from which they had to be separated before they could be used in the following experiments. The film was removed from its support by cutting the emulsion along the three edges of the glass plate or the celluloid support. The emulsion was then slowly peeled off to about one inch from the edge. It was found that blowing moist air over the emulsion that was being very slowly stripped reduced the amount of fog caused by static charges produced by fast stripping.

In the first attempt at using Kodak Special Stripfilm, the film was cut to a size slightly larger than a 3 x 1" glass slide. The strip was applied, emulsion side down, to the histological preparation and the combination then lowered into a dish of cool distilled water (20°C.) for approximately 30 seconds. When the wet preparation was retrieved from its bath, the stripfilm was gently pressed to the slide to insure close contact and to smooth out any irregularities. Regardless of the smoothing process, however, irregularities often occurred. Folding of the emulsion was common and non-adhering areas gave cause for disappointment in the use of the method.

Another source of difficulty was the fact that the film was prepared on a plastic base from which it could not be severed. Since the base was impermeable to developing solutions, acetone

was used as a dissolving medium for the plastic so that the solutions might penetrate and exert their action on the emulsion. One minute in acetone was found to be inadequate for complete removal of the plastic since upon development, areas of emulsion which were known to contain latent images showed no deposition of black silver granules. Five minutes in acetone, on the other hand, removed all the plastic base present but in addition caused a stippling of the emulsion, thereby defeating the purpose of the stripfilm, that is, altering the standard thickness allowing the technique to give quantitative results. Several subsequent attempts using NTB Stripfilm and different intervals in acetone were made but the results discouraged the use of this procedure as a practical method.

If the strip were to be applied so that the plastic base would be in contact with the slide, the emulsion would then be free to be developed. This procedure obviously increased the distance between the radioactive source and emulsion, i.e., interspace (See Theory). However, where the plastic base was present in any strip emulsion, this procedure had to be adopted for prestained tissue sections.

The availability of Ilford Half-Tone Stripping Plates (6" x 8") (emulsion thickness 40 μ) in which the base of the strip emulsion was gelatin instead of cellulose acetate promised more satisfactory results. Now it was possible to apply the emulsion strip with the emulsion directly over the celloidin protected histological slide. To determine whether there was any difference

in the resolution depending on which side of the emulsion strip was in closest proximity with the slide, eight autographic preparations were made. Four of the slides were covered so that the part of the emulsion strip that was originally in contact with the glass support was now in contact with the histological slide; the other four slides were covered so that the part of the emulsion strip that had originally been in contact with air was now in close proximity with the histological slide. Examination of the developed autographs revealed that although the resolution was good in both groups, a slightly better definition was obtained with the four in the second group. This suggested that either of the emulsion strip sides could be placed next to the section but that the side which did not adhere to the plate originally was to be preferred.

To overcome the difficulty caused by incomplete contact of the strip with the slide, the strip was allowed to soak in distilled water for two minutes after which time it was floated onto the histological slide (87). As the slide with its overlying emulsion strip was lifted from the dish of water, the excess water was allowed to drain off the sides of the slides. The overlapping edges of the emulsion were then folded under on the base of the slide and the preparation was allowed to dry in a calcium chloride atmosphere before being stored in the cold.

Although contact between slide and emulsion seemed fairly stable during this procedure, upon development it was very often

noted that the emulsion strip became disengaged and was left floating in the various solutions after the carrier had been lifted to proceed to its next immersion. This occurrence resulted in a complete displacement of the image even when the floating strip was reunited with the glass slide and superimposition was attempted. It was, therefore, necessary to devise a method of application of the strip to the slide which would eliminate the floating-off phenomenon. This work was attempted mainly with Ilford Half-Tone Stripping Plate Emulsion.

Immediately prior to development, paper clips were applied to the sides of the slide to hold the emulsion strip fixed to the slide. This resulted in a cumbersome preparation which nevertheless often showed a displacement phenomenon. Since this may have resulted from mechanical movement caused by the manipulation with the paper clips, this method was abandoned.

By cutting larger emulsions strips, it was possible to make the overlapping edges meet when folded underneath the slide. The close adhesion between the two edges as well as to the back of the slide kept the strip from floating off during development. Examination of the preparation under a low magnification, however, revealed areas in which displacement of the emulsion had obviously occurred.

This displacement was of two types: 1) a generalized slight displacement in which most of the autographic images in the emulsion did not coincide with the structures from which they

were known to have arisen and 2) a localized slight displacement in which only few areas showed this displacement, the remaining ones coinciding faithfully with their points of origin.

The first type was explained as being due to areas in the emulsion which had not soaked up a maximum amount of water during the soaking stage and had, therefore, not expanded to their fullest capacity. The strips were then allowed to dry and set. Upon development, during which time the preparation was in an aqueous medium for approximately 30 minutes, the emulsion was again allowed to expand, but this time to its maximum. When it was allowed to dry at this atage, a general shift resulted to accommodate for the extra areas now occupied by the originally undersoaked ones.

Incomplete soaking was not the likely cause in the second type of displacement. The fact that displacement occurred in some areas and not in others on the same slides was attributed to the possibility that tiny folds of emulsion formed in certain areas. When the slide was developed, the folds swelled and may have shifted slightly upon drying. It was, therefore, advisable during the application to test the smoothness of the film before and after it had dried completely on the slide.

To test the effect of soaking time as well as the effect of folding of edges underneath the slides on the amount of displacement in autographs, slides of histological sections of

thyroids containing I^{131} were divided into four groups. It was decided to use 1% dupanol (lauryl sulfate), a spreading agent, in the distilled water since this would allow a more even, faster, and more thorough penetration of the water into the emulsion. Dupanol was seen to have no effect on the sensitivity of the emulsion.

a) The first group was covered with strip emulsion that had soaked for <u>two</u> minutes in distilled water containing dupanol. The emulsion strip edges were folded under the slides on <u>three</u> sides.

b) The second group was covered with strip emulsion that had soaked for <u>five</u> minutes in the water-dupanol bath. This emulsion strip was again folded under the slides on <u>three</u> sides.

c) and d) The third and fourth groups were similar in soaking time to group one and two respectively but only <u>two</u> side edges of the emulsion strip were folded.

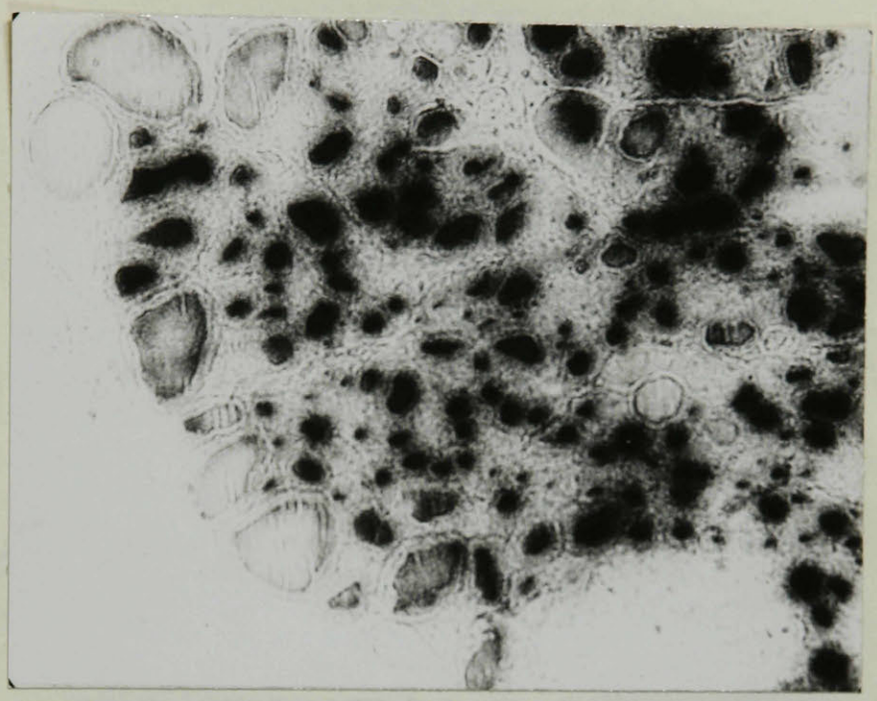
The results seen in Table VII and Fig. 12 indicated that two minutes was not sufficient soaking time for Ilford Strip Emulsion and that folding on two sides only did not give consistent results. It was concluded, therefore, that Ilford Strip Emulsion soaked for <u>five</u> minutes in a dupanol-distilled water solution and applied so that three flaps could be folded under to meet on the undersurface of the slide would prevent any type of displacement.

TABLE VII

EFFECT OF SOAKING TIME OF STRIP EMULSION ON AMOUNT OF DISPLACEMENT

Number of slides	Time in distilled water and dupanol	Number of edges folder under	Number of slides in which Displacements occurred
6	2 minutes	3	3
6	2 minutes	2	6
6	5 minutes	3	0
6	5 minutes	2	2

FIG. 11



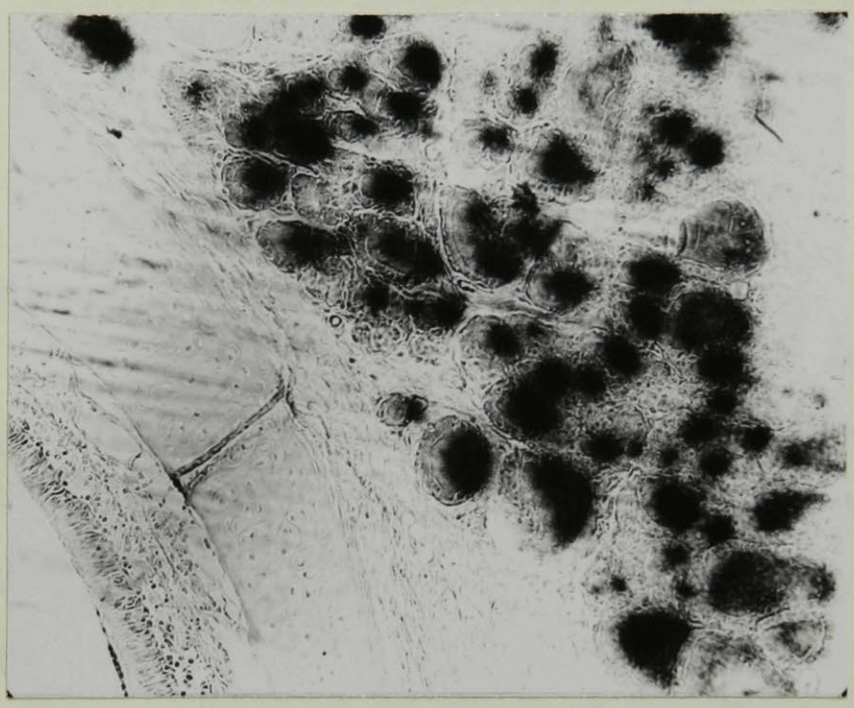
Strip coated autograph of a thyroid gland from a rat injected subcutaneously with I^{131} and sacrificed 24 hours after the injection.

The autograph was prepared by soaking Ilford Half-Tone Stripping Plate Emulsion for five minutes in distilled water and dupanol. Three of the emulsion strip edges were folded under the slide.

Note that the autographic reaction is predominantly in the colloid except at the periphery of the gland where the reaction is in the apex of the cells.

Compare with Fig. 12.

FIG. 12



Strip coated autograph of a thyroid gland from a rat injected subcutaneously with I^{131} and sacrificed 24 hours after injection.

The autograph was prepared by soaking the emulsion strip for two minutes and folding the edges of the strip under two sides of the slide.

Note that the autographic reaction does not correspond exactly with the histological details of the colloid indicating a displaced autograph.

Compare with Fig. 11.

This method has been adopted in this Laboratory for use with stripfilm.

Recently Kodak NTB_2 Stripping Plates (4" x 5") have been prepared in which the emulsion is also set on a gelatin base. The general procedure for the preparation of autographs with this strip emulsion is similar to that of Ilford Stripping Plates.

2. Storage of Strip Covered Autographs

Strip covered autographs were stored in boxes and jars as described in the section on Storage in the Coated Technique section (p.46). In addition to the precautions already described for this procedure, it was necessary to guard against the wet strip adhering to the box or to the corrugated paper in the box. If adhesion did occur, the possibility of tearing the strip from the slide was great when the slide was being removed for development. If a tear did not occur, there was a likelihood that any slight pulling on the strip during removal of the slide might cause a displacement if not a breaking of the contact between the strip and the slide.

The manner in which these hazards were avoided involved the complete drying of the slides in a dessicator or in a calcium chloride atmosphere. All surfaces of the slide which were covered with emulsion were allowed to remain free and in contact with air only. This was accomplished by using a slide carrier which allowed the two long edges of the slide to remain

free. A projection on either side of the slide carrier served as a rest on which the two narrow edges of the slide could fit. In this way, only a minute portion on the back of one narrow edge of the slide was in contact with another surface. When the strip was completely dry it was carefully removed to be arranged in the storage boxes. The dry emulsion did not adhere to the box but slid easily into the corrugated slot.

Storage of Ilford Strip covered preparations during exposure was in the deep-freeze. The low temperatures did not seem to have an adverse affect on the results of the autographs obtained. It was found, however, that when Kodak NTB_1 Stripping Film or NTB_2 Stripping Plates were used, the extremely cold temperatures caused the strip to peel away from the slide at times ripping the tissue section off the slide. It was, therefore, necessary to keep all but the Ilford Strip Film autographs in an ice box at temperatures not lower than $0^{o}C$.

3. Development and Fixation of Exposed Slides.

The strip autographic slides were developed for one and a half minutes in D-72 developer, rinsed, and fixed in acid fixer. In contrast to the liquid coated autographs, these required a considerably longer fixation period. Fifteen minutes was the minimum length of time taken for the emulsion to clear which meant that a minimum of 25 minutes was necessary for complete fixation. This was due to the fact that all the strip emulsions used were highly concentrated with silver bromide, consequently a

longer time was needed to dissolve out all the undeposited silver bromide. To insure complete removal of the acid fixer, a minumum of 30 minutes was allotted for washing. A shorter washing time resulted in a yellow coloration of the autographs.

