solution was used in all injections.

The method of assay has been described previously.[3] A scale from 0 to 4 has been established to estimate the intensity of a given effect. A negative sign indicates a lightening with respect to an appropriate control, a positive sign indicates a darkening.

Results. A typical experiment using the drop technic is summarized in Table I and an example of the test-tube method is illustrated in Table II. In both cases it is quite clear that the body-lightening factor (CBLH) can be separated without any significant content of the tail-darkening principle (CDH). The solvent used in the experiment of Table I, however, is not exceedingly volatile. Using isopropyl alcohol as the extracting solvent, a cleaner separation was effected. With methyl alcohol, which vaporized very rapidly, assays usually show a slight amount of tail-darkening in the collected drops of solvent. It was quite obvious that water was condensing on the slide during the dropping of the methyl alcohol and the water must have carried along a little of the tail-darkening factor, for when the closed-tube technic was adopted (Table II) none of the darkening factor appeared in the alcohol extracts. Apparently the darkening factor has no significant solubility in the organic solvents used.

Fig. 1 illustrates the action of a sea-water extract of a tritocerebral commissure which contains both CBLH and CDH, and the action of only the alcohol-insoluble fraction of the commissure which contains CDH alone.

Summary. The tritocerebral commissure of the shrimp, *Crago,* has been chemically fractionated to separate 2 mutually antagonistic chromatophorotropins influencing body-coloration, a general darkening one (CDH) and a body-lightening one (CBLH).

15778

Radioautographs in Which the Tissue is Mounted Directly on the Photographic Plate.

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In early methods of determining the location of radioactive elements in tissue, the microscope slide bearing the tissue section was placed face down on a photographic plate.[1-4] After a suitable exposure to the radiation the plate was removed and developed. The resulting image, or autograph, was then compared with the tissue section (after staining etc.) to locate the radioactive material. The efficacy of this method is limited by (1) lack of sharpness and (2) difficulty in matching the autograph with the corresponding histologic detail in the tissue.

Recently, Bélanger and Leblond[5] have described a method which reduces these objections. The photographic emulsion from a lantern slide is removed and spread over the tissue section. After the radiation exposure the autograph is developed, fixed and washed. The tissue sections are then stained, cleared and the entire preparation mounted in balsam.

For some time the writer had been considering the feasibility of mounting tissue sections directly on the photographic emul-

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1 Lacassagne, A., and Lattes, C., *J. Radiol. et Elect.*, 1925, **9**, 1.

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

4 Hamilton, J. G., *Radiology*, 1942, **39**, 541.

5 Bélanger, L. F., and Leblond, C. P., *Endocrinology*, 1946, **39**, 8.

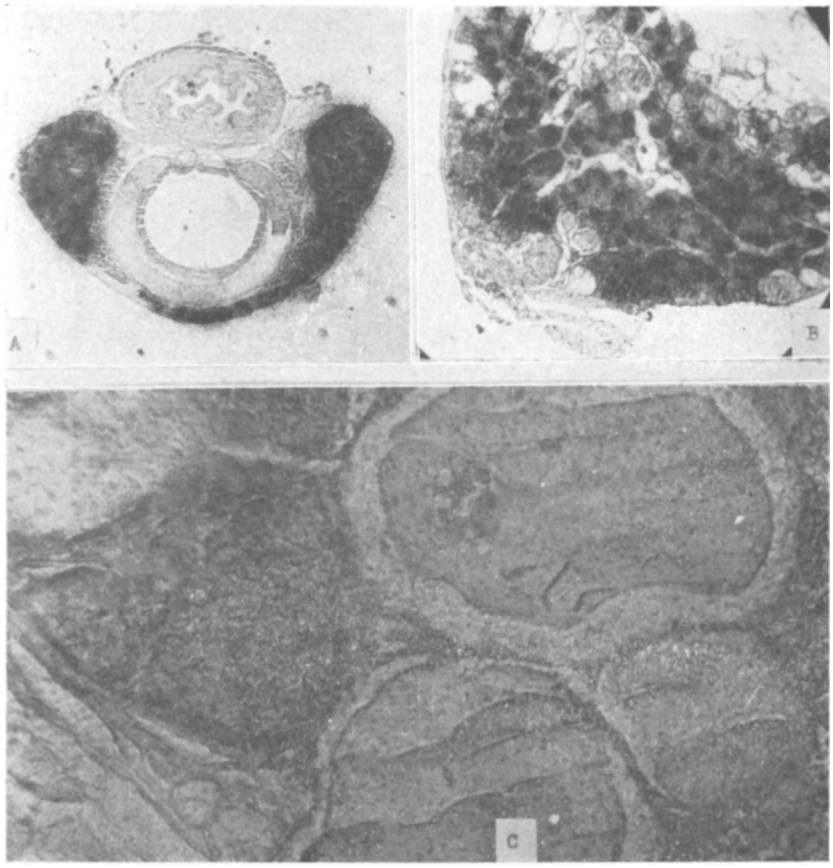

PLATE 1.

