infection, showing abortive fevers of one to 2 days duration, in contrast with the afebrile course of the animals treated with the antiserum. The serological analysis of these "normal" sera gave evidence of lysins against sheep cells with titers of 1:20 or 1:40, and in some cases as high as 1:200. This has been recorded by other workers and attributed either to constitutional genetic factors (Witebsky and Neter[6]), or to past infections of heterogeneous origin (Hyde[7]). Attempts to investigate the possible role of the hemolysins by their absorption from both normal and immune sera with boiled sheep cells do not suggest correlation of heterophile antibodies with the observed protective action.

Experiments with guinea pig antiserum No. 91 also showed protective qualities when challenged with the highly virulent spotted fever, namely, abortive fever reactions, or complete absence of fever were observed. No greater protection was afforded by the globulin fraction of antiguinea pig serum (Complement fixation titer 1:300; hemolysin titer 1:1250) used in dilution 1:10. Slight elevations of body temperatures not exceeding 40°C for 1-2 days were recorded in 5 guinea pigs of the 10 used in the series. The temperatures of the other 5 animals remained normal over the 18 days observation. On the contrary, the 3 untreated controls developed typical spotted fever. As observed in the typhus series, the course of spotted fever was occasionally attenuated by certain "normal" rabbit sera.

Conclusions. Antiguinea pig rabbit immune sera, when injected intradermally into guinea pigs inhibited the clinical manifestations of typhus and spotted fever after the infective material was inoculated at the site of the serum administration. Highly protective properties against typhus and spotted fever, previously absent in the serum of untreated rabbits, were demonstrated in the antisera of the same animals after their immunization with guinea pig spleen and bone marrow. However, an attenuation of typhus and spotted fevers occurred when serum of certain "normal" rabbits was used. The presence of Forssman antibodies in the rabbit immune sera and of sheep cell lysins in the "normal" sera seems of no significance. Among the factors involved in the observed phenomena, the barrier effect of the skin and its modified permeability are probably decisive. The role of the spreading factor in the invasiveness of rickettsiae is under systematic experimental study.

The authors make no suggestions as to the possibilities of utilizing the methods and results described as a practical application for protection against or treatment of typhus or spotted fever.

[6] Witebsky, E., and Neter, E., *J. Exp. Med.*, 1935, **61**, 489.

[7] Hyde, R. R., *Am. J. Hyg.*, 1928, **8**, 205.

16209

Autographic Localization of Radio-Iodine in Stained Sections of Thyroid Gland by Coating with Photographic Emulsion.*

C. P. LEBLOND, W. L. PERCIVAL, AND J. GROSS. (Introduced by J. S. L. Browne.)
From the Department of Anatomy and Department of Experimental Surgery, McGill University, Montreal, Canada.

Radioactive materials emit radiations which have the same effect as light on a photographic emulsion. This property has been used to locate radio-elements in histological sections of tissues, and especially radio-iodine in the thyroid gland.[1] The radioactive section is usually placed in contact with a photographic film or plate.[2,3,4] The image or "autograph"

* This work was supported by a grant from the National Research Council of Canada.

[1] Gross, J., and Leblond, C. P., *Canad. Med. Assn. J.*, 1947, **57**, 102.

obtained by this method is rather hazy and cannot be easily compared with the tissue section. These disadvantages are obviated by coating the section with fluid photographic emulsion and staining it after development.[5] A similar modification consists of mounting the sections directly on photographic plates.[6] These technics, however, resulted in erratic staining and some dissolution of silver granules in the staining reagents. The method, was, therefore, modified to permit staining of the section before application of the emulsion. This procedure, described below, results in an autographic image which may be observed under the high powers of the microscope (Fig. 1 and 2).

Technical Details. The thyroids of animals treated with radioactive iodine are fixed in Bouin's fluid or neutral formalin; mercury-containing fluids, such as Susa or Helly, cannot be used. The tissues are embedded in paraffin, sectioned at 3 to 5 micra and stained with either hematoxylin and eosin or Masson's trichrome. The stained sections, dehydrated through graded alcohols, are left for 1 minute in a 1% celloidin solution and dried. In the case of the trichrome stain, however, the slides should be quickly dipped into 1% celloidin for a few seconds, dried and dipped again. All celloidin-coated sections are then dried for at least 6 hours in order to harden the celloidin coating and thus protect the stains from the action of developer and fixer.

Subsequent operations are carried out in the darkroom at 2 to 3 feet from a No. 1 Wratten Safelight. The Kodak medium lantern slide plates used for the preparation of the coating emulsion are sampled for freedom from fog. The plates are soaked in distilled water for 10 minutes at 19° ± 1°C. The softened emulsion is then scraped into a 50 cc beaker, using the edge of another plate as a