4. Dehydrating and Mounting

The method of dehydrating and mounting was that described for the liquid coated techniques. In order to obtain an esthetically satisfactory preparation, the excess emulsion on the back of the slide was removed. It was found that if the superfluous emulsion strip were trimmed away before the preparation was completely mounted, the possibility of slight displacement or even total lifting of the emulsion strip increased. This was avoided by allowing the balsam mounted autograph to dry for 24 hours after which time the emulsion lying outside of the region of the coverslip and on the back of the slide was trimmed away with a razor blade. It was considered advisable to allow at least one centimeter of emulsion to remain outside all the margins of the coverslip.

5. Description of the "Strip Coated" Technique

1. Ilford Special Half-Tone Stripping Plates, Kodak NTB_2, or (Eastman Kodak) NTB_1 Stripping Films are used. The emulsion strip is removed from its support by cutting the emulsion along three edges of the plate or film and slowly peeling off the emulsion to one inch from the edge.

2. The emulsion with the side that formerly adhered to the glass facing upward (Ilford) or downward (NTB_1) is put into a shallow dish containing distilled water and dupanol at 18-20°C. for five minutes.

3. The stained histological section on a slide is slipped underneath the emulsion and the slide with the emulsion is lifted out of the water. The emulsion is trimmed to a little larger than the size of the slide so that the edges may be folded under the histological slide to insure adherence.

4. The slides are kept in a dessicator for 15 minutes before they are stored in light-tight boxes in light-tight jars containing a drying agent. The jars are then transferred to an ice box at 0°C. for the duration of exposure.

5. Development and fixation is identical with the procedure for coated autographs.

All the above steps are carried out in a darkroom.

III. Descriptions of Other Autographic Techniques

During the course of this study it was found necessary to use other autographic techniques as well as those already described in detail. Of these other methods, the contact method was the one used most routinely while the mounted method was used only in comparison studies. The details of several modifications of the mounted method and inverted methods that have recently been reported will be included for completeness. The methods used here were:

1) Coated Autographic Method.

2a) Mounted Autographic Method.

The modifications to be described that have been used elsewhere are:

2b) Dry Mounted Method as suggested by Williams (17).

3) Coated Inverted Method as suggested by Bélanger (8).

4) Inverted Method as described by McDonald, Cobb, Solomon, and Steinberg (75).

1. Contact Autographic Method

A. Preparation of Tissues

In the case of soft tissues, paraffin or celloidin sections were prepared in the routine histological manner and dehydrated with or without staining. The thinner the section, the better the resolution. For practical pruposes, 5 μ paraffin sections and 10 μ celloidin sections seemed to be most convenient.

In young animals, it is possible to cut bones and even teeth after celloidin embedding according to Bloom's method (14). However, the tissues such as mature tooth or bone must be ground to a smooth surface on a fine revolving stone. Examples of such grinding machines may be found in the literature (53,60,100). In this Laboratory, the sample was glued with Duco cement on a microtome mount, which was then clamped on a microtome facing a motor-driven emery stone. After polishing one side with a fine stone cooled by a stream of water, the sample was removed from the mount, glued to a microscope slide, polished on the other side and then placed in contact with a photographic plate for exposure.

Large specimens of soft tissues may be frozen, ground in the same way or sectioned with a chilled blade or fine electric saw while being kept in the frozen state, and thus applied to the photographic emulsion. This technique has been used in the localization of radio-elements in amputated limbs, whole mice, etc. (53).

B. Autographic Procedures

1. The Contact Method (Figs. 14-16)

The steps of the "contact" method used here were as follows:

1) The sections were coated in celloidin by being dipped twice in a 1% ether-alcohol solution after staining and passage through 95% and absolute alcohol. The sections were then dried overnight to insure hardening of the celloidin.

2) The surface of the section to be autographed was examined under a dissecting microscope for dust granules and particles that may make for uneven contact.

3) In the darkroom, using a suitable safelight, the section on the slide was placed next to a photographic plate or film (E.K. contrast lanter slide, X-ray film, etc.). A very close contact was obtained by applying equal pressure to both sides of the plate and slide with the help of light-tight X-ray pressure cassettes. For macro-specimens a sheet of cellophane may be interposed between specimen and film. The cassette is stored at 1-2°C. for the duration of the exposure.

4) After exposure, the photographic plate or film is developed and fixed according to routine photographic procedures.

The resulting autograph (Fig. 16, p. 115) may be superimposed on the section (Fig. 15) and both are examined simultaneously under the microscope. It was found useful to cover the autograph with a histological coverslip after deposition of a drop of Canada balsam on the dry emulsion.

2a. The Mounted Method (Figs. 17-19)

The following are the details of the technique as adapted for use in this Laboratory:

1) Unstained strips of tissue sections were floated in a 40°C. water bath to remove wrinkles from the tissue. They were then transferred to a bath of distilled water at 18-20°C., and all subsequent work was carried out in the darkroom at more than three feet from a Wratten No. 1 Safelight. From the water bath the sections were floated onto photographic plates (Medium, Contrast and Contrast Lantern Plates, NTB films, etc.). When the excess water drained off, the section adhered to the emulsion.

2) The plates were stored at 1-2°C. in light-tight jars containing a drying agent for the duration of exposure.

3) The sections were deparaffinated prior to development by being placed for one minute in each of two changes of absolute xylol. The xylol was allowed to evaporate completely in a well-ventilated darkroom. Fifteen minutes was usually sufficient.

4) The plates were developed and fixed according to routine photographic procedures, all solutions being kept at 18-20°C.

5) After being washed in running water, below 20°C. for 30 minutes, the plates were placed in dilute solutions of hematoxylin stain in the coldroom (1-2°C.) and left overnight.

The use of dilute solutions prevented a heavy staining of the emulsion, obscuring the autograph. Recently a mixture of metanil yellow and iron hematoxylin has been recommended as being less absorbed by the emulsion than routine hematoxylin (39). For staining prior to development, hot carbol fuchsin-neutral red has been found to give stained sections which resist the destaining action of the developing reagents (30).

6) The plates were then dehydrated in 95% alcohol, absolute alcohol, alcohol-xylol, and three changes of xylol. After immersion in a dilute solution of balsam for at least five minutes, the section was mounted in balsam under a coverslip.

2b. Dry Mounted Method

Williams (17) has devised a method for dry mounting paraffin embedded soft tissues, to avoid the pulling of colloidal particles into the emulsion by the wet emulsion (17). The method is as follows for soft tissues:

1. A 1 1/4 x 1/2" strip of lens paper is centered at the end of a No. 1 1.22 x 60 mm. coverglass, the lens paper is wetted with 50% Kodak Stripping Film Cement (diluted with acetone), the ends of the lens paper are turned back over either side of the coverglass and allowed to dry thoroughly.

2. A paraffin section of a frozen-dehydrated tissue is placed on the lens paper and rolled flat with a rubber roller. The section adheres to the lens paper.

3. In the darkroom, the coverglass is placed on the photographic plate with tissue side next to the emulsion and the end of the coverglass on which there is not tissue is lined up with one end of the plate. This end is put in the alignment clamp and the screw is tightened so that both the coverglass and photographic plate are held securely.

4. The tissue is clamped next to the emulsion with a No. 110 Parrot Binder Clip and stored in the presence of a dessicant in a light-tight box during exposure.

5. After exposure, the Binder Clip is removed, and the coverglass is bent away from the emulsion by prying under one corner of the free end with a razor blade.

6. A cellophane envelope is slipped over the coverglass to protect the tissue from contact with the development solution.

7. The photographic plate, and the covered, bent coverglass in the developing clamp is placed so that the tissue is held away from the emulsion to permit photographic processing.

8. The developing clamp, photographic plate and covered coverglass is submerged in the developer, fixed and washed, being careful that the level of the solutions is not above that of the cellophane envelope at all times.

9. After processing and while the emulsion is wet, the cellophane envelope is removed.

10. The plate and coverglass is removed from the developing clamp, being careful not to disturb the alignment clamp screw, and the coverglass is allowed to return to its original position. The tissue and the image are now in perfect alignment.

11. The wet emulsion wets the lens paper. The lens paper is allowed to become thoroughly wet and then partially dry on the emulsion so that it will stick to the emulsion when the coverglass is removed.

12. The alignment clamp screw is loosened and the coverglass is removed first and then the lens paper by lifting them away from the emulsion. Pulling sideways should be avoided.

13. The paraffin section is allowed to dry thoroughly on the emulsion making permanent and intimate contact with the image.

3. Coated Inverted Method

The inverted method allows the histological section to be stained after being autographed without affecting the emulsion. This method is of great value in autographing bones that contain radio-elements that are easily removed or displaced by acid stains. The details of the technique are as follows:

1. Two drops of distilled water are applied to a chemically clean (no egg albumen) histological slide on a hot plate at 40-45°C. An unstained tissue section is mounted in the water to remove wrinkles in the tissue. The water is allowed to evaporate completely.

2. The tissue on the slide is deparaffinated, then dipped in a 1% solution of celloidin and allowed to dry completely for 12 hours.

3. Kodak Matrix Emulsion is placed in distilled H_2O for one minute after which time it is transferred (emulsion side up) to a glass plate. The emulsion is now scraped into a beaker, melted as in the "coated" technique. A Blanchard brush (made of Bandage gauze on a glass slide held down by adhesive tape) is saturated with emulsion and carried across the slide with a uniform slow motion applying minimum pressure.

The slides are placed on a level table to dry, stored and left to expose. The slides are developed in Kodak Dektol (1:1) at 10°C. for 10 minutes, rinsed 20 times, fixed in Kodak acid fixer for 16 minutes. They are then washed in cold (10°C.) running water for 30 minutes.

4. After proper development and fixation, the emulsion-celloidin complex is lifted from the slide under water carrying the section on its undersurface. To facilitate the removal, the edges of the celloidin on the slide are cut with a sharp razor.

5. The complex is then inverted and affixed on a clean slide, section side up. The free edges are sealed with 1% celloidin. The celloidin is allowed to dry completely. The section is now ready for routine staining. The celloidin protects the emulsion from being stained.

6. Dilute basic stains give the best results. The slides are then dehydrated in alcohol and xylol and mounted with balsam under a coverslip.

4. Inverted Technique using Strip Film

A method similar in principle to Bélanger's method has been used by McDonald, Cobb, Solomon and Steinberg (75). The details of the procedure they use are as follows:

1. Stained paraffin sections are prepared in the conventional manner, using the desired stain, stopping in the absolute alcohol stage. The slide is kept in the alcohol until the film is mounted unless a delay greater than two hours is expected. In this case, the section is taken to xylol. Being sure to remove all xylol, the section is returned to absolute alcohol before the film is mounted. If staining after development, the procedure omits Step 20.

2. Frozen sections are prepared as follows:

a. Immediately on removal, tissues are frozen in iso-pentane at liquid nitrogen temperatures, according to the technique of Linderstrom-Lang and Mogensen. To prevent rolling of the sections as they come off the blade, the "window" devised by A. H. Coons is used.

b. Tissues are kept at -10 to -15°C. throughout subsequent handling. Even brief thawing will mar histological details.

c. The microtome is set up in the coldroom or cold box andequilibrated at -10 to -15°C. together with all other equipment to be used in preparing sections: slides, tweezers, camel's hair brush, teasing needle.

d. A drop of water is placed on the mounting block and the tissue is firmly pressed against it until it freezes in place.

e. Glass slides are prepared in advance by coating each with the conventional 1:1 egg albumen-glycerine mixture diluted with water to half strength and containing 0.01% phenol.

f. Sections are cut 8 to 10 microns in thickness.

g. With a teasing needle or camel's hair brush, each section as cut is transferred to a slide and melted in place by touching the undersurface with the bare hand. Once dried onto the slide, sections may be handled at room temperature.

h. Slides are transferred to the darkroom for mounting of stripping film.

3. In the darkroom under appropriate safelights a supply of 1" x 3" strips of film is cut. The supply is covered with a dark box or placed in a light-tight drawer until used.

4. Paraffin sections are removed from alcohol and placed side up on the desk. The alcohol is allowed to evaporate almost but not quite completely. Frozen sections once dry are ready for Step 6.

5. A small drop of 1% celloidin in methyl alcohol is placed directly on the tissue section and another drop on either side of it. Too little celloidin leads to a "tacky" preparation; too much leads to oozing onto the emulsion and wrinkling.

6. The emulsion base combination is partially stripped from the support, starting the stripping in either of two ways;

a. Stripping is started at a corner by stroking the emulsion away from the support with the thumb, or

b. One end of the film is placed on the table top, emulsion side down; the support is partially cut through with a razor blade and the breaking is completed leaving the small end tab attached to the film to aid stripping from the main portion of the support.

7. The partially stripped film is applied to the section completing the stripping of the film at the same time.

8. The transparent support is laid over the emulsion and the preparation is smoothed with the fingers.

9. A second microscope slide is placed on top of the support. Pressing the two slides firmly together, they are bound with Scotch tape.

10. Each preparation is wrapped individually in opaque paper and placed immediately under a pressure of eight pounds per square inch for 24 hours.

11. After 24 hours the preparation is transferred to the coldroom or dry ice box and stored in the presence of a dessicant at freezing temperature for duration of exposure.

12. The autograph is unwrapped in the darkroom, the Scotch tape is cut along the edges of the "sandwich" with a razor blade and the guard slide and the support are removed. (Unwinding the Scotch tape causes intolerable blue static discharge.)

13. The bottom slide, bearing the tissue and stripping film <u>in toto</u> is placed in a test tube containing 50 cc. of fresh D-19 at 20°C.

