increased linearly from 155 at 10°C to 210 at 37°C. Published values for ratios at temperatures below 37°C are questionable for chloroform and not available for methoxyflurane. In general, a rise in temperature in the range 10° to 40°C causes a slight increase in the solubility of volatile compounds in oil and a decrease of their solubility in water, resulting in a rise in their oil-water distribution ratios.

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16. We thank Linus Pauling for his interest and generous advice, T. R. Sato for many suggestions, and P. Dacre, D. Davies, C. Leonard and V. Walker for technical assistance. The work reported in this article (contribution No. 3058) was supported by grants to the California Institute of Technology made by the Ford Foundation and the National Institutes of Health.

1 May 1964

Endocrine Control of Tanning in the Crayfish Exoskeleton

Abstract. *Tanning of the newly formed exoskeleton of the dwarf crayfish,* Cambarellus shufeldti, *depends in part upon the presence of a substance from the eyestalk. Injection of eyestalk extract enhances tanning in a specimen with its eyestalks removed, whereas the exoskeleton of an untreated crayfish without eyestalks will not become as dark as that of an intact individual.*

The molting process in Crustacea has been reviewed by Passano (*1*). The eyestalks contain a molt-inhibiting hormone, and removal of the eyestalks has long been known to result in increased molting. The eyestalk is the site of important neurosecretory centers and contains the sinus gland, a neurohemal organ. After the old exoskeleton of higher crustaceans has been shed, hardening of the new one begins, inorganic salts and organic material being deposited. The organic matter participates in a tanning process that is the result of interactions between quinones and proteins (*2*). Fraenkel and Hsiao (*3*) recently showed that tanning in insects is regulated by an endocrine substance which is secreted by neurosecretory cells in the brain. This substance is neither the prothoracicotropic hormone nor the gonadotropic hormone. Insofar as we know, no one has investigated the possibility that tanning in crustaceans is controlