per 100 ml at the start of the observation period. The average hemoglobin level closely paralleled that of the xanthopterin-treated anemic rats during the 12-day observation period. Only 1 rat was alive at the end of 12 days. Weight and leukocyte changes also were similar to those noted in the xanthopterin-treated rats.

Discussion. Norris and Majnarich[15] have not indicated the mechanism of production of the anemia they observed in rats fed a purified diet containing sulfathiazole. Sulfathiazole has been reported by Richardson[17] to cause hemolytic anemia and the hemolytic anemia-producing properties of related sulfonamides have been studied by Latven and Welch.[18] That such a hemolytic process operated in the rats of Norris and Majnarich[15] is indicated by the production of an anemia within 22 days after the diet was started, and by the rapid fall in erythrocyte counts noted after the brief rise following therapy. Against a hemolytic process are the apparently very low reticulocyte levels which were reported by them; it would seem certain, however, that a measurable erythrocytic response following therapy in any type of severe anemia would produce greater reticulocyte responses than the 0.8 and 1% maximum values that they observed. It is suggested that the reticulocyte counts reported by Norris and Majnarich[15] are in error.

The effects shown here of PGA and xanthopterin in rats made anemic by feeding a purified diet containing sulfathiazole do not confirm the observations of Norris and Majnarich.[15] No positive leukocytic responses were noted after therapy with xanthopterin, although marked responses occurred after the administration of PGA. Doses of xanthopterin larger than the doses they used failed to produce the distinct increases in circulating erythrocytes that they recorded. Highly variable results also were obtained when PGA was given to those rats fed a purified diet containing sulfathiazole; however, those PGA-deficient rats made anemic by hemorrhage, and then given PGA, all showed nearly equal, marked erythropoietic responses.

Summary. 1. A purified diet containing sulfathiazole produced a hemolytic anemia of variable intensity which could not be completely reversed either by pteroylglutamic acid or by xanthopterin in any of the doses used.

2. Rats fed a purified diet containing succinylsulfathiazole developed leukopenia and an inhibition of growth which responded to PGA, but not to xanthopterin.

3. In similar rats fed a purified diet containing succinylsulfathiazole, but made anemic by repeated hemorrhage, PGA induced definite erythropoietic, leukopoietic and growth responses, while xanthopterin was inactive.

17 Richardson, A., *J. Pharmacol. and Exp. Therap.*, 1941, **72**, 99.

18 Latven, A. R., and Welch, A. D., *J. Pharmacol. and Exp. Therap.*, 1944, **81**, 301.

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Stripping Film Technics for Histological Autoradiographs.

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There are now three general technics of autoradiography. The first method developed was that of placing the specimen in contact with a photographic plate, removing the specimen, and developing the plate. London[1] studied anatomical specimens in 1904 and Lacassagne and Lattes,[2] histological specimens

1 London, E. S., *Arc D'elec. Med.*, 1904, **12**, 363.

2 Lacassagne, M. A., and Lattes, J. S., *Compt. rend. Acad. Sci.*, 1924, **178**, 488.

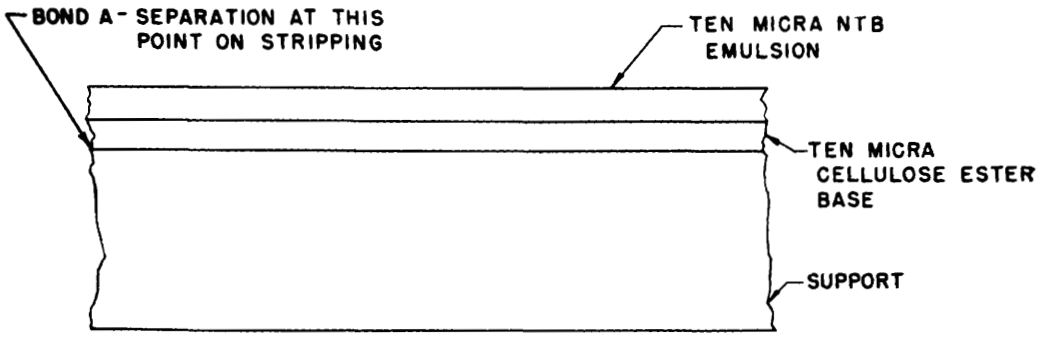

FIG. 1.
Schematic illustration of Eastman NTB stripping film used for autoradiographs.