14. Development is for five minutes, inverting the tube every 30 seconds.

15. The developer is gently poured off and 1% acetic acid stop bath at 20°C. for 15 seconds is added immediately.

16. The acetic acid is poured off and Kodak F-5 fixing-hardening solution at 20°C. is added. Fixing is for five minutes.

17. The autograph is transferred to gently flowing wash water for 5 to 10 minutes.

18. One end of the film is lifted and the film is steadily pulled laterally, with the tissue adhering, off the slide.

19. The delicate film with tissue adhering is stored in a beaker of distilled water until mounted.

20. Mounting is as follows:

a. The stripping film is taken up through 50%, 80%, 95%, and absolute alcohol to xylol.

b. The excess film around the tissue section is trimmed away.

c. Mounting is with balsam and a cover slip, as with an ordinary tissue section. The preparation after drying through the alcohols is very markedly wrinkled. Therefore, it is necessary to place it under a weight until the balsam dries in order to get a flat field. If the image is very dense, the tissue is mounted uppermost.

21. If the tissue is to be stained after development, Step 20 is omitted and procedure is as follows: The stripping film, emulsion side down, is cemented onto a clean side with Kodalith

Stripping film cement or with 1% celloidin in ether alcohol. It is particularly important to seal the stripping film to the slide along the edges so that the emulsion is protected in the subsequent staining procedures.

IV. Comparison of Autographs Made by Various Autographic Techniques

Although it was immediately obvious that the coated and the mounted methods gave far better results than did the contact method, it was nevertheless desirable to compare all the available techniques under standard conditions. This meant that sections with comparable geometry and radioactive distribution were necessary.

For this purpose twelve male albino rats of comparable weights were injected with I^{131}. The rats were divided into three groups of four animals.

1) Group 1 was injected with one million counts per animal.
2) Group 2 was injected with 0.3 million counts per animal.
3) Group 3 was injected with 0.1 million counts per animal.

To test whether the thyroid glands of each animal had taken up the same percentage of the injected dose after 24 hours, one lobe of each thyroid gland was digested with NaOH for Geiger counter studies, while the other lobe of each animal was taken for autographic studies.

Geiger counter studies of the thyroids revealed that each thyroid had picked up approximately 20% of the injected dose, that is,

Group 1 contained approximately 200,000 counts per thyroid gland (10)

Group 2 contained approximately 60,000 counts per thyroid gland (3)

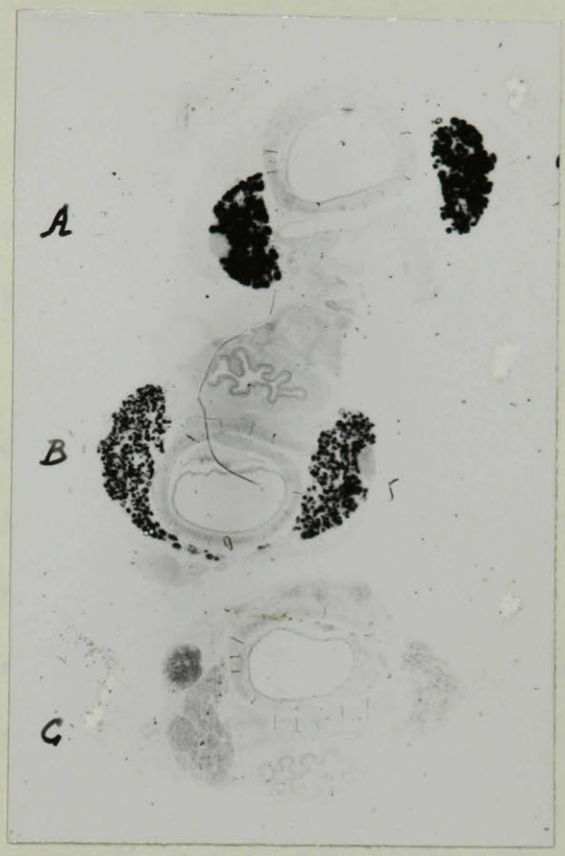
Group 3 contained approximately 20,000 counts per thyroid gland (1)

To see the variation in density of autographs containing different amounts of radioactivity, one thyroid from each group was mounted on the same histological slide, thus allowing the three thyroids containing logarithmic doses (10:3:1) to be coated, exposed, developed, and fixed for the same period of time. Since the photographic density of medium emulsion increases logarithmically as the number of incident β-particles per cm^2 (106) it was expected that graded degrees of densities could be obtained in this way. Although densitometric readings were not made, it was obvious that a graded degree of blackening did occur as can be seen in Fig. 13.

At the same time that medium emulsion coated slides were prepared, autographs of similar sections as above were prepared by the:

1) contact method, using a contrast process lantern slide plate;
2) mounted method, using a medium contrast lantern slide plate;

FIG. 13



Liquid medium emulsion coated autograph of sections of thyroids from rats injected with logarithmic doses of I^{131}. The sections were mounted on the same histological slide and therefore were coated, exposed, developed and fixed in an identical manner.

A. Autograph from a rat injected with one million counts.

B. Autograph from a rat injected with 0.3 million counts.

C. Autograph from a rat injected with 0.1 million counts.

Note the gradations in the intensity of the autographic reactions.

3) liquid coated technique, using thinned medium emulsion; and

4) strip coated technique, using Ilford Half-Tone Stripping Plate Emulsion.

The techniques were those described in the preceding sections.

This entire experiment was then repeated and the results of both experiments were found to be identical.

Representative portions of the autographs obtained using the various techniques can be seen in Plate 1 . The first group of figures, of a contact autograph, represents the maximal useful magnification for this type of autograph. This immediately suggested the superiority of the other methods.

The advantage of the contact method was the relative simplicity with which autographs were obtained. It is especially useful to detect radioactivity in hard structures that cannot be cut with a microtome. The main criticism of the technique is the poor resolution, directly related to the size of the interspace. Also, comparison between tissue section and autograph is difficult, except at low magnifications. Techniques have been suggested to facilitate the comparison, but these are rather cumbersome.

A sample autograph obtained by the mounted method is seen in Figs. 18 and 19. Although the section and autograph were intimately united and could, therefore, easily be viewed together, several disadvantages to this method were apparent:

a) the failure of the developer to penetrate the tissue uniformly, leaving some exposed areas underdeveloped (Fig. 18); b) the tendency of the gelatin to stain with the tissue dyes obscuring the autograph to some extent (Fig. 18); and c) the occurrence of emulsion blackening due to the chemical action of the tissue. It must be emphasized, however, that the preparations obtained by this technique were often more satisfactory under high than under low magnifications (Fig. 19).

Another type of artefact resulted from the fact that the section was stained (post-stained) after the autographic image had been obtained. By placing a drop of hematoxylin stain on a developed exposed photographic plate, it was found that some of the deposited black silver granules were removed, that is, the density of the photographic plate was decreased. When the densities of a stained and an unstained mounted autograph which had been prepared at the same time were compared, it could be seen that the intensity of the autograph of the unstained preparation was greater than that of the stained one. This dissolving out phenomenon would lead to erroneous results if the counting of grains in quantitative work was attempted. Only if the section were allowed to remain unstained could quantitative work be possible with this method.

In general, with the mounted autographs it was not possible to obtain regularity in the results. Variations occurred

in both the autographic reaction and the staining of the overlying section. Much of the difficulty was apparently due to uneven swelling of the emulsion which caused slight irregularities of its surface.

In our experience the technique of coated autographs was easier than the mounted autographic method and lent itself better to the production of a large series of slides. Reproducible intensity and staining reactions were also possible. It was felt that the coating technique permitted the emulsion to flow into a more intimate contact with the section than was possible by the mounted method. This decreased the amount of diffusion and gave more intense autographs. Also, as can be seen by comparing Fig. 19 with Fig. 22 the amount of background fog was greater with the mounted method than with the coated method although the same photographic emulsion was used for both.

The results obtained with the liquid coated method (Figs. 21, 22) and the strip coated method (Figs. 24,25) are somewhat comparable. The advantage of the strip coated method is that the emulsion strip is presumably of a standard thickness, and, therefore, allows quantitative work to be attempted with more confidence. However, the liquid coated technique offered a more intimate contact than did the strip coated method. Since the emulsions used in both variations of the coated technique were of different concentration and grain size, a true comparison

of the results was difficult to make.

One serious disadvantage of the coated method is the removal or displacement of some radioactive elements from the tissue section by the prestaining method (e.g., P^{32} in bone). Both the mounted and the inverted methods overcome this difficulty. The inverted method as described by Bélanger sometimes results in the tearing of the tissue section during the inversion procedure. In the method described by McDonald et al. the task of mounting the developed emulsion strip and the tissue section on a histological slide (step 20) is indeed a formidible one. It has been found that when a strip coated autograph became loose and floated away from the slide, the strip could not be mounted since uneven ridges with the emulsion formed in the dehydrating solutions. The resulting preparation could, therefore, not be viewed in one plane. In addition, the procedure itself, does not lend itself to mass production.

Conclusions

1. In our experience, the coated technique offered the most satisfactory resolution of the techniques tested.

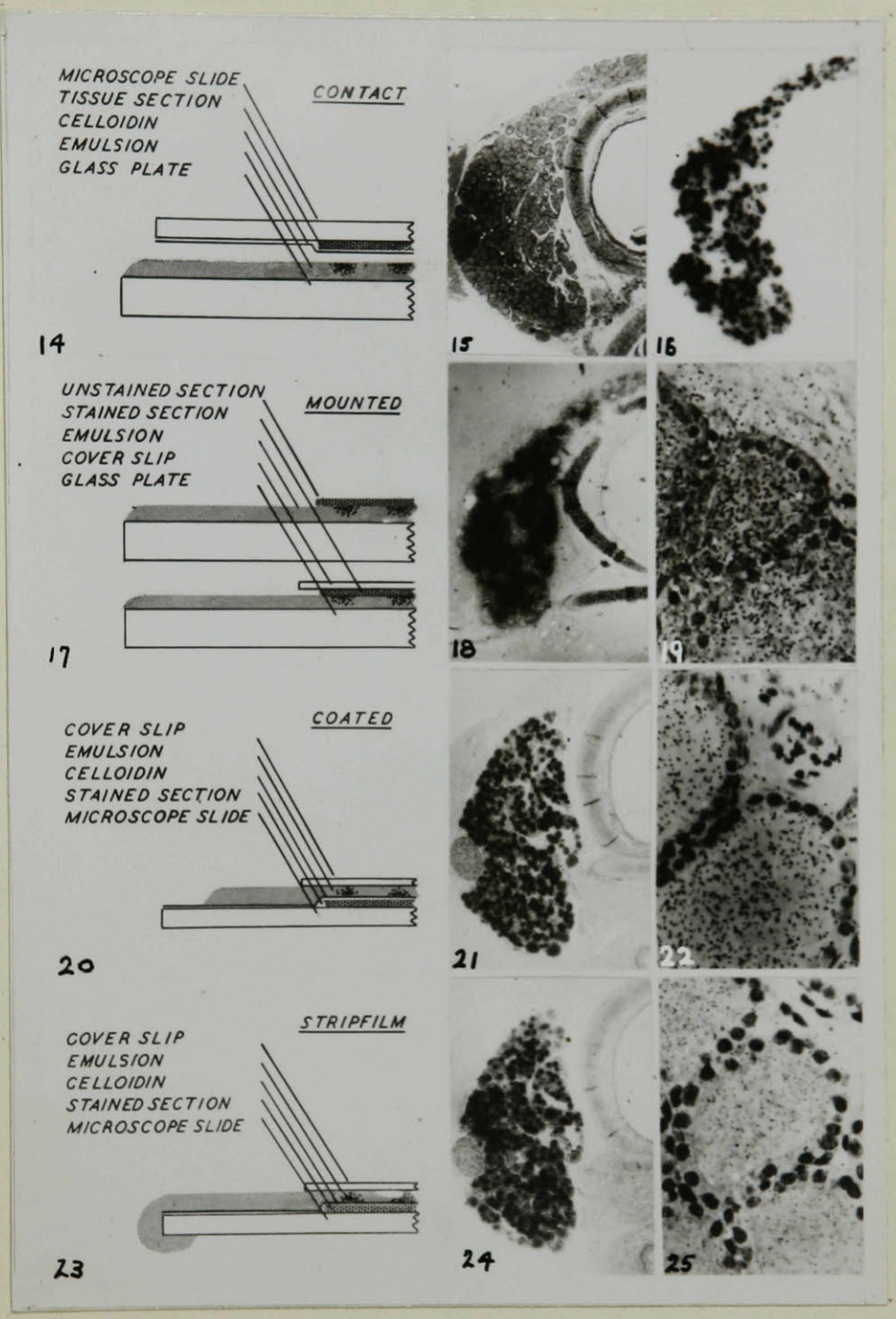
2. The contact method was the simplest to do while, of the newer techniques, the coated method was the easiest and gave the most reproducible results.

3. Both the liquid coated and the strip coated methods gave comparable results although the liquid coated method gave a

far neater and more esthetically satisfactory preparation.

Many autographs made with the use of the different techniques have appeared in the literature. If the autographs that appear in plates 2 and 7 are compared, it can be seen that the conclusions reached above are substantiated since the autographic results are optimum for the techniques used in each case. Some of the autographs that have been prepared by the author are included. Thus, Figs. 51 are contact autographs while in plate 3 all but Fig. 40 are liquid coated autographs as are Figs. 58,67,71,72,79,80,81,82. A descriptive legend for all the autographs in the following plates has been included to illustrate the sites of localization of radioactive compounds.

PLATE 1



This plate illustrates the various types of commonly used autographic methods. The schematic drawings on the left indicate the relationship of radioactive tissue section and photographic emulsion. The photographs on the right represent preparations made by the various methods from the same rat thyroid. The animal was sacrificed 20 hours after injection of 25 μc. of carrier-free radioiodine. Descriptive legends appear on the following pages.

Fig. 14 Schematic drawing indicating the relationship of the radioactive tissue section to emulsion in the contact method.

The section is placed in close contact with, but not attached to the emulsion. The two groups of black granules indicate the response of the emulsion to two radioactive areas in the tissue.

Fig. 15 Section of thyroid from the experimental animal. H. & E. Stain x 25

Fig. 16 Contact autograph obtained by placing the above section (Fig. 15) in contact with an EK contrast lantern slide plate. x 25

A comparison with the section show spot-like reactions, each one of which is due to a thyroid follicle. The parathyroid (middle left) does not react. This picture represents the maximal useful magnification for contact autographs. To obtain a resolution sufficient to distinguish the individual reactions of follicles as small as those of the rat, the preparation must be in closer contact with the emulsion.

