bacterial growth. Except where involved by adjacent inflammatory processes, the muscles showed surprisingly little change. The lack of muscle changes rules out to some extent a vitamin E deficiency.

Summary. When ANTU was fed chronically to rats for 2 years in concentrations of 50 to 800 ppm in the diet, the concentrations from 100 to 800 ppm were toxic. Externally, the rats were stunted and showed spectacle eyes, thinning and coarsening of the hair and deformities of the legs and feet. Histopathological changes consisted of hyperplasia of the thyroid, hyperplasia of the splenic pulp, hyaline changes in the cytoplasm of the centrolobular hepatic cells, a slight to moderate decrease in the thickness of the adrenal cortex, calcified tubular casts in the renal medulla, slight myeloid hyperplasia of the bone marrow, and slight modifications in bone structure. The degree of injury in each case was related to the concentration of the ANTU. The concentrations of 600 and 800 ppm ANTU produced a marked tolerance.

15777

Separation of Two Mutually Antagonistic Chromatophorotropins from the Tritocerebral Commissure of *Crago*.

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From the time of Koller's original contention that he had discovered in the rostral region of the thorax of the shrimp, *Crago*, a new endocrine source, producing a principle which blackened white-adapted specimens,[1] repeated attempts to confirm this conclusion have been generally unsuccessful at the hands of other investigators. More recently it has been shown that the circumoesophageal connective region,[2] or more specifically the tritocerebral commissure, of *Crago*[3] possesses very potent chromatophorotropic activity. Extracts of the commissures in sea-water, injected into eyestalkless specimens produce within 5 minutes striking lightening of the body and simultaneously blackening of the tail-fin (telson and uropods). It has been postulated upon the basis of such observations as the following, that the tritocerebral commissure of *Crago* possesses 2 mutually antagonistic principles for general body-coloration: (1) extracts of commissures from different individuals show variation in their relative influences in body-lightening and tail-fin-darkening, at times even darkening the body instead of lightening it,[3] (2) stimulation of the stubs of the eyestalks in eyestalkless animals results in body-lightening and tail-fin-darkening when the stimulus is strong, and, overall blackening if the stimulus is weak,[4] (3) comparative studies of crustacean nervous systems show some to possess only body-lightening activity (*e.g.* the crab, *Uca*), others predominantly body- and tail-fin-darkening activity (*e.g.* the lobster, *Homarus*).[5]

A clear proof, however, that the observed results of the action of extracts of *Crago* tritocerebral commissures are due to 2 hormones (1) a body-blackening hormone, and (2) an antagonistic body-lightening one obviously requires the chemical fractionation of the *Crago* commissure into 2 portions, each possessing only one of these 2 actions.

1 Koller, G., *Z. vergl. Physiol.*, 1928, **8**, 601.

2 Brown, F. A., Jr., and Ederstrom, H. E., *J. Exp. Zool.*, 1940, **85**, 53.

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TABLE I.
Fractionation of Principles of *Crago* Commissure by Drop Method.
Absolute Ethyl Alcohol as Extracting Solvent.

Extraction No.	No. of drops of solvent	Quantity of sea water to take up residue cc	Size of injection, cc	Assay	
				Body	Tail
1	5	.06	.03	—4	0
2	5	.06	.03	—4	0
3	5	.06	.03	—3	0
4	5	.06	.03	—1	0
5	5	.06	.03	—1	0
6	—	.06*	.03	—2 i† +4 f	+4

* Water was added directly to the slide and the smeared tissue was scraped off and thoroughly mixed with the liquid.
† i means initial; f means final.

TABLE II.
Fractionation of Principles of *Crago* Commissure by Equilibrium Method.
Absolute Methyl Alcohol as Extracting Solvent.

Extraction No.	Quantity of solvent in test tube, cc	Quantity of sea water to take up residue, cc	Size of injection, cc	Assay	
				Body	Tail
1	.50	.06	.03	—3	0
2	.50	.06	.03	0	0
3	—	.06	.03	—1 i†	+4
1	.50	.06	.03	—3	0
2	.50	.06	.03	—1	0
3	.50	.06	.03	0	0
4	.50	.06	.03	0	0
5	—	.06*	.03	0 i† +1 f	+3

* Water was added directly to the slide and the smeared tissue was scraped off thoroughly and mixed with the liquid.
† i stands for initial; f stands for final.