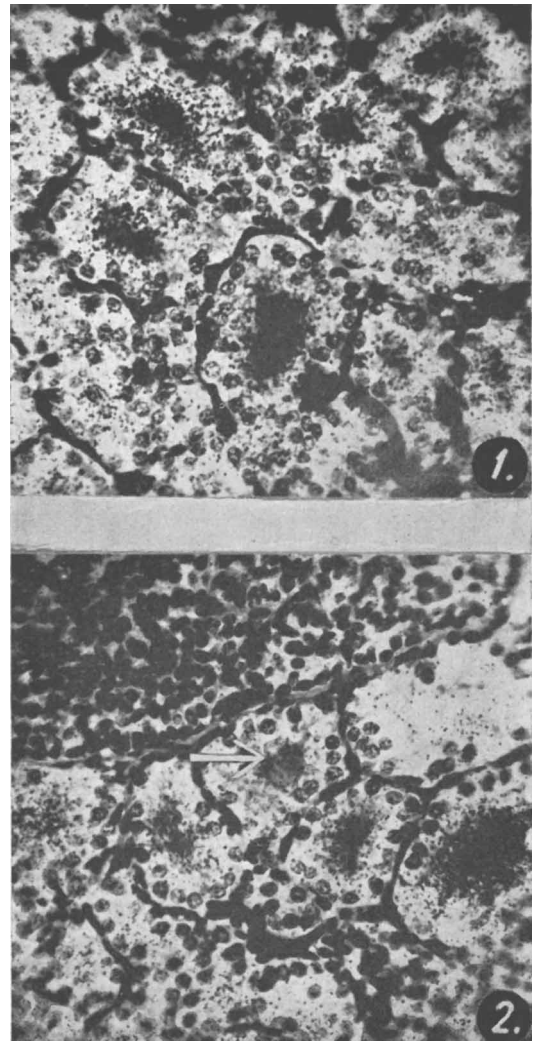

FIG. 1.

Section of thyroid of rat given radioactive iodide. The dark granules of silver, indicative of the presence of radioactivity, are seen in greatest density over the colloid contained in the thyroid follicles. Note that the thyroid epithelium shows a reaction only in the parts of the cells close to the colloid (as a result of the diffusion of the colloid image). The rest of the cell shows only background fog. The black outlines of the follicles represent the deeply staining red blood cells in the vessels. (H. & E., green filter, ×250.)

FIG. 2.

Section of parathyroid (top) and thyroid of rat treated as above. Only a slight background fog is seen in the parathyroid. Thyroid reacts as in Fig. 1. The central arrow indicates a follicle where the colloid may be easily seen through the silver granules. (H. & E., green filter, × 250.)

[2] Hamilton, J. G., Soley, M. H., and Eichorn, K. B., *Univ. Calif. Publ. Pharmacol.*, 1940, **1**, 339.

[3] Leblond, C. P., *J. Anat.*, 1943, **77**, 149.

[4] Leblond, C. P., *Stain Techn.*, 1943, **18**, 159.

[5] Belanger, L. F., and Leblond, C. P., *Endocrinology*, 1946, **39**, 8.

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

scraper.† One lantern slide plate yields sufficient fluid emulsion to coat 3 or 4 microscope slides. The beaker is then placed in a water bath kept between 38° and 39°C. After 15 minutes in this bath, the emulsion becomes sufficiently liquid for application. A uniform emulsion coating is obtained through the use of a leveling table heated at one end to about 38°C and left at room temperature at the other end. This table was made of a plate glass top, with a heating element under one end, adjusted to give the required temperature. The whole is set on leveling screws and made horizontal with the aid of a spirit level.

The stained slides, after being warmed on the leveling table, are taken in one hand. Four to 8 drops of emulsion are applied with a medicine dropper held in the other hand. The air must be expelled from the dropper before dipping it into the emulsion, in order to prevent the occurrence of bubbles. About 2 drops of emulsion are applied per square inch of slide covered. The drops are spread evenly and quickly with a camel's hair brush. The slide is then rotated from side to side along its long axis to make the emulsion flow gently from edge to edge and thus obtain an even film. This may be seen by the red light reflected from the safelight on the surface of the emulsion.

The slides are returned to the warm side of the leveling table for 30 to 60 seconds and then gently slid to the cool side of the plate. The emulsion gels in about 15 minutes. The slides are then stored at 0° to +2°C in light-tight containers kept dry with P_2O_5. Test slides are developed at various time intervals after coating to determine the proper length of exposure. Development is in Kodak D-72 for 2 minutes at 19° ± 1°C with fixation in acid fixer for 10 minutes. The slides are then washed for 20 minutes, the water being kept below 20°C to prevent buckling and peeling of the emulsion. After passage through alcohol and xylol the sections are mounted in balsam. Five minutes in each bath is necessary for complete dehydration. After placing the coverslips on the sections, these should be left to dry at room temperature. Throughout development and mounting, slides should be kept horizontal.

Discussion. A satisfactory autographic method should permit the visualization of histological details through the silver granules in the gelatin emulsion and thus enable a precise localization of these granules with reference to the stained structures in the section.

This requires the reduction of background fog to a minimum. Normally, unexposed lantern slides will show a certain amount of fog which increases with subsequent manipulations. This increase is avoided by careful control of temperature, minimum safe-light exposure, and storage at 0° to 2°C. In autographs with very little fog, it becomes possible to identify even minute accumulations of silver granules as resulting from the action of the radioactivity. Under these conditions, the length of exposure can be shortened; and the rather small reactions thus obtained make it easier to see the stained structures through the silver granules at the sites of radioactivity.

The photographic reaction due to the β-rays of I^{131} is maximal in the zone located immediately above a site of radioactivity and decreases as the square of the distance from this point. When such a reacting zone is examined through the microscope it appears circular; extending far from the source with prolonged exposure, but being more closely limited to it with controlled exposure. Indeed, with a short exposure, it is possible to limit the image to a zone almost directly above the emittor and thus obtain a nearly cytological localization (Fig. 1 and 2).

Summary. The autographic technic has been modified to permit staining of histological sections before coating with photographic emulsion. With this method, the diffusion of the image is sufficiently decreased to permit examination of the autograph under the high power of the microscope.

† Occasional batches of lantern slides are unserviceable as the emulsion does not soften in water and remains stringy and elastic.