Fig. 17 Two schematic drawings illustrating the mounted method. The upper diagram represents the section mounted on the emulsion, after development of the autographic granules. The lower diagram represents the final preparation after the section has been stained and protected with a coverslip. It is apparent that an intimate union between emulsion and section is obtained. Note that the developer must penetrate the section to reach the emulsion.

Fig. 18 Mounted autograph (EK Medium Lantern Slide) of thyroid from the experimental animal. H. & E. Stain x 25

The occasional round light areas are due to lack of development of the emulsion under certain follicles due to the failure of penetration of the developing solution. The greyish background is due to the staining of the emulsion gelatin

Fig.19. High power of a portion from Fig. 18. x 425

The concentration of silver granules over the colloid of the follicle is clearly visible. This concentration should be compared to that of the background fog, as seen over the unreactive tissue in the upper left hand corner.

Fig.20. Schematic drawing of a preparation made by the liquid coated method.

The stained radioactive tissue section is coated with a layer of liquid emulsion. A thin protective coat of celloidin between the section and the emulsion preserves the stain from effects of developer and fixer.

Fig.21. Coated autograph (EK Medium Lantern Slide Emulsion diluted 1 in 8 with 1% dupanol) of thyroid from the experimental animal. H. & E. Stain x 25

There are no processing artefacts. The histological detail of the section is visible through the unstained emulsion.

Fig.22. High power of a portion from Fig. 21. x 425

The reaction is localized in the colloid, with little fog being apparent over the unreactive tissue in the upper left corner.

Fig.23. Schematic drawing of a preparation made by the strip coated method.

The principle is the same as that of the coated autograph with strip emulsion being used instead of melted emulsion.

Fig.24. Strip coated autograph (Ilford Half-Tone Stripping Plate Emulsion) of thyroid from the experimental animal. H. & E. Stain x 25

The appearance at this magnification is very similar to that of the coated autograph.

Fig. 25. High power of a portion from Fig. 24.

It can be seen here that the granules of this type of emulsion are much smaller than those of the medium lantern slide emulsion (Fig. 22.).
H. & E. Stain x 425

PLATE 2

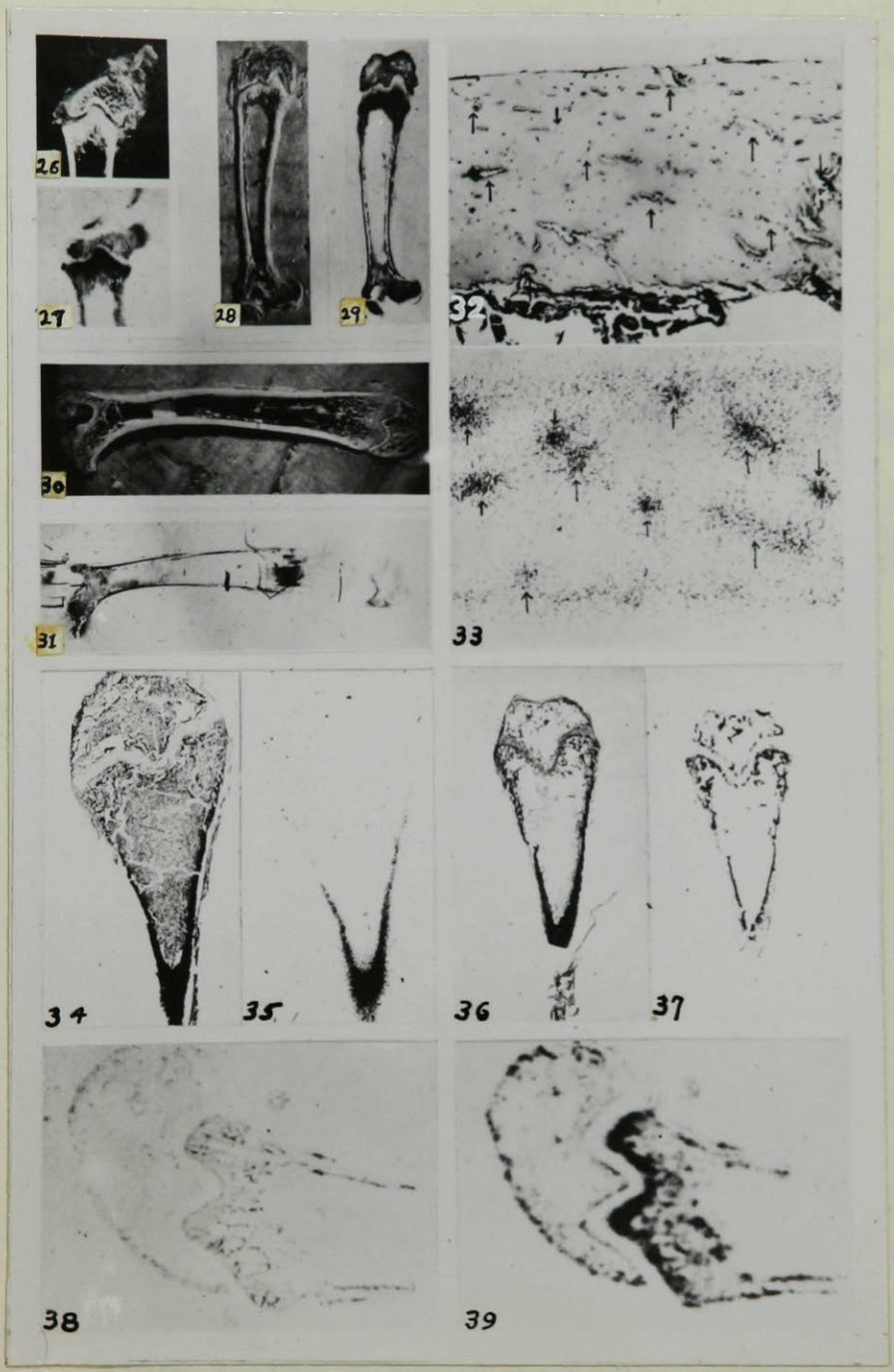


Fig. 26. Longitudinal section of distal end of femur of a growing rat injected intraperitoneally with 1.5 μc. of Ur^{233}, and sacrificed four hours after injection.

Fig. 27. Contact autograph (E.K. X-ray Plate) of Fig. 26 .

The most intense autographic reaction occurs on the diaphyseal edge of the epiphyseal plate, with a lesser one of the subepiphyseal spicules. Some reaction is also noted in the diaphyseal bone, as well as the trabeculae of cancellous bone in the head of the epiphysis.

Fig. 28. Longitudinal section of the femur of a growing rat injected with 1.5 μc. of Ur^{233} and sacrificed five days after injection.

Fig. 29. Contact autograph (Eastman X-ray Plate) of Fig. 28.

The autographic reactions are now slight on the diaphyseal edge of the epiphyseal plate and intense on the underlying spicules. The diaphyseal reaction seems mostly periosteal. A reaction is present in the cancellous bone of the epiphyses.

Fig. 30. Longitudinal section of the femur of a growing rat injected with 1.5 μc. of Ur^{233} and sacrificed 40 days after injection.

Fig. 31. Contact autograph (E.K. X-ray Plate) of Fig. 30.

The areas of bone fixing uranium soon after injection were not resorbed, thus the large spot visible in the marrow cavity on the middle right is due to unresorbed uranium-bearing spicules. At the proximal end of the shaft, bone has been deposited externally to the periosteal line of uranium deposition but has failed to resorb at the marrow surface, hence the thickening of the diaphyseal wall. (Fig. 30) (Figs. 26-31 from Neuman and Neuman (82).

Fig. 32. Shaft of bone of a rat injected intramuscularly with 10 μc. of carrier-free $Cm^{242}Cl_3$, and sacrificed seven days after injection. Stained. x 38

The arrows point to small blood vessels.

Fig. 33. Contact autograph of Fig. 32 .

The autographic reaction indicates the deposition of curium in the region of the small blood vessels. x 38

(Fig. 32-33 from Scott, Axelrod, and Hamilton (94))

Fig. 34. Femur of a rat weaned on a phosphorus-deficient diet at three weeks, injected with 5 μc. of Sr^{89} at five weeks and sacrificed one week later. Stained with hematoxylin, eosin and silver nitrate. x 10

Fig. 35. Contact autograph (No screen X-ray film) of Fig. 34 .

The autographic reactions indicate the deposition of Sr^{89} in the bone salt remaining in the shaft, and practically none in the unmineralized osteoid matrix below the epiphysis.

Fig. 36. Femur of a rat weaned on phosphorus deficient diet at three weeks, injected intramuscularly with 20 μg. plutonium at five weeks, and sacrificed one week later.

Stained with hematoxylin, eosin and silver nitrate. x 5

Fig. 37. Contact autograph of Fig. 36 .

The autographic reactions indicates heavy deposits of plutonium in the uncalcified osteoid matrix below the epiphysis and the endosteal region of the shaft.

(Figs. 34-37 from Copp. Axelrod, and Hamilton (22)).

Fig. 38. Femur of a young rat injected intravenously with 0.2 mg./g. of alizarin and 0.015 μc./g. of plutonium, and sacrificed 24 hours after injection. Undecalcified. x 8

The dark accumulations correspond to the areas of alizarin deposition.

Fig. 39. Contact autograph of Fig. 38.

The autographic reactions due to plutonium show a pattern of uptake similar to that of alizarin. The heaviest deposition is in the epiphyseal region just below the plate, with a less intense deposition on the subepiphyseal spicules and in the periosteal and endosteal regions.

(Figs. 38-39 from Bloom (13)).

PLATE 3

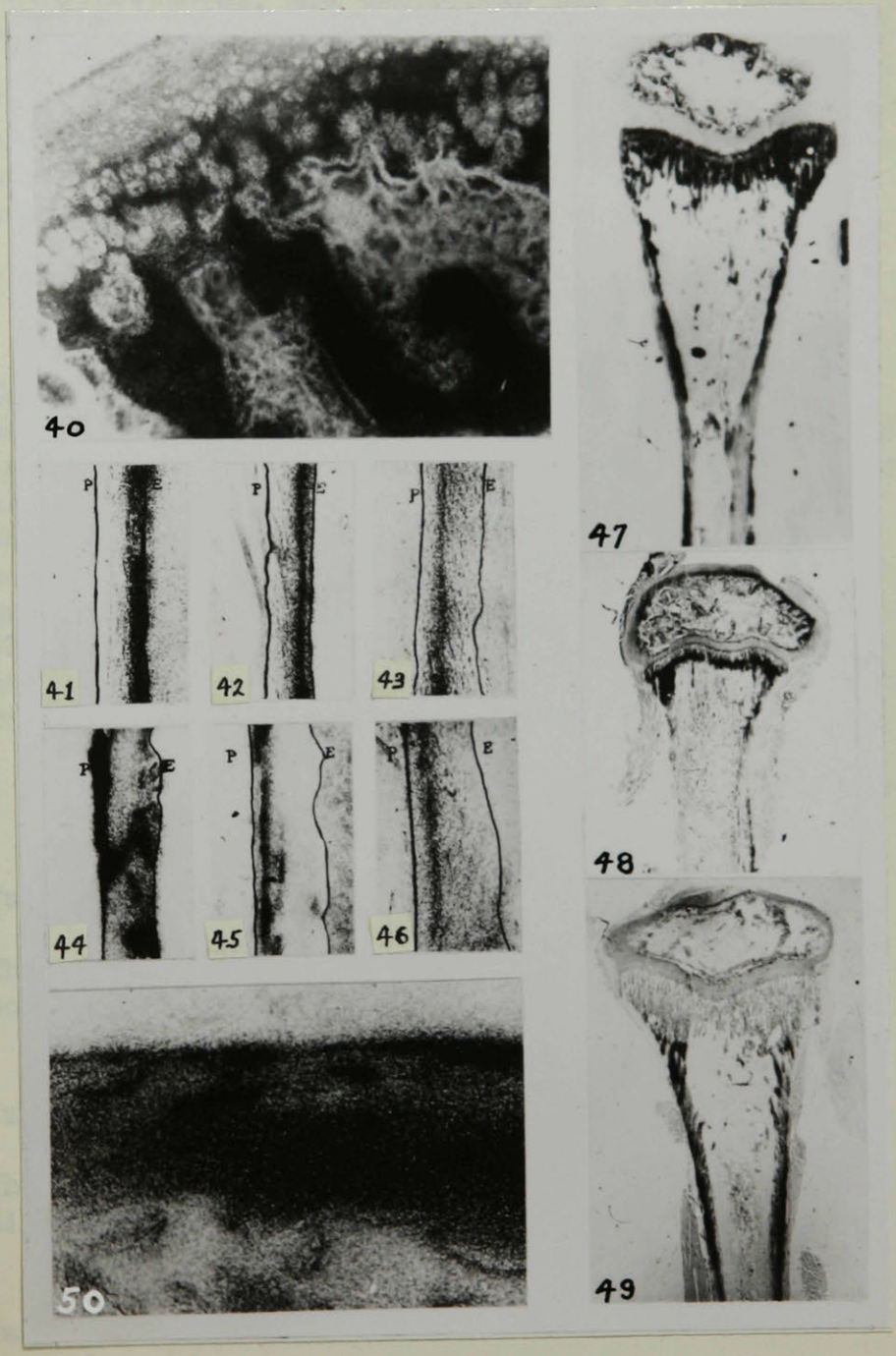


Fig.40. Inverted autograph (E.K. Matrix Emulsion) of endochondral ossification in the proximal tibial epiphysis from a 50 g. rat given a subcutaneous injection of carrier-free radiophosphorus, and sacrificed one hour later. Hematoxylin. x 100

The radio-phosphates deposit in the area of hypertrophic cartilage, on the trabeculi separating the enlarged cartilage cells. Its presence is also noted in the endochondral bone spicules.

(from Bélanger (8)).