Photomicrographs of rat thyroid tissue (hematoxylin and eosin) mounted on the photographic emulsion in which the autograph was later produced directly under the regions containing radioiodine.

A. Thyroid region of newborn rat. Autograph and tissue on commercial film. Magnification ×18. Autograph developed 4 days after tissue containing the radioiodine had been attached. Three days before this babe was born, the mother had been injected with 30 μc of I^{131}. The foetus was able to concentrate the iodine in its thyroid, in competition with the mother, as can be seen by the heavy blackening which is limited to the thyroid regions.

B. Portion of thyroid of young adult rat (wt. 171 g) which had been sacrificed 24 hours after an injection of 25 μc of I^{131}. Magnification ×25. Medium contrast lantern slide, 6-day exposure. The indication of presence of radioiodine is most pronounced in the smaller follicles.

C. Small region of same thyroid shown in B. ×188. Deposition of the reduced silver is heavy underneath the one triangular follicle, whereas that under the others is only slightly greater than background (*i.e.*, lower left corner).

sion. When the paper of Bélanger and Leblond appeared, their method was tried. The preparations, although better than those obtained with the older methods, were not completely satisfactory. The emulsion was uneven and contained bubbles, the autograph was not sharp enough for high power study, the "fog" was heavy, and staining was complicated by the gelatin over the tissue, which became colored and lifted away from the slide. No doubt, some of the difficulties were due to inexperience and would be eliminated in time. It seemed advisable, however, to try our first plan, *i.e.* to mount the tissue directly on the plate. This method has been satisfactory and gives much better resolution than was possible formerly.

Fixation, dehydration, embedding and sec-

tioning are done according to the usual histologic technics. The paraffin ribbons are then cut into strips of 2-4 sections and allowed to expand on the surface of water at 42°C in a small petri dish. This is dropped into a larger bowl which allows the sections to float out into cold water. The photographic plate (lantern slide, or film not sensitive to red light) is then slipped under the tissue sections in dark room, in front of red safelight, one corner of the section is held against the emulsion of the plate and the plate plus the tissue removed from the water. The preparation is then dried in front of a fan and placed in a light-tight box for exposure to the radiation. Before developing the photographic plate the paraffin is removed with xylol and when this has evaporated the plate is developed in Eastman D-11 or D-72 solution. After fixation in acid hypo, the preparation is washed and allowed to dry. If it is desired that the background (and also the image) be reduced, this can be done by dipping the preparation, before the tissue is stained into a 0.2% permanganate solution.[6]

The tissue is stained with Harris' hematoxylin (over stain, acid water, alkaline water) and aqueous eosin, then dehydrated, cleared and mounted in permount or balsam. The plate should be dried as often as possible. Swelling and reticulation of the emulsion must be avoided.

Several methods of avoiding heavy staining of the photographic film, which is more of a problem than when plates are used, have been employed successfully. One method is staining *in toto* (acetocarmine, Harris' hematoxylin, or eosin when absolute alcohol is the fixative). Another, is to place ferric alum in with the fixative[7] in order that the tissue may be selectively stained when the preparation is immersed in iron hematoxylin. Care must be exercised in the second use of ferric alum since it will reduce the autograph as well as the nuclear stain.

Tissue prepared in the usual way may be used to check detail and staining properties. Some of the tissue should also be used for autographs using the older method, to insure that the general distribution of the radioactive material appears to be the same by both methods.

Tissue-autograph preparations offer many advantages over the older method of preparing the two separately. The entire section may be studied simultaneously for histologic detail and for localization of the radioactive element. The resolution is better and studies can be made under either the low or high power of the compound microscope (Plate 1). These findings have aided materially the study of experimental animals, of thyroid adenomas, and of thyroid carcinoma metastases.

Summary. The tissue section containing radioactive material is mounted directly on the photographic emulsion in the dark room. After suitable exposure, the plate is developed, and the tissue stained etc. The preparation may be studied microscopically as the autographic image is in place just below the tissue.

[6] Liebermann, L. N., Barschall, H. H., *Rev. Scien. Instr.*, 1943, **14**, 89.

[7] Kupperman, H. S., and Novack, C. R., *Science*, 1943, **98**, 591.