Investigations of the solubility of the active commissural material in organic solvents[3] indicated that one of the factors is preferentially soluble in polar organic substances, such as ethyl alcohol. A fractionation method based on an extraction procedure was thus suggested. Craig has shown[6] that the method of successive extractions can be used very successfully in fractionating substances of biological interest. Unfortunately his procedure deals with quantities of the order of 20 μg or more. A rough measurement of the size of the glandular tissue of the commissure as determined from histological serial sections indicated that the quantity of active principle available must be much smaller than even one microgram. Considering the region of the commissure possessing the gland as approximately a cylinder, one finds for an average specimen a diameter of about 0.005 cm, a length of about .05 cm, and hence a volume of about 1×10^{-6} cc. Assuming a density of about 1, one obtains 1×10^{-6} g for the mass of this structure. A very generous estimate of the actual glandular tissue in the commissure would be about 25%. The concentration of active substance within the glandular tissue could hardly exceed 33% so that a mass of 0.1 μg is probably an upper limit to the total quantity of active material present. It is this 0.1 μg which must be fractionated into 2 components.

Experimental. A commissure was carefully excised from a medium sized (1 g) specimen of the shrimp, *Crago*, as previously described[3]

[6] Craig, L. C., *J. Biol. Chem.*, 1944, **155**, 519.

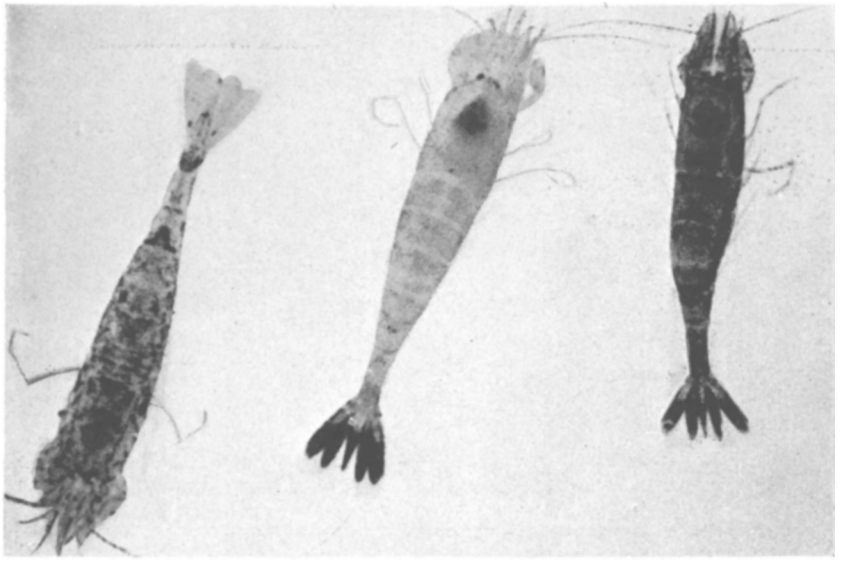

FIG. 1.
From left to right: 1. A sea-water-injected eyestalkless *Crago* (control); 2. an eyestalkless specimen receiving an injection the equivalent of one-sixth of the sea-water-soluble contents of one tritocerebral commissure; 3. an eyestalkless specimen receiving an injection the equivalent of one-sixth of the alcohol-insoluble contents of a tritocerebral commissure. The injections were made about nine minutes before the photograph was taken. All 3 animls were matched and resembled specimen one at the beginning of the experiment.

and deposited on a microscope slide or cover-glass. The surrounding water was allowed to dry for about 2 minutes. The specimen was then smeared over an area of about 0.5 cm^2 with the aid of a blunt glass rod or cover-glass. Examination under the microscope indicated that the maximum thickness of the smeared specimen was about 5 μ and that numerous valleys and ridges were present to facilitate penetration by the solvent.

Two alternative procedures were used to carry out the extraction. One consisted of dropping the organic solvent onto the tilted slide bearing the smeared specimen and collecting the liquid on a depression slide. The liquid was collected in groups of 5 or 10 drops. An interval of a few minutes was allowed between each group so that the specimen slide could dry.

This procedure was found to have the objectionable feature, particularly with very volatile solvents, that rapid evaporation of the solvent cooled the slide appreciably and led to the condensation of water vapor on the specimen. The water could dissolve both active principles and hence nullify the effort at fractionation. This difficulty was overcome at least partially by drying between groups of drops, but some doubt always remained.

An additional objection to this drop procedure lies in the difficulty of carrying out a physico-chemical analysis of the results since the establishment of equilibrium between smear and solvent can be seriously questioned.

To overcome the objections mentioned a modified approach was also used. The cover-glass containing the smear was cut down to approximately the area occupied by the tissue and then inserted into a 3 cc test tube. 0.50 cc of solvent was added to the tube, which was then stoppered and shaken carefully for about 10 minutes. The closed tube minimized evaporation and the long time of contact between tissue and solvent insured the attainment of equilibrium.

With either procedure the solvent was evaporated in the open air and the residue was taken up in sea water. The sea-water

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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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.