in 1924 in this manner. In recent years Bélanger and Leblond[3] developed the procedure of painting the tissue section with a photographic emulsion. Endicott[4] and Evans[5] independently worked out a third technic of permanently laying a tissue section on the emulsion of a plate or film. Each of these methods has its advantages and disadvantages, some of which have been discussed elsewhere.[6]

The use of stripping film has certain advantages. With the tissue mounted on the emulsion of the film stretched over a frame, back scattering is eliminated, and thus, in theory, resolution is increased. We have not as yet proved that this is of practical value. Another advantage is the ability to make autoradiographs of blood and bone marrow smears on microscope slides by the simple procedure of laying the film on the smear. With the histological specimen mounted on the photographically insensitive and water impervious base, it is separated by 10 μ from the autograph. This enables one to prevent harm to the tissue while developing and fixing and to eliminate staining of the emulsion. This separation of tissue and emulsion also enables one to identify histological bodies coincident with an alpha sunburst. Also, the separation opens the way to a semi-quantitative estimation of beta emitters by counting the grains which can be seen beneath the darkly stained nuclei because of the difference in the focal plane. And since the tissue is not in contact with the emulsion chemical fogging is eliminated, an application which will probably prove to be most valuable.

Beginning in the early fall of 1947, we have developed methods of using stripping film to achieve these advantages. During this work there appeared the paper of Pelc[7] on the use of stripping film.*

Description of the film. The stripping film was made in experimental quantities for this work by the research laboratory of the Eastman Kodak Company. Nuclear Track Emulsion B (NTB) was used as this had been found to be the most sensitive to beta particles for its grain size. It can also be used for alphagraphs. The sensitivity and fogging qualities of the emulsion on plates will be discussed in another paper. The stripping film comprises a thick cellulose ester support to one side of which has been bonded a thin film (approximately 10 μ) of another cellulose ester which serves as the base for the emulsion. A diagrammatic sketch is shown in Fig. 1. The thicker cellulose ester serves merely as a rigid support for the manufacture and transport of the base-emulsion combination, the latter being the film which is stripped.

[3] Bélanger, L. F., and Leblond, C. P., *Endocrinology*, 1946, **39**, 8.

[4] Endicott, K. M., and Yagoda, H., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 170.

[5] Evans, T. C., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 313.

[6] Evans, T. C., *Nucleonics*, 1948, **2**, 52.

* While this paper was in the process of declassification there appeared another paper on stripping film: "Radioautograph Technic with C^{14}," A. M. MacDonald, Jock Cobb, and A. K. Solomon, *Science*, 1948, **107**, 550.

[7] Pelc, S. R., *Nature*, 1947, **160**, 749.

The base, *i.e.*, the 10 μ cellulose ester film, is required to hold the low rigidity emulsion. This is especially true of the heavily loaded NTB emulsion with its low gelatin content. The rigidity of the emulsion has been further reduced in comparison to the ordinary emulsion by having been reduced in thickness to increase resolution of the betagraphs. Most of the satisfactory emulsions which we have used have been approximately ten micra although other thicknesses can be obtained.

The background grain problem. While the NTB emulsion on plates has an extremely low fog background, the same emulsion when stripped from the support may contain considerable fog, depending upon the stripping conditions. This appears as long streaks of single grain width, streaks of multiple grain width, and splotches. The streaks of single grain and multiple grain width are always on the surface and can be differentiated from nuclear tracks which dive at various angles into the emulsion. Some nuclear tracks do occur occasionally on the surface, but their length and grain density, as determined by those that do dive into the emulsion, differentiate them from the surface streaks.

However, the splotches cannot be differentiated from a random distribution of grains produced by beta particles. Therefore, if one wished to use stripping film for betagraphs, every precaution must be taken to prevent fog development. While we do not yet know how to eliminate all background grains, the following hints will aid in reducing them.