Figs. 41-46. Coated autographs of various parts of the tibial diaphysis of 50 g. rats at various time intervals after injection of 350 µc. carrier-free P^{32}. The periosteal and endosteal surfaces of the diaphysis have been outlined with India ink, the letter P marking the periosteum on the left and the letter E the endosteum on the right. The first row (Figs.41-43) illustrates the reaction of the funnel-like subepiphyseal region, the second row (Figs. 44-46), that of the cylindrical region of the shaft. Unstained. x 30

(from Leblond, Wilkinson, Robichon and Bélanger (70)).

Fig. 41. Funnel at five minutes after P^{32} injection.

The reaction is located on the endosteal surface of the bone.

Fig. 42. Funnel at two days after P^{32} injection.

The reaction is located in the bone substance at some distance from the endosteal surface.

Fig. 43. Funnel at eight days after P^{32} injection.

The reaction is located in the bone substance and farther from the endosteum than in Fig. 42.

Fig. 44. Cylinder at five minutes after P^{32} injection.

The reaction is located on the periosteal surface of the bone. The spots in the bone substance correspond to blood channels.

Fig. 45. Cylinder at two days after P^{32} injection.

The reaction is located in the bone substance at some distance from the periosteal surface.

Fig. 46. Cylinder at eight days after P^{32} injection.

The reaction is located in the bone and farther from the periosteum than in Fig. 45.

Fig. 47. Coated autograph of the cylindrical region of the shaft from the tibia of a 50 g. rat subcutaneously injected with P^{32} and sacrificed two hours after injection. Safranin stain. x 100

At this early time interval, a diffuse reaction extends throughout the bone.

Fig. 48. Coated autograph of a longitudinal section of a tibia from a 50 g. rat subcutaneously injected with 350 µc. carrier-free P^{32} and sacrificed four hours after injection. Safranin stain. x 8

The lower edge of the epiphyseal plate (hypertrophic cartilage) shows an intense dark line, beneath which a light reaction of spicules may be seen. This is followed below by a fairly intensely-reacting area.

Fig. 49. Coated autograph of a longitudinal section of a humerus from a 50 g. rat subcutaneously injected with 350 µc. carrier-free P^{32} and sacrificed two days after injection. Safranin stain. x 8

The lowest part of the spicules shows a reaction, which appears as a dark line extending across the spicules.

Fig. 50. Coated autograph of a longitudinal section of a tibia from a 50 g. rat subcutaneously injected with 150 µc. carrier-free P^{32} and sacrificed eight days after injection. Safranin stain. x 8

The apparent downward motion of the dark line continues only on the spicules which are close to the wall of the diaphysis, as the most central and most peripheral spicules have been resorbed.

PLATE 4

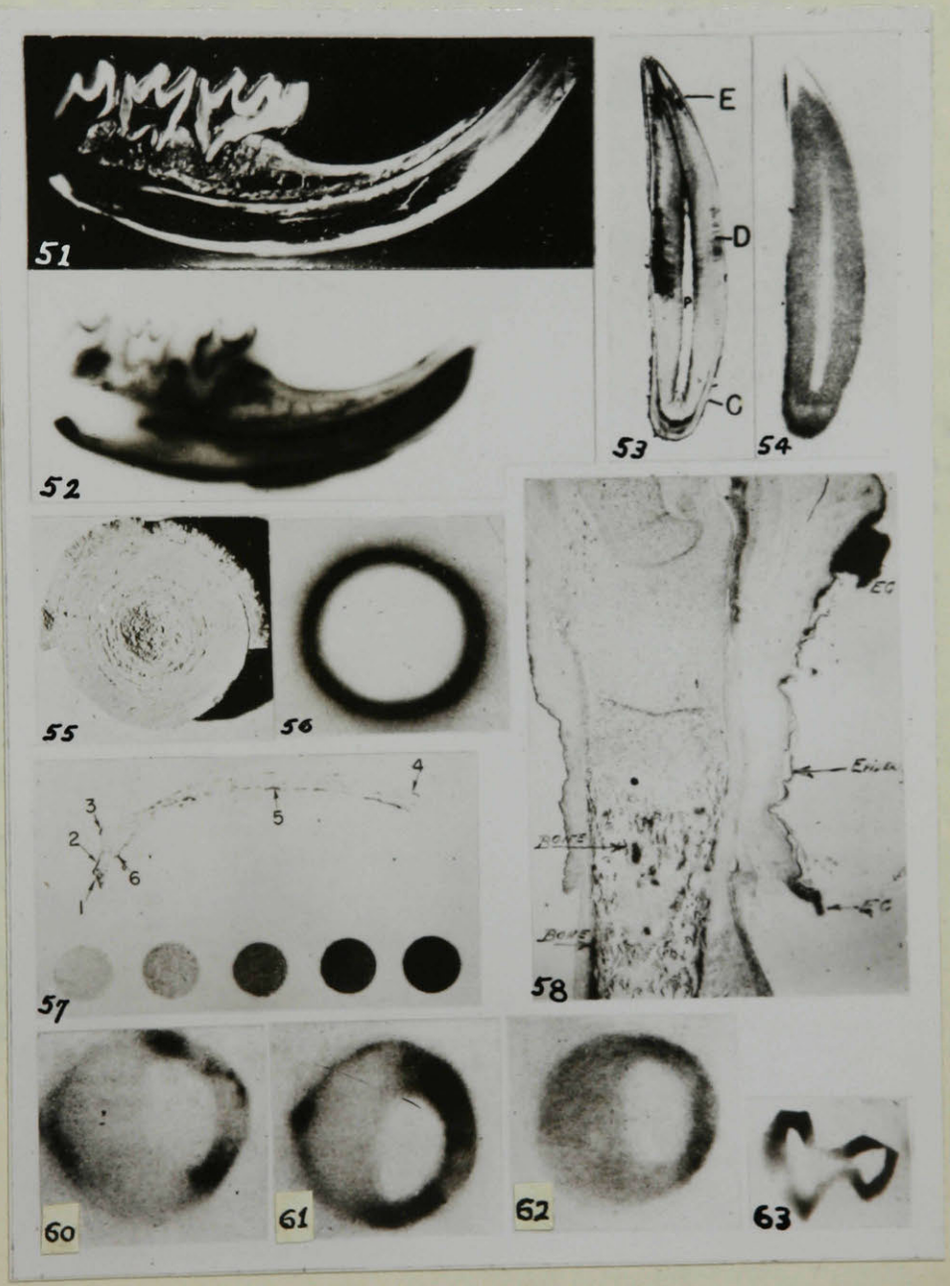


Fig. 51. Ground section of a lower jaw of a 70 g. rat injected with 0.5 mc. radiophosphorus containing 1 mc. carrier phosphate and sacrificed two hours after the injection. The jaw was dried without fixation. ca. x 8

The section shows the incisor tooth cut along its length showing solid dentine on the right hand side and the roots surrounding the pulp on the left. A thin line at the lower edge of the dentine represents the enamel. Three erupted molars are visible on the upper left.

Fig. 52. Contact autograph (E.K. Plate) of Fig. 51.

The dentine reacts only in the portion adjacent to the pulp. In the enamel organ only the newly formed enamel shows a reaction. Some reaction is also visible at the surface of the bone spicules of the mandible.

Fig. 53. Ground section of the canine of an adult cat, intraperitoneally injected with radio active iodine (0.5 mc/kg. of body weight) and sacrificed 12 hours after injection. Unstained. E, enamel; C, cementum; D, dentine; P, pulp.

Fig. 54. Contact autograph (Ansco No-Screen X-Ray Film) of Fig. 53.

Radioactivity is present in dentine and cementum and perhaps also in the enamel. The pulp area shows no reaction due to loss of the pulp in grinding. In the dentine there is an even density.

(Figs. 53-54 from Bartelstone, Mandel, Oshry, and Seidlin (4).)

Fig. 55. Prostatic calculus removed from a man who had received radioactive phosphorus sixty one days previous to the removal of the calculus.

Fig. 56. Contact Autograph of Fig. 55.

The radioactivity of the phosphorus is seen to be deposited at the outermost periphery of the calculus. No radioactive phosphorus was deposited in the central portion.

(Figs. 55 and 56 from Cristol, Bothe, and Grotzinger . (26))

Fig. 57. Contact autograph (E.K. No-Screen X-Ray) of a resected rib of a dog given radiocalcium orally, and sacrificed 21 days after administration. The circles of graded blackening are calibration exposures increasing in magnitude by factors of 2, produced from plaster of Paris blocks containing known amounts of Ca^{45}.

(From Dudley and Dobyns (31).)

Fig. 58. Coated autograph (E.K. Medium Lantern Slide Emulsion) of a section of bone from a newborn rat injected with carrier-free radiocarbon (as sodium carbonate) and sacrificed 24 hours after injection. Unstained.

Most intense reactions are found in the bone spicules located below the epiphysis and periosteum (lower left) as well as in the epidermis. The two dark spots, labeled EC, are pieces of epidermis cut parallel to the surface. Cartilage shows a moderate reaction, muscle a less intense one and connective tissue a slight one.

Figs.60-62. Contact autographs (dental X-ray film) of frozen slices of eyes from rabbits sacrificed at various time intervals after intraperitoneal injections of 0.6 to 0.8 mc. of radiosodium per kg. of body weight.

Fig. 60. 30 minutes after injection.

The radioactivity is predominantly in the ciliary body, choroid and optic nerve.

Fig. 61. One hour after injection.

The radioactivity increases in the same three sites as in Fig. 60 with some diffusion of the radioisotope into the vitreous. Diffusion in the anterior chamber seems to occur from the capillaries in the ciliary body of the iris.

Fig. 62. Forty-eight hours after injection.

The radioactivity is uniformly distributed throughout the eye, with the exception of the substance of the lens.

(Von Sallman, Evans, and Dillon (92)).

Fig. 63. Contact autograph (E.K. Spectroscopic Plates) of a ground section of the molar tooth of a dog injected with 20 mc. of radiosodium and sacrificed four hours after injection.

The autographic image shows the presence of sodium in the tissue of the tooth.

(From Berggren (11)).

PLATE 5

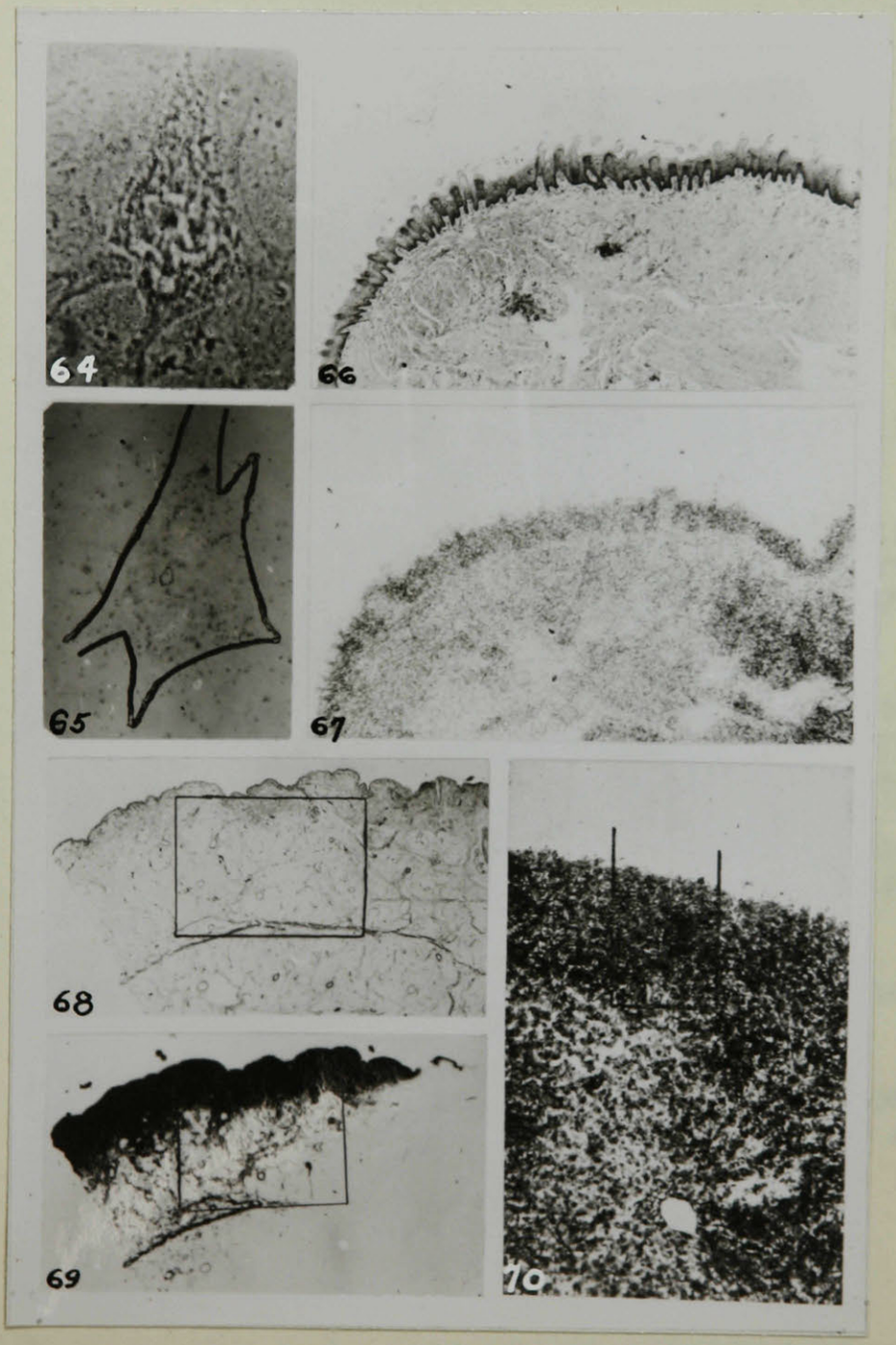


Fig. 64. Chick fibroblast cultivated in a drop of fowl plasma to which was added one drop of radiophosphorus solution (100 µc. carrier-free radiophosphate in 3 cc. sterile saline) and incubated for 48 hours. Unstained.

This cell is said to be in early prophase.

Fig. 65. Strip autograph (Ilford) of Fig. 64. The outline of the cell and the position of the nucleolus are drawn in.

The autographic reaction indicates an appreciable concentration of P^{32} in the cytoplasm and none in the nucleolus.

(Figs. 64 amd 65 from Pelc and Spear (Unpublished)).

Fig. 66. Photomicrograph of the tongue of a rat injected with 2 µc. P^{32} carrier-free and sacrificed two hours after injection. H. E. stain. ca. x 100

Fig. 67. Coated autograph (E.K. Medium Lantern Slide Emulsion) of section of rat tongue (serial section in Fig. 66.) injected with 2 µc. of carrier-free radiophosphate, and sacrificed two hours after injection. The histological section was treated with ribonuclease. Unstained. ca. x 100

The autographic reactions are mostly due to acid soluble phosphate, since inorganic and ribonucleic acid phosphate were removed by the histological procedure and desoxyribonucleic acid and protein incorporation of radiophosphorus was relatively small. Both the epithelium and the striated muscle show a reaction.

(Figs. 66-67 Stevens and Leblond (101)).

Fig. 68. Section of skin from pig exposed to mustard gas containing radiosulphur and killed 24 hours after exposure. H.-E. stain

Fig. 69. Contact autograph of Fig. 68.

The autographic reaction indicates the accumulation of mustard gas predominently in the epidermis and dermis where it infiltrates along connective tissue septa.

(Figs. 68-69 from Axelrod and Hamilton (3)).

Fig. 70. Inverted autograph (E.K. Type M Stripping Film) of rabbit liver incubated for two hours in vitro with C^{14} labeled sodium bicarbonate in the presence of pyruvate substrate. H.-E. stain. x 110

The black granules predominate at the periphery of the lobule (Fig. 66 from McDonald, Cobb, and Solomon (75)).

PLATE 6

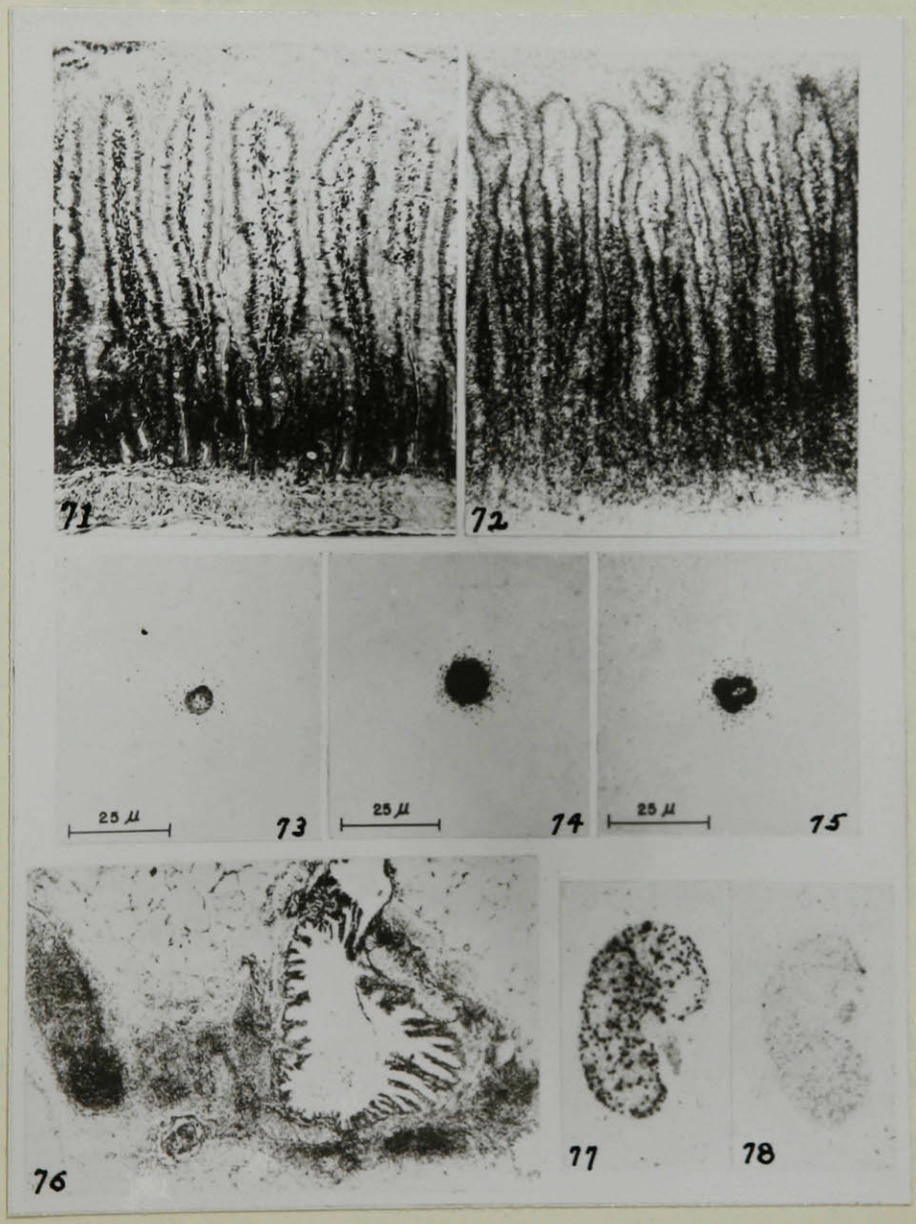


Fig. 71. Coated autograph (E.K. Medium Lantern Slide Emulsion) of the duodenum of a rat injected with 2.4 mc. of nearly carrier-free radiophosphate and sacrificed two hours after injection. Ribonuclease treatment. H.-E. stain. x 125

An intense reaction is located over the crypts of Lieberkuhn.

Fig. 72. Coated autograph of the duodenum of a rat injected with 2.4 mc. of nearly carrier-free radiophosphatase and treated as Fig. 71. x 125

An intense reaction is located over the nuclei in the epithelium of the villi up to two thirds of their height. Compare with Fig. 71.

(Figs. 71 and 72 from Leblond, Stevens and Bogoroch (69)).

Fig. 73. Mounted autograph (E.K. NTB Emulsion) of an erythrocyte from the venous blood of a rat given three intraperitoneal injections of 1 µc. of radiocarbon labeled glycine. Blood was withdrawn 25 hours after injections, smeared and stained with Wright's stain. x 950

The presence of an autographic reaction over the erythrocyte indicates the incorporation of glycine in the course of its formation.

Fig. 74. Mounted autograph of a lymphocyte prepared as in Fig. 73. x 950

Glycine is incorporated in proteins of the lymphocyte.

Fig. 75. Mounted autograph of polymorphonuclear leucocyte prepared as in Fig. 73. x 950

Glycine is incorporated in the proteins of this leucocyte.

(Figs. 73-75 from Boyd, Casarett, Altman, Noonan, and Salomon (19))

Fig. 76. Mounted autograph of a bronchus from a sensitized pig injected intravenously with about 8 mg. of radioiodine-labeled bovine gamma globulin which caused death from anaphylactic shock in about five minutes.
Hematoxylin x 50

The large blood vessel at left is filled with antigen. Antigen is also seen concentrated in the fibrous tissue around the bronchus. The smooth muscle immediately beneath the mucosa is conspicuously lacking in activity.

(Fig. 76 from Warren and Dixon (105)).

Fig. 77. Contact autograph (Ansco X-Ray No-Screen Film) of a kidney from a mouse injected with about 17 μc. of radioiodinated globulin fraction of anti-mouse kidney serum. The animal was sacrificed five days after injection.

Punctate autographic reactions are visible. These were attributed to an accumulation of the material in the glomeruli of the kidney cortex, where presumably specific antibodies are present.

Fig. 78. Contact autograph (Ansco No-Screen X-Ray Film) of a kidney from a mouse injected with 17 μc. of radioiodinated globulin fraction of anti-mouse plasma serum and sacrificed five hours after injection.

A diffuse autographic reaction is present throughout the tissue presumably due to content of the plasma in kidney.

(Figs. 77-78 from Pressman, Hill and Foote (90)).

PLATE 7

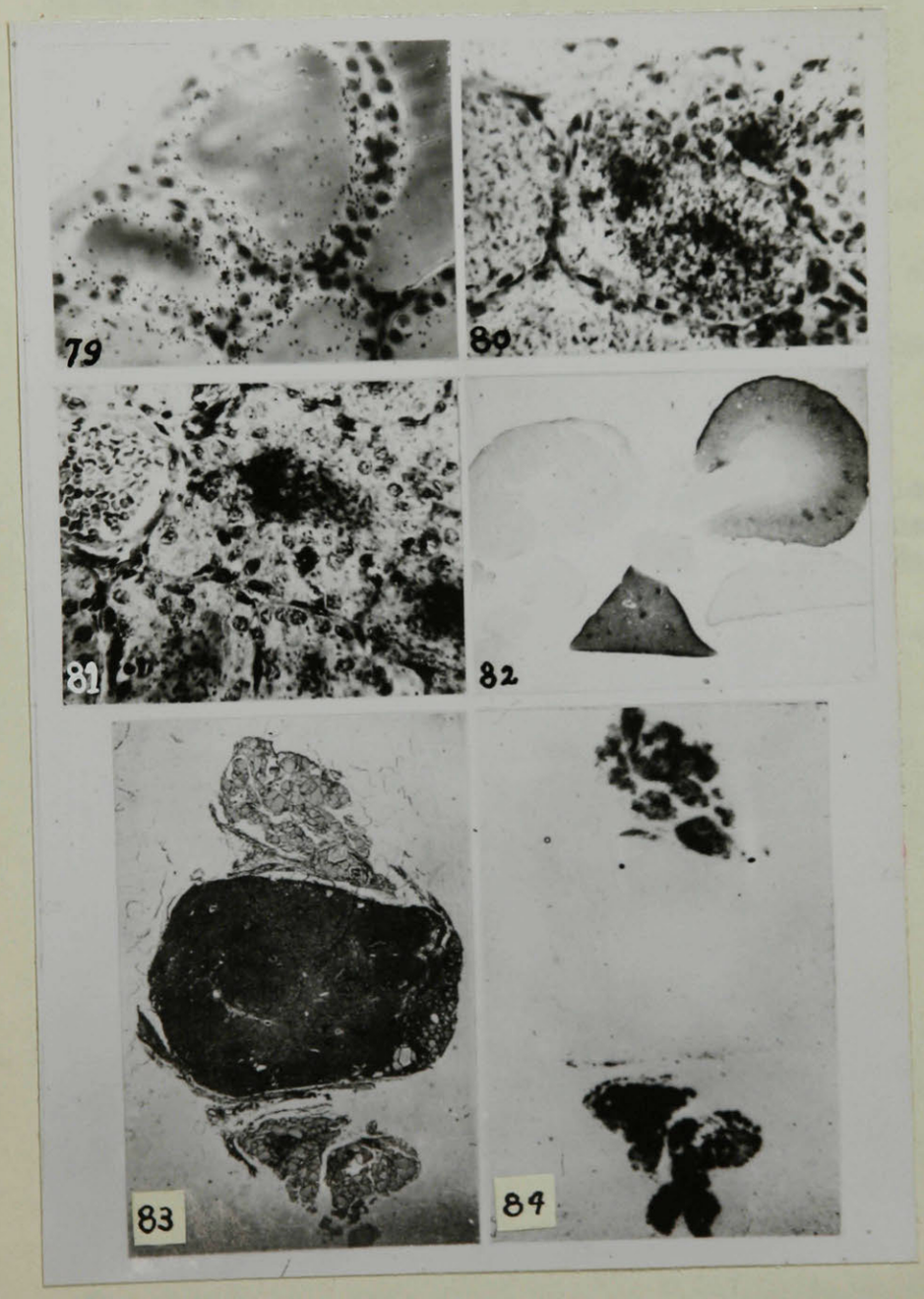


Fig. 79. Coated autograph (E.K. Medium Lantern Slide Emulsion) of a section of thyroid from a rat on a high iodine diet (22 µg. daily), injected with 20 µc. of carrier-free radioiodide and killed one hour after injection. H.-E. stain. x 600

The radioiodine is indicated by the black granules in the apical portions of the cells.

Fig. 80. Coated autograph as in Fig. 79 except that the animal was sacrificed 24 hours after injection. H.-E. stain. x 600

The radioiodine is now located uniformly throughout the colloid.

Fig. 81. Coated autograph (E.K. Medium Lantern Slide Emulsion) of a section of thyroid from a rat on a iodine-deficient diet, injected with 20 µc. carrier-free radioiodide and sacrificed one hour after injection. H.-E. stain. x 400

The radioiodine is concentrated evenly throughout the colloid. Only granules of background fog can be detected in the cells. This indicates the very rapid secretion of thyroglobulin from the cells into the colloid. Compare with Fig. 79. x 400

(Figs. 79-81 from Leblond and Gross (66)).

Fig. 82. Coated autograph of sections of organs (kidney, spleen, liver, pancreas, ovary and cross section of ventricules of the heart. The pituitary may be found between the kidney and the liver) from a rat injected with 100 µc. of radioiodide and sacrificed 48 hours after injection. Unstained. x 4

The reactions, are believed to be, at least, in part due to the radio-thyroxine formed from radioiodine by the thyroid. The strongest reaction is found in the liver and kidney. The remaining tissues show a diffuse concentration throughout their parenchymae. In the kidney most of the reaction is confined to the cortex while the medulla shows a lighter diffuse reaction.

Fig. 83. Photomicrograph of a section of undifferentiated embryonal adenoma of a thyroid from a patient given an oral administration of radioiodine. H.-E. stain

Fig. 84. Contact autograph of Fig. 83 The absence of an autograph in the area of the adenoma indicates the lack of function of that adenoma. The areas of blackening coincide with the normal thyroid tissue.

(Figs. 83 and 84 from Dobyns and Lennon (28)).

GENERAL DISCUSSION

Of the many methods used today by the biologist to meet the challenge offered by the problems of plant and animal physiology, those involving the use of radioisotopes are among the most recently developed. With these tools many hurdles, hithertofore apparently insurmountable, have been overcome by the research scientist.

