

UCA - Hormonal Control of Chromatophores.

1. Examine the black and white chromatophores in a normal Uca. You will find these chromatophores most in evidence upon the under side of the largest segment of the walking legs.
2. Similarly examine the black and white chromatophores of Uca which have been kept in darkness for two or three hours.
3. Now examine the same chromatophore types in animals from which the eyestalks have been extirpated for twenty-four hours or more.
4. You have now seen the full range of activity of at least one of the principal chromatophore types of Uca. Considering that a fully dispersed pigment mass be designated as (5) and that a fully concentrated pigment mass be designated as (1), visualize stages which are intermediate and which you could call respectively (4), (3) and (2). These numbers you will now use to indicate the state of chromatophores in any animal under controlled types of experimental situations. However, first describe the states of the chromatophore types which you observed in parts 1, 2 and 3 above.
5. Prepare the following extracts of Uca organs in sea water.
 - (a) 16 eyestalks in 2 cc. of sea water.
 - (b) 3 brains in 1 cc. of sea water.
 - (c) 6 circum-oesophageal commissures in 1 cc. of sea water.
 - (d) 3 thoracic ganglionic masses in 2 cc. of sea water.
 - (e) a comparable sized fragment of muscle in 2 cc. of sea water.
10. Boil briefly each of these extract samples in a cotton plugged test tube and set them aside in a refrigerator to be assayed in future experiments for specific effects upon Uca chromatophore types.
11. To what extent can sinus gland extract be diluted and still be effective upon crayfish isolated red chromatophores? Here it is suggested that one sinus gland be extracted in 1 cc. of van Harreveld's solution and then diluted a number of times by steps of one-half down to approximately 1/4096 of the original concentration. Place a drop of each concentration in a depression upon the 18-depression slide and determine the last sample showing effect upon red chromatophores.

6. Assay each of the extracts (eyestalk extract diluted to about one-half) upon two eyestalkless UCA by injecting 0.05 cc. into animal at base of fourth or fifth walking leg. Record average stage of black and white chromatophores with the aid of a dissecting microscope as 5 (fully discussed) above) and 4, 3, 2 and 1. These records should be made at the beginning of the experiment and at 15 min. intervals following injection.
7. Determine the maximum dilution effective. Do this only for eyestalk extract.
8. Are comparable materials present in:
Crayfish eyestalk and nervous system
Cockroach head and nervous system
Isopod head
Fly head

Are their effects the same upon both chromatophore types as any of the UCA extracts?
9. Localization of glandular sources of hormones.
 - a. Remove according to directions the sinus glands from the eyestalks of a large crayfish. Grind and extract these two in .5 cc. of seawater. Make an eyestalk extract of comparable strength (two eyestalks to .5 cc.) Now compare these two extracts with respect to the quantitative aspects of their effects upon UCA black chromatophores.
 - b. Remove and extract the endocrine glands, corpora alata and corpora cardiaca, from the cockroach. Extract each in the same amount of salt solution as in the case of the cockroach head in #8 above. Compare the actions of these extracts with that of the cockroach head extract.
10. Using the isolated chromatophore technique (oral directions will be given), compare the responses to various extracts of crayfish red and white chromatophores, to what you have learned from UCA.
11. To what extent can sinus gland extract be diluted and still be effective upon crayfish isolated red chromatophores? Here it is suggested that one sinus gland be extracted in 1 cc. of van Harreveld's solution and then diluted a number of times by steps of one-half down to approximately $1/4096$ of the original concentration. Place a drop of each concentration in a depression upon the 12-depression slide and determine the last sample showing effect upon red chromatophores.

If time permits, do one or more of the following:

12. Effect of sinus gland extract upon retinal pigment migration.
13. Effect of sinus gland and of nervous tissue extract upon heart rate.
14. Sinus glands and viability and molt.
15. The influence of a principle from the corpora cardiaca upon cockroach heart rate.
16. Any other project which the student desires to do after consultation with instructor.