The usual precautions must be taken against light leakage and the presence of fogging chemicals in both the darkroom and dark storage boxes. For example, it is imperative that dark boxes, even though made of 1" wood, be painted inside and out with black paint. Unpainted wood will transmit sufficient light to fog these emulsions. Obviously, one should not touch the emulsion with the hands. Abrasion fogging can be prevented by lifting the film out of the box instead of pulling it out edgewise and by preventing contact of any non-yielding object with the emulsion. It should be emphasized that the technics of ordinary amateur or scientific photography will not suffice for precise beta autoradiography. A few fog grains which would not greatly influence interpretation of a spectrographic plate or a photomicrograph of a histological section can lead to error in the interpretation of a betagraph under high magnification. In the latter there must either be no background fog or a known and constant quantity if one wishes even a semiquantitative estimate of the beta emitter in a histological section.

Stripping. The stripping of the base from the support is one of the greatest sources of fog. In the breaking of the bond between the base and the support, static charges are produced. If the film is stripped rapidly, these charges produce a light visible to the dark-adapted eye. This, though small in amount, will fog the emulsion as can be determined by examination under the microscope with a 1.8 mm objective. If the film is stripped slowly so that no light is observed, the fog is reduced but not eliminated. Even with this precaution, however, the static charges are retained by the film. Apparently the charges either produce latent images directly or indirectly by unperceived photons. The charges can be reduced by stripping in a humid atmosphere.

The technic used in our laboratory is as follows: A section of the film of the required size is protected in some suitable manner with a light opaque shield in addition to the usual darkroom precautions. It can be wrapped in black paper if care is taken to prevent the stiff paper from scratching the emulsion. Another method is to use a dark box with a slot in one side, the slot being covered with velvet. In either case about 1 cm is exposed. With the exposed end on the table top, emulsion side down, cut deeply but not completely through the support. (The position of the emulsion to be used for the autograph will be some distance removed from this point and protected from radiation by the shield.) Break only the support at the cut, *i.e.*, do not break the support and the base. On the break, photons are generated but the shield protects the emulsion. After the break the shield is removed and the base is stripped slowly while blowing the humid

breath onto the point of bondage to discharge the static electricity. As the base-emulsion combination is stripped it should make as small an angle with the support as possible to avoid mechanical fogging due to creasing of the emulsion.

Preparation of autograph with the tissue on the emulsion. Autographs can of course be made by placing the tissue either on the emulsion or on the cellulose ester base. In the latter case, some resolution is lost because of the spatial separation of the tissue from the emulsion. For the highest resolution, the tissue is placed on the emulsion, thus decreasing to a minimum the distance between the origin of the ionizing particles and the emulsion; and at the same time the stripping film on a frame eliminates backscattering.

A 2″ x 6″ section of the stripped film is floated on water, the emulsion side down. It is then transferred to a Lucite frame 2″ x 6″ x ⅛″ by placing the frame in the water beneath the film. In the center of the frame is a rectangular opening 1″ x 3″ which is covered with the film, exposing the emulsion inside the ⅛″ well and the cellulose base on the other side. The tissue is placed in this area on either side.

The tissue section has been prepared before entering the darkroom as follows: One or more sections from a ribbon of paraffin-imbedded tissue is floated on water in a petri dish on a hot plate.[5] The water is warmed until the wrinkles have disappeared. This is now picked up by inserting a section lift under the edge of the paraffin and lifting it from the water while the remaining portion of the section hangs from the edge. The angle at which the section lift is pulled out of the water as illustrated in Fig. 2 is necessary to enable the tissue to hang freely. This manipulation is facilitated by drilling a large number of holes in the section lift to permit the water to drain from under the section. If the water drains over the edge of the lift, the current will carry the tissue section back into the petri dish.

The free end of the tissue section is placed on the wet emulsion immediately after the film is placed on the Lucite frame. With a horizontal motion the tissue is freed from the section lift and laid flat on the film. Any excess water is permitted to drain off or is picked up by a small hand syringe. The preparation is placed in a lead lined dark box in the refrigerator for exposure.

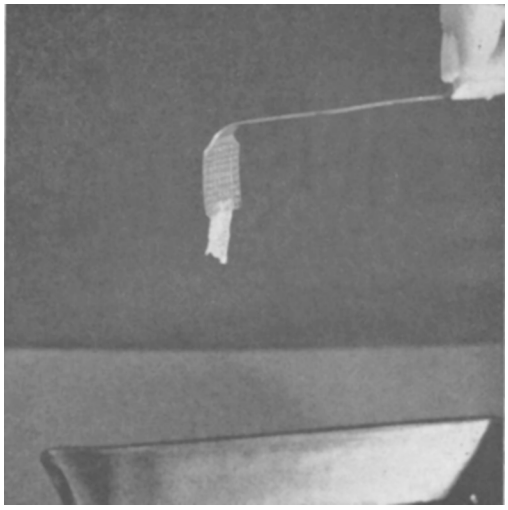

FIG. 2.