Before radioactivity could be used in biological research, it was obviously essential that techniques be devised whereby the activity could be detected. The Geiger counter represents one of the methods for the detection of such activity in a substance, be it non-living or living. For the histologist, however, it was necessary to have a means of determining the relation of the radioactivity to the tissue structures. The technique of radio-autography answered this need.

After the discovery of Becquerel in 1895, when he noticed that a piece of uranium ore caused a blackening of a photographic plate in the dark, workers in biological research attempted to determine whether or not radioactivity in animal tissues would have a similar effect on photographic emulsions.

Lacassagne in 1924 injected polonium into experimental animals and prepared sections of various tissues and organs. He placed these sections in contact with the emulsion of a photographic plate and after some time found that upon photographic development a blackening of the emulsion occurred over those sites containing radioactivity which had been in contact with the emulsion.

The main improvements in the technique were not made until 1946 when Bélanger and Leblond, using the coated "autographic technique", and Yagoda and Endicott as well as Evans, using the mounted "autographic method" improved and refined the localization of radioactivity in animal tissues. It therefore became possible to associate the photographic densities caused by radioactivity with minute tissue structures.

In this Laboratory and in the Laboratory of Pelc, the theoretical considerations involved in the autographic techniques were examined. These studies suggested that the conditions necessary for optimum resolution in an autograph were 1) a thin tissue section, 2) a thin layer of emulsion, and more importantly, 3) a minimum distance between section and emulsion(interspace). Both the mounted and coated methods and their variants reduced the interspace; but only the liquid coated method allowed for the emulsion thickness to be varied at will. In this study it was shown that the coated technique did, in fact, produce an autograph of more superior resolution than did those of other techniques.

Theoretically, both the coated and the mounted methods should give comparable results since emulsion and section are intimately related. However, because of uneven penetration of the developer through the overlying tissue section, as well as the removal of deposited silver granules from the photographic emulsion by histological stains (post-staining), reproducible results are difficult to obtain. Nevertheless, this method is being used with fair success by many workers.

The most serious objection to the coated method is that prestaining of tissue sections may cause the radioactive element that is being detected to be removed or displaced whenever it is in a form that may be soluble in the acids or alkalis of certain stains. It would be advisable, therefore, to prepare unstained controls at the same time that stained autographs are prepared. In this way any shift in localization or any decrease in intensity of reaction due to removal of the radio-element will readily be noted.

Grain size, grain sensitivity, and grain concentration were also found to affect the resolution. Hence, it was necessary to study photographic emulsions in an effort to determine which of the available photographic emulsions could be used to give the most satisfactory results with the coated method. By comparing the autographs obtained by using emulsions in which the above factors varied, the theoretical assumptions were found to apply in practice. Thus, it was found that Ansco A emulsion, with the largest grain size gave the poorest resolution, while emulsions such as Ilford Stripping Plate and NTB, with very small grain size, gave the best resolution. Emulsions such as Eastman Kodak Contrast Plate and NTB pellicles, because of their lack of uniformity in melting, could not easily be used for liquid coated autographs.

Many authors still prefer to use other techniques rather than the coated one because of the rigid controls that

are necessary for each step involved. If all the techniques are compared, however, it is obvious that to obtain satisfactory results even in the simple contact method, rigid control and care is necessary for every step in every technique or variant thereof. The routine for coated autographs, once established, is relatively simple and may be rapidly carried out. In experienced hands 60 autographic preparations may be coated in one hour.

It is true that the liquid coated autographic method requires more elaborate apparatus than does the contact or mounted methods. If fine localization is not sought, the contact method may be used. This method is useful in clinical studies in cases where the uptake or lack of uptake of a radio-element by a tissue is to be ascertained, when radioiodine is given to patients with thyroid malignancies. This is the only known method available for hard tissues, but is, at best, very crude.

From the viewpoint of tracing radioactive substances from structure to structure in the animal organism, the aim of much biological research, the method which has to date best accomplished this has been the coated method of autography. A review of the recent literature using autographic methods verifies this point for it was by using the liquid coated method that Leblond and Gross were able to elaborate the theory of the mode of action of the thyroid gland and by this same method Leblond, Wilkinson, Robichon, and Bélanger were able to

substantially extend the theory of bone formation.

To date, the only method that can be used to obtain autographs of blood has been the mounted method described by Boyd et al. (19). However, since the blood cells are directly in contact with the photographic emulsion the possibility of chemical fogging is an important factor to consider. Indeed, Morse (107) found that by exposing non-radioactive blood cells to NTB_2 emulsion, and using the mounted method, an autographic image could be obtained. This chemical fogging factor makes it important to be extremely cautious in interpreting results obtained from using this technique for the detection of radio-elements.

The possibility of diluting emulsions so that a thin layer of emulsion may be used to coat a histological preparation is greatly responsible for an increase in the resolution of liquid coated autographs. This increase in resolution is to a considerable extent due to the relative reduction in the amount of background fog. Since the fog is distributed uniformly throughout the emulsion while the autographic reaction is most intense in an area in the emulsion closest to its site of origin, for β-particles of average energies, the decrease in emulsion thickness does not greatly affect the intensity of the autographic reaction.

For this reason, too, the coated method can give quantitative results, especially by using granule counts.

Densitometric studies require emulsion of uniform thickness which it is hoped will soon be achieved. Fairly satisfactory results have already been obtained. Therefore, for both fine localization and quantitative work, the coated method is at present the method of choice.

It is hoped, and indeed it is expected, that the improvements in the technique that have been presented will be superseded by more and better improvements and that an even better localization will become possible. Many important discoveries have appeared using the techniques as they exist today. Many more discoveries can and most likely will be made with these techniques - at present the only means available to obtain minute localization of radioactive substances.

From the experience gained in this Laboratory a summary of Emulsions and Techniques recommended for specimens containing various amounts of radioactivity has been compiled in Table VIII.

TABLE VIII

EMULSIONS AND TECHNIQUES RECOMMENDED FOR SPECIMENS CONTAINING VARIOUS AMOUNTS OF RADIOACTIVITY

SPECIMEN AND PURPOSE	METHOD RECOMMENDED	EMULSION RECOMMENDED FOR HIGH ACTIVITY	EMULSION RECOMMENDED FOR LOW RADIOACTIVITY
I. A. Gross Sections B. Sections too hard for cutting (e.g., bone, tooth) C. Frozen Sections (to retain labile, radioactive component)	Contact	a) EK Medium Lantern Slide Plate b) EK Contrast Process Plate	a) X-ray Film b) Ansco No-Screen c) Kodak No-Screen
II. Histological Sections - for fine localization	1. Liquid Coated (standard of thin emulsion)	a) EK Medium Lantern Slide Plate b) Ilford Half-Tone Stripping Plate c) Kodak NTB	Radioautographic Ansco A
	2. Strip Coated	a) Ilford Half-Tone Stripping Plate b) Kodak Stripping Film c) Kodak NTB_1 (Heineke) d) Kodak NTB_2 Stripping Plate	
	3. Mounted	a) EK Medium Lantern Slide Plate b) Kodak NTB_1 (Heineke) c) Kodak NTB_2 Stripping Plate	X-ray film
	4. Inverted	Eastman Kodak Matrix Film	
III. Histological Sections (unstained - for quantitative studies	1. Contact	a) EK Medium Lantern Slide Plate b) EK Contrast Process Plate	X-ray film
	2. Strip Coated	a) Ilford Half-Tone Stripping Film b) Kodak NTB_1 c) Kodak NTB_2	

SUMMARY AND CONCLUSIONS

1. A theoretical and technical study of the autographic method has been made in an effort to improve the localization of radio-elements in histological material.

2. The theoretical studies suggested that the most satisfactory localization would be obtained from an autograph in which:

a) the tissue section was thin, (5 μ),

b) the interspace between section and emulsion was minimal,

c) the emulsion coat was thin, (5-20 μ),

d) the silver bromide grain of the emulsion was small (about 1 μ) but had a high sensitivity,

e) the silver bromide grains of the emulsion were uniform in cases where the radioactivity was distributed relatively uniformly within a tissue, and

f) background fog was minimal.

3. Experiments to improve the liquid coated technique have been made by studying and improving each step involved in the technique.

4. A comparison of autographs prepared with the use of different emulsions led to the conclusion that thinned emulsions with small grains such as Ilford Half-Tone Stripping Plate Emulsion and NTB emulsions gave the best results.

5. Studies to remove background fog gave disappointing results. It was found that the most satisfactory way to reduce the amount of fog was to use thin emulsion coats.

6. One coat of 1% celloidin and one coat of 1% resin were found to be the thinnest protective medium of all those tested that would completely protect the stain in prestained tissue sections.

7. Each step in the preparation of the strip coated autograph has been examined and a method for its preparation has been described.

8. The details of the methods of other autographic techniques (the contact, the mounted, and variants such as the dry mounted, and inverted methods), has also been described.

9. A comparison of autographs made by various techniques has been made. It was concluded that the techniques which gave the most satisfactory autographs were those which best fulfilled the requirements suggested by theory, i.e., that the interspace between tissue section and emulsion should be minimal. Thus the coated and the mounted methods gave the best results.

10. The coated technique afforded the most satisfactory resolution of the techniques tested, and gave the most reproducible results.

11. Both the liquid coated and the strip coated technique gave comparable results although the liquid coated method gave a far neater and more esthetically satisfactory preparation.

BIBLIOGRAPHY

1. ASLING, C. W., D. A. HELLER, D. H. COPP, AND J. G. HAMILTON: Deposition of radioactive strontium and the rare earth promethium in the skeletal tissues of the rat. Anat. Rec., 103: 4, 1949.

2. AXELROD, D. J.: The radioautographic technique. U. S. Naval M. Bull., March-April (Suppl.) 122, 1948.

3. AXELROD, D. J., AND J. G. HAMILTON: Radio-autographic studies of the distribution of lewisite and mustard gas in skin and eye tissues. Am. J. Path., 23: 389, 1947.

4. BARTELSTONE, H. J., I. P. MANDEL., E. OSHRY,, AND S. M. SEIDLIN: Use of radioactive iodine as a tracer in the study of the physiology of teeth. Science, 106: 132, 1947.

5. BECQUEREL, H.: Sur les radiations invisible émises par les corps phosphorescents. Compt. rend. Acad. d. Sc., 122: 501, 1895.

6. BEHRENS, B., AND A. BAUMANN: Weiters Untersuchungev über die Verteilung des Bleis mit hilfe der methode der Autohistoradiographie. Ztschr. f. d. ges. exper. Med., 92: 241, 1933.

7. BEHRENS, B., AND A. BAUMANN: Die Beziehung zum der Bleiablagerung Calciumztoffwechsel. Ztschr. f. d. ges. exper. Med., 92: 241, 1933.

8. BELANGER, L. F.: A method for routine detection of radio-phosphates and other compounds in tissues: The inverted autograph. Anat. Rec., 1950. (In press).

9. BELANGER, L. F., AND C. P. LEBLOND: A method for locating radioactive elements in tissues by covering histological sections with a photographic emulsion. Endocrinology, 39: 8, 1946.

10. BELANGER, L. F., AND C. P. LEBLOND: Mineralization of the growing tooth as shown by radiophosphorus autographs. Proc. Soc. Exper. Biol. & Med., 1950. (In press).

11. BERGGREN, H.:: The mineral metabolism in the dental hard tissues. Tracer experiments *in vivo* with P^{32} and Na^{24} (Preliminary report). Acta radiol., 27: 248, 1946.

12. BLOOM, W.: Deposition of C^{14} in the metaphysis of long bones of young rats. Anat. Rec., 103: (Suppl.), 9, 1949.

13. BLOOM, W.: Histopathology of irradiation: From External and Internal Sources. McGraw-Hill Book Co., Inc., New York, Toronto, London, first ed., 1948.

14. BLOOM, W., H. J. CURTIS, AND F. C. McLEAN: The deposition of C^{14} in bone. Science, 105: Jan. 10, No. 2715, 1947.

15. BOYD, G. A.: The physical principles and techniques of autoradiographs. J. Biol. Photogr. A., 16: 65, 1947.

16. BOYD, G. A.: A comparison study of NTB and Medium Lantern Slide Emulsions for autoradiographs. AECD - 2106, May 25, 1948.

17. BOYD, G. A.: Personal communication.

18. BOYD, G. A., AND F. A. BOARD: A preliminary report on histochemography. Science, 110: 586, 1949.

19. BOYD, G. A., G. W. CASARETT, K. I. ALTMAN, T. R. NOONAN, AND K. SALOMON: Autoradiographs of C^{14} incorporated in individual blood cells. Science, 108: 529, 1948.

20. BOYD, G. A., AND H. LEVI: Carbon 14 beta track autoradiography. Science, 111: 58, 1950.

21. BOYD, G. A., AND A. I. WILLIAMS: Stripping film techniques for histological autoradiographs. Proc. Soc. Biol. & Med., 69: 225, 1948.

22. COPP, D. H., D. J. AXELROD, AND J. G. HAMILTON: The deposition of radioactive metals in bone as a potential health hazard. Am. J. Roentgenol., 58: 10, 1947.

23. COLFER, H. F., AND H. E. ESSEX: Radiopotassium autography of the rat's brain. Proc. Soc. Exper. Biol. & Med., 63: 243, 1946.

24. COUCEIRO, A.: Deposição do radiofósforo em tecidos osseo e dentário demonstrado pela tecnica autográfica. Mem. Inst. Oswaldo-Cruz, 41: 541, 1944.

25. COUCEIRO, A., L. G. VIEIRA, AND J. DE MORAES: Ação da tiouracila sobre a fixação de iodo pela tiroide. Rev. brasil. biol., 4: 173, 1944.

26. CRISTOL, D. S., A. E. BOTHE, AND P. W. GROTZINGER: Radioactivity and urinary tract calculi. New England J. Med., 239: 427, 1948.

27. DEMERS, P.: New photographic emulsions showing improved tracks of ionizing particles. Canad. J. Research, 25: A, 223, 1947.

28. DOBYNS, B. M., AND B. LENNON: A study of the histopathology of physiologic function of thyroid tumors using radioactive iodine and radioautography. J. Clin. Endocrinol., 8: 732, 1948.