Removing a tissue section from water with a section lift, the perforated spoon held vertically to prevent the tissue from curling around the edge. The bottom of the tissue is touched to the photographic emulsion with the lift in the same vertical position after which the spoon is backed away horizontally from the tissue, permitting it to lie on the surface.

As the film dries it becomes taut and smooth over the 1″ and 3″ well and the gelatin of the emulsion bonds the film to the Lucite frame. Occasionally after long exposure the film becomes loose at the end of the frame and curls. Paper clamps can be used to prevent this, but they should not be put in place until after the film has dried. If placed on earlier, the contraction of the gelatin in drying may break the film.

The paraffin is removed in the usual manner before development[5] which is accomplished by passing the frame, film, and tissue as a single unit through the solutions. After development in D-19 at 20°C for 2 minutes, fixing in 30% hypo and washing for about 10 minutes each, the 1″ x 3″ film is cut out and placed on a microscope slide, the emulsion side up. When thoroughly dry the cellulose ester base usually adheres to the glass. If it

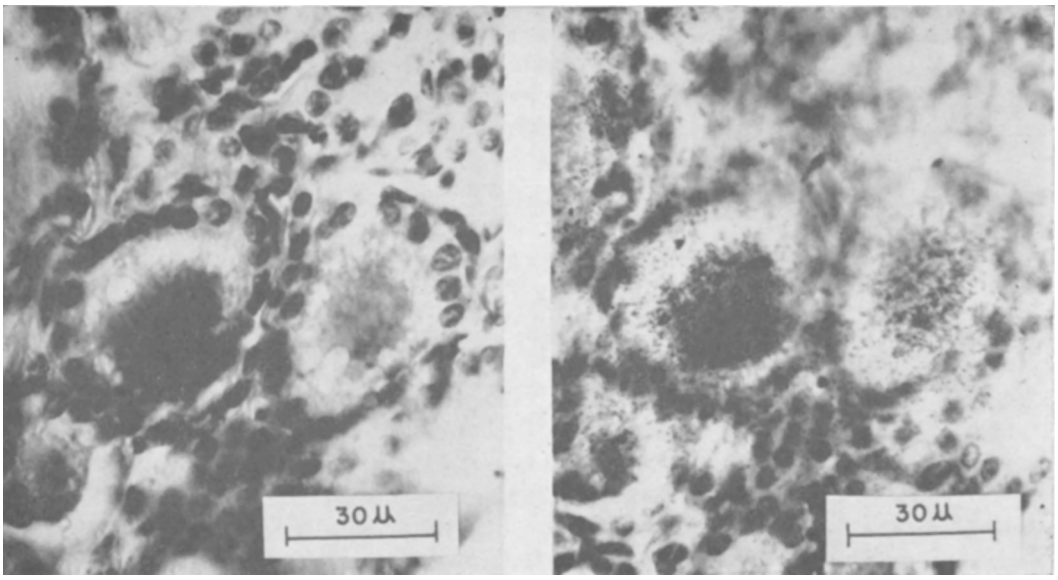

FIG. 3.
a. (left) Tissue level. b. (right) Grain level.
Photomicrographs of a thyroid tissue-autograph. A 7 μ section containing I^{131} from a rat sacrificed 24 hours after injection of 500 μc, exposed 17 hours on NTB stripping film.

fails to bond, the glass can be painted with egg albumin or Kodak Stripping Film Cement. The excess film can now be cut away from the tissue and autograph area if desired. The entire unstained tissue and film can be mounted in Clarite, Permount, etc., and examined with the dark phase microscope.

However, since the developing and fixing solution slightly changes the refractive index differential of the histological bodies, *i.e.*, clears the tissue optically, the phase examination does not always give the best results. In this case the tissue and emulsion after having been placed on the slide can be stained in the usual manner. Some stain is taken up by the gelatin of the emulsion, but the gelatin content in ten micra of heavily loaded emulsion is low and the consequent uptake of hematoxylin and eosin is low in comparison with 7 μ of tissue. The background stain is insignificant and color contrast good at high magnification.

Fig. 3 shows a thyroid autograph taken with stripping film. The tissue was placed on the emulsion and later stained with hematoxylin and eosin. Fig. 3-a shows the tissue focal plane, originally photographed with a 1.8 mm objective and a 10x occular. The grain focal plane is shown in photograph 3-b. These illustrate a difficulty which, while it is not impossible to overcome, requires careful handling of the film. It will be seen that the entire field of each is not in good focus. This is due to uneven laying of the film on the glass slide.

Preparation of the autograph with the tissue on the cellulose ester base. If it is desired to eliminate all staining of the emulsion, the tissue can be placed on the cellulose ester base. In this procedure the base is painted with egg albumin to seal the tissue and prevent it from coming off in the developing, fixing, or staining solutions. However, as one or 2 days, is required for complete drying of the albumin, we have found it expedient in exposures under 2 days to develop and fix by placing the solutions in the $\frac{1}{8}$" well of the Lucite frame, removing them by tipping to one side and draining. This prevents the washing off of the tisue. The preparation is dried well before placing in the staining solutions.

Since in this method the paraffin is not removed before development, the tissue is protected and clearing is prevented. After development the tissue-emulsion area is cut out

as described above and placed on a microscope slide, emulsion side down. The tissue is now deparaffinated in xylol and taken through the alcohol-water solutions to water and then the edges of the film sealed by dipping in molten paraffin. The tissue is then stained. The paraffin around the edges of the film is removed and the tissue-film combination mounted as described previously.

The emulsion which is protected by the aqueous-impervious paraffin and the cellulose ester is not stained, thus giving a high color differential between the tissue and the gelatin. The phase microscope can also be used without staining. The tissue intensity differentials as seen by the phase microscope have not been reduced by partial clearing, since the tissue has been protected in the developing and fixing solutions by the paraffin.

Stripping film for blood smears. The ideal blood smear for an autoradiograph has the cells separated by several micra so that the autograph of the beta emitters of one cell will not be confused with those of adjoining cells. It is difficult to make such a smear on an emulsion since the gelatin imbibes the water so quickly that the cells will not flow easily.

A thin smear is made on a microscope slide in the usual manner, stained for bright field illumination or left unstained for phase examination. In either case it is fixed for about 3 minutes with absolute methyl alcohol. After drying thoroughly, it is flooded with water and a piece of the film, emulsion side up, is floated over the smear. The water is then drawn out from under the film with filter paper, permitting the film to settle uniformly and without wrinkles over the smear. Since the fixing solution destroys the stain properties of the leukocytes, the procedure of paraffin dipping the edges is used to protect the smear during the developing and fixing steps. After washing and drying the paraffin is cut away with a razor blade and the smear, covered with the film, is mounted under a glass slip.

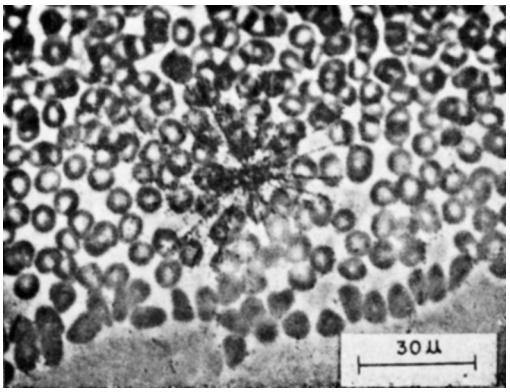

FIG. 4.
Sunburst of alpha tracks centered on a red cell.

Fig. 4 shows a concentration of a source of alpha particles coincident with one erythrocyte as evidenced by the center of an alpha track sunburst. This is a dark contrast phase photomicrograph of an unstained smear.

It should not be inferred from the photomicrograph that the alpha emitter is attached to the cell. It cannot yet be definitely stated whether the element concentrate is associated with the red cell in the blood stream or whether it has settled upon the cell in the smearing procedure. Some concentrations of alpha tracks have been found unassociated with cells. The exact nature of this phenomenon is being investigated and will be reported at another time.