29. DOLS, M. J. L., B. C. P. JANSEN, G. J. SIZOE, AND G. J. VAN DER LAS: Distribution of phosphorus in the leg bones of chickens. Nature, 142: 953, 1938.

30. DONIACH, D., AND S. R. PELC: Private communication.

31. DUDLEY, R. A., AND B. M. DOBYNS: The use of autoradiographs in the quantitative determination of radiation dosages from Ca^{45} in bone. Science, 109: 327, 1949.

32. DUDLEY, H. C., AND G. E. MADDOX: Deposition of radio gallium (Ga^{72}) in skeletal tissues. J. Pharmacol. & Exper. Therap., 96: 224, 1949.

33. DUIJN, C., Jr. VAN: The reduction of negatives. Am. Photogr., 41: 31, 1947.

34. ENDICOTT, K. M., AND H. YAGODA: Microscopic historadiographic technic for locating and quantitating radioactive elements in tissues. Proc. Soc. Exper. Biol. & Med., 64: 170, 1947.

35. EVANS, F. C. : Radioautographs in which the tissue is mounted directly on the photographic plate. Proc. Soc. Exper. Biol. & Med., 64: 313, 1947.

36. ERDBACHER, O., AND E. WANNEMACHER: Zur Frage von Stoffwechsel-vorgangen in den Zahnbartgewehen. Deutsche Zahn-, Mund u. Kleferheilk., 8: 201, 1941.

37. FINDLAY, D., AND C. P. LEBLOND: Partial destruction of rat thyroid by large doses of radio-iodine. Am. J. Roentgenol., 59: 387, 1948.

38. FINKEL, M.: Transmission of radio-strontium and plutonium from mother to offspring in laboratory animals. Physiol. Zool., 20: 405, 1947.

39. FITZGERALD, P. J., AND F. W. FOOTE: The function of various types of thyroid carcinomas as revealed by the radioautographic demonstration of radioactive iodine (I^{131}). J. Clin. Endocrinol., 9: 1153, 1949.

40. FITZGERALD, P. J., F. W. FOOTE, Jr., AND R. F. HILL: Concentration of I^{131} in thyroid cancer, shown by radioautography. A study of 100 consecutive cases showing the relation of histological structure to the function of thyroid carcinoma. Cancer, 3: 86, 1950.

41. FRANZ, V. K., E. QUIMBY, AND T. C. EVANS: Radioactive iodine studies of functional thyroid carcinomas. Radiology, 51: 532, 1948.

42. GORBMAN, A.: Radioautography in biological research. Nucleonics, 2: 30, 1948.

43. GORBMAN, A., AND C. W. CREASER: Accumulation of radio-active iodine by the endostyle of larval lampreys and the problem of homology of the thyroid. J. Exper. Zool., 89: 391, 1942.

44. GORBMAN, A., AND H. M. EVANS: Correlation of histological differentiation with beginning of function of developing thyroid gland of frog. Proc. Soc. Exper. Biol. & Med., 47: 103, 1941.

45. GORBMAN, A., AND H. M. EVANS: Beginning of function in thyroid of fetal rat. Endocrinology, 32: 113, 1943.

46. GROSS, J.: Formation and fate of thyroid hormone. Doctoral Dissertation, McGill University, 1949.

47. GROSS, J., R. BOGOROCH, N. J. NADLER, AND C. P. LEBLOND: The theory and methods of the autographic localization of radioelements in tissues. Am. J. Roentgenol., 1950. (In press).

48. GROSS, J., AND C. P. LEBLOND: Histological localization of radioactive elements. A review. McGill M. J., 15; Dec., 1946.

49. HAMILTON, J. G.: The metabolism of the fission products and the heaviest elements. Radiology, 49: 325, 1947.

50. HAMILTON, J. G., AND M. H. SOLEY, AND K. B. EICHORN: Deposition of radioactive iodine in human thyroid tissue. Univ. Calif. Pub. Pharmacol., 1: 339, 1940.

51. HEVESY, G.: Radioactive indicators. Interscience Publishers, New York and London, 1948.

52. HEVESY, G., AND F. H. PANETH: A manual of radioactivity. Oxford University Press.

53. HOECKER, F. E., AND R. G. ROOFE: Structural differences inbone matrix associated with metabolised radium. Radiology, 52: 856, 1949.

54. JAMES, T. H., AND G. KORNFIELD: Reduction of silver halides and mechanism of photographic development. Chem. Rev., 30: 1, 1942.

55. KATZAREFF, A.: Photography with radium emanation. J. de radiol. et d'elect., 6: 131, 1922.

56. LACASSAGNE, A., AND R. TERROUX: Nouvelle demonstration de l'absence de fixation elective de l'émanation du radium sur les tissus cancéreux. Compt. rend. Soc. de biol., 93: 1356, 1925.

57. LACASSAGNE, A., AND J. LATTES: Methode autohisto-radiographique pour la detection dans les organes du polonium injecté. Compt. rend. Acad. d. Sc., 178: 488, 1924.

58. LACASSAGNE, A., AND J. LATTES: Mise en evidence par l'autoradiographic des organes, des localization histologiques du polonium injecté dans l'organisme. Bull. d'histol. appliq. à la Physiol., 1: 279, 1924.

59. LACASSAGNE, A., J. LATTES AND J. LAVEDAN: Etude expérimentale des effects biologiques au polonium introduit dans l'organisme. J. de radiol. et d'elect., 9: 1, 1925.

60. LAUDE, P., R. C. JANES AND J. D. BOYD: A simplified machine for making ground sections of teeth or bone. Anat. Rec., 104: 11, 1949.

61. LAWRENCE, J. H.: The new neuclear physics and medicine. Caldwell Lecture, 1941. Am. J. Roentgenol., 48: 283, 1942.

62. LAWRENCE, J. H.: The use of isotopes in medical research. J. A. M. A., 134: 219, 1947.

63. LEBLOND, C. P., L. F. BELANGER, R. BOGOROCH, C. E. STEVENS, J. GROSS AND G. W. WILKINSON: Detection of radio-elements by various radio-autographic techniques. Anat. Rec., 103: (Suppl.), 161, 1949.

64. LEBLOND, C. P., L. F. BELANGER, J. GROSS, J. ROBICHON, R. BOGOROCH AND R. D. JACOBS: Technical improvements of the "coating" autographic technique for demonstration of I^{131} and P^{32} in tissues. Anat. Rec., 100: (Suppl.), 138, 1948.

65. LEBLOND, C. P., M. B. FERTMAN, I. D. PUPPEL AND C. M. CURTIS: Radioiodine autography in studies of human goitrous thyroid glands. Arch. Path., 41: 510, 1946.

66. LEBLOND, C. P. AND J. GROSS: Thyroglobulin formation in the thyroid follicle visualized by the "coated autograph" technique. Endocrinology, 43: 306, 1948.

67. LEBLOND, C. P. AND A. LACASSAGNE: Specific action of polonium on the lymphatic system as shown in adrenalectomized animals. Am. J. Roentgenol., 50: 801, 1943.

68. LEBLOND, C. P., W. L. PERCIVAL AND J. GROSS: Autographic localization of radio-iodine in stained sections of thyroid gland by coating with photographic emulsion. Proc. Soc. Exper. Biol. & Med., 67: 74, 1948.

69. LEBLOND, C. P., C. E. STEVENS AND R. BOGOROCH: Histological localization of newly-formed desoxyribonucleic acid. Science, 108: 531, 1948.

70. LEBLOND, C. P., G. W. WILKINSON, L. F. BELANGER AND J. ROBICHON: Radio-autographic visualization of bone formation in the rat. Am. J. Anat., 86, 1950. (In press).

71. LIEBERMANN, L. N. AND H. H. BARSCHALL: A method for removing microscopic fog from photographic plates. Rev. Sc. Instr., 14: 89, 1943.

72. LONDON, E. S.: Etude sur la valeur physiologique et pathologique de l'emanation du radium. Arch. d'élect. méd. et de physiotherap. du cancer, 12: 363, 1904.

73. MARINELLI, M. A., F. W.,FOOTE, R. F. HILL AND A. F. HOCKER: Retention of radioactive iodine in thyroid carcinomas. Histopathologic and radio-autographic studies. Am. J. Rontgenol., 58: 17, 1947.

74. MacDONALD, A. M., J. COBB AND A. K. SOLOMON: Radioautograph technique with C^{14}. Science, 107: No. 2786, May 21, 1948.

75. MacDONALD, A. M., J. COBB, A. K. SOLOMON AND D. STEINBERG: Stripping film technic for radioautographs. Proc. Soc. Exper. Biol. & Med., 72: 117, 1949.

76. MEES, C. E. K.: The theory of the photographic process. The MacMillan Company, 1946.

77. MÜLLER, J. H., von: Zur Klinischen Bedeutung des autoradiographischen Verfahrens bei der Verwendung von Kurzlebigen Kunstlichen radioactiven Isotopen. Experientia, 4: (fasc. 10), 406, 1948.

78. MÜLLER, J. H., von: Contribution à l'étude au métabolisme du Zinc, par la méthode des indicateurs radio-actifs (utilization de l'isotope radioactif artificiel Zn^{65}). Bull. Acad. suisse d. Sc. méd., 3: 56, 1947-48.

79. MÜLLER, J. H., von: Ueber die Verwendung von künstlichen radioaktiven Isotopen zur Erzielung von lokalisierten biologischen Strahlenwirkungen. Experientia, 2: (fasc. 9), 1946.

80. MÜLLER, J. H., von: AND P. H. ROSSIER: De l'emploi d'isotopes radioactif, dans le but d'exercer un effet radio-biologique localisé. Experientia, 3: (fasc. 2), 75, 1947.

81. NACHMAN, H. M., V. CRAWFORD AND I. A. BIGGER: Radioactive iodine (I^{131}) in the diagnosis of lingual thyroid. J. A. M. A., 140: 1154, 1949.

82. NEUMAN, M. W. AND W. F. NEUMAN: The deposition of uranium in bone. II. Radioautographic studies. J. Biol. Chem., 175: 711, 1948.

83. PALM, E.: On the phosphate exchange between the blood and the eye. Experiments on the entrance of radioactive phosphate into the aqueous humor, the anterior uvea and the lens. Acta ophth., Suppl. 32, 1948.

84. PALM, E.: The exchange of phosphate between the blood and the eye, studied with the aid of radio-autographs. Acta ophth., 27: (fasc. 2), 267, 1949.

85. PERCIVAL, W. L.: Rapid exchange of the bone salts in the skeleton of new born animals. Master of Science Dissertation, McGill University, 1948.

86. PERCIVAL, W. L. AND C. P. LEBLOND: Rapid exchange of the bone salts in newborn rat as demonstrated with radioactive phosphorus. Rev. canad. de biol., 7: 217, 1948.

87. PELC, S. R.: Autoradiograph technique. Nature, 160, 749, 1947.

88. PELC, S. R.: Calculations of factors affecting blackening and resolution. (Private communication).

89. PECHER, C.: Biological investigations with radioactive calcium and strontium. Univ. Calif. Publ. Pharmacol., 2: 117, 1942.

90. PRESSMAN, D., R. F. HILL AND F. W. FOOTE: The zone of localization of anti-mouse-kidney serum as determined by radioautographs. Science, 109: 65, 1949.

91. SALLMANN, L., von AND B. DILLON: Studies of the eye with radioiodine autographs. Am. J. Ophth., 33: 429, 1950.

92. SALLMANN, L., von, T. C. EVANS AND B. DILLON: Studies of the eye with radiosodium autographs. Arch. Ophth., 41: 611, 1950.

93. SCOTT, K. G., D. J. AXELROD, J. CROWLEY AND J. G. HAMILTON: Deposition and fate of plutonium, uranium and their fission products inhaled as aerosols by rats and man. Arch. Path., 48: 31, 1949.

94. SCOTT, K. G., D. J. AXELROD AND J. G. HAMILTON: The metabolism of curium in the rat. J. Biol. Chem., 177: 325, 1949.

95. SCOTT, K. G., D. H. COPP, D. J. AXELROD AND J. G. HAMILTON: The metabolism of americium in the rat. J. Biol. Chem., 175: 691, 1948.

96. SIEGBAHN, K.: The disintegration of Na^{24} and P^{32}. Phys. Rev., 70: 127, 1946.

97. SIFFERT, R. S.: The demonstration of P^{32} in bone by radioautography. Science, 108: 445, 1948.

98. SIRI, W. E.: Isotopic tracers and nuclear radiations, with application to biology and medicine. McGraw-Hill Book Company, Inc., New York, Toronto, London, First ed., 1949.

99. SMITH, E. AND P. GRAY: The distribution of copper in the early chick embryo. Anat. Rec., 99: (Suppl.), 52, 1947.

100. SOGNNAES, R. F., J. H. SHAW, A. K. SOLOMON AND E. HARVOLD: A method for radioautography of specimens composed of both hard and soft structures. Anat. Rec., 104: 319, 1949.

101. STEVENS, C. E.: Radio-autographic reactions in tissues other than bones and teeth after injection of radio-phosphorus. Anat. Rec., 103: (Suppl.), 93, 1948.

102. THOMAS, H. E. AND F. H. BRUNER: Chronic radium poisoning in rats. Am. J. Roentgenol., 29: 641, 1933.

103. TREADWELL, A., B. V. A. LOW-BEER, H. L. FRIEDELL AND J. H. LAWRENCE: Metabolic studies on neoplasms of bone with the aid of radioactive strontium. Am. J. M. Sc., 204: 521, 1942.

104. VAN CLEAVE, C. D.: A radioautographic study of the localization of radioactive europium in certain soft tissues of the rat. Anat. Rec., 103: (Suppl.), 98, 1949.

105. WARREN, S. AND F. J. DIXON: Antigen tracer studies and histologic observations in anaphylactic shock in the guinea pig. Am. J. M. Sc., 216: 136, 1948.

106. YAGODA, H.: Radioactive measurements with nuclear emulsions. John Wiley and Sons Inc., 1949.

107. MORSE (Unpublished).