Two types of fog patterns interfering with interpretation can be pointed out in this photomicrograph. In the lower right-hand corner of the photograph is a fog streak. It is obviously different from the straight alpha tracks of one grain width. Neither can it be a beta track as it is too dense and straight. Nor does it fit the description of other nuclear particle tracks.[8] This type of streak appears in the NTB stripping film and not in the NTB plate. We suggest that they originate in the stripping operation. In addition to the streaks the random fog grains should be noted. While these do not detract from the interpretation of an alphagraph, they do detract from that of a betagraph.

Stripping film to permit identification of histological bodies above sunbursts. Fig. 5-a and 5-b show alphagraphs which have been made by placing a liver section containing

[8] Powell, C. F., Occhialini, G. P. S., Nuclear Physics in Photographs, Oxford at the Clarendon Press, 1947.

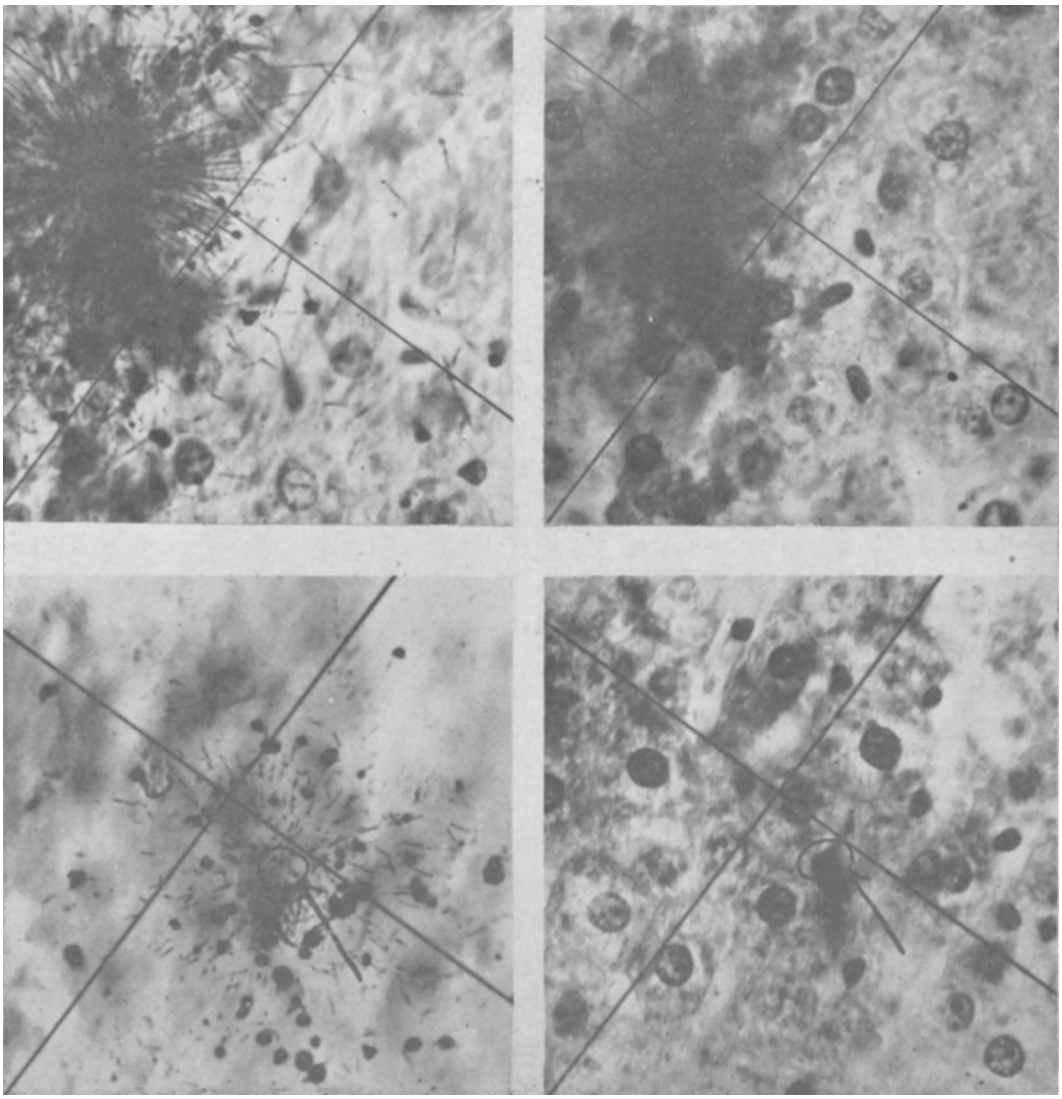

FIG. 5.

a (top left); b (top right); c (lower left); d (lower right).

Illustration of stripping film technic in identifying a histological body of rat liver containing an alpha emitter. In a and b the tissue section is on the emulsion of an NTA plate; a, track level; b, tissue level. In c and d the tissue is on the cellulose ester base of NTB stripping film; c, end of tracks in emulsion showing center of burst under a von Kupffer cell indicated in d.

an alpha emitter directly on an NTA (Nuclear Track A) plate, the former being a photograph of tracks in the emulsion, *i.e.*, just below the tissue-emulsion interface, and the latter is of the tissue directly above the tracks. Because of the high concentration of silver grains in the center of the sunburst there is insufficient illumination from below to permit observation or photography of the histological body directly above. Since this body presumably contains the collection of the radioactive element it is of interest to identify it.

When another section from the same liver specimen was placed on the cellulose ester support of a stripped film, photographs 5-c

and 5-d were obtained. Only the low energy ends of the tracks are seen in 5-c, the concentration of silver grains being prevented by the insensitive layer of cellulose ester. Fig. 5-d shows sufficient illumination to observe and photograph the histological body directly above the center of the alpha track ends. This body (circled) appears to be a von Kupffer cell. This is a tentative conclusion awaiting further checking.

Stripping film to permit estimating relative amounts of beta emitters in tissue. The estimation of relative quantities of an alpha emitter is relatively easy since an alpha track representing a single disintegrated atom is a definite grain pattern and can easily be recognized. If the geometry and exposure time and half-life are known, a fair estimate of the concentration of an alpha-emitting element can be made. Certain precautions have to be taken. For example, the exposure must not be so long as to give a sunburst density as shown in Fig. 5-a in which the tracks cannot be counted. Also, if an optically dense histological body should override an alpha track, its chances of detection would be diminished.

These difficulties are magnified for the estimation of a beta emitter since no recognizable pattern of grains is produced. Instead, single grains must be counted. In the case of the thyroid, for example, where the colloid is reasonably transparent, the grains underneath the colloid can be easily distinguished one from the other. However, when one attempts to count the grains beneath the follicular epithelial cells, the grains are obscured by the optical density of the stained cells and their nuclei especially. This is illustrated in Fig. 3.

Stripping film offers an escape from this impassé. The tissue is mounted on the cellulose ester, 10 μ thick, instead of on the emulsion. Examining with a 1.8 mm objective having a field depth of about 0.5 μ and focusing the microscope 10 μ beneath a section to observe the grains, the optically dense bodies of the tissue are sufficiently out of focus and thereby transparent to permit counting the grains immediately beneath them. However, this advantage is not gained without some loss in resolution due to the 10 μ separation of the tissue from the emulsion. But this is a small loss compared with the gain in resolution of the NTB emulsion over that of medium lantern slide emulsions.

An additional advantage of the stripping film technic is a method of laying a uniformly thin emulsion layer close to a single cell or tissue. Either the emulsion or the 10 μ base can be placed in contact with the specimen. The latter differs from the emulsion-painting technic of Bélanger and Leblond[3] in that the emulsion is separated from the tissue by the thickness of the base. The stripping film has the advantage over the painting technic in that the base and emulsion are standardized at the factory by those experienced in the art of making photographic emulsions.

Another advantage of the stripping film is its use in the prevention of chemical fogging. As emulsions become more sensitive to beta particles we will be able to detect smaller amounts of beta emitters in a tissue section. However, as the technic becomes more sensitive, the problem of eliminating fogging due to various naturally occurring chemicals in the tissue looms large. The mounting of sections on the cellulose ester base instead of directly on the emulsion will eliminate this difficulty.

Summary. An autoradiographic technic, using stripping film, is described by which back scattering, emulsion staining, chemical fogging, and photographic-developer damage to the tissue are eliminated; blood smear autographs can be made; histological bodies above intense collection of grain can be identified; and grain counting for quantitation is permitted.

We appreciate the valuable assistance of Mr. Robert Hay in the preparation of the final photographs for this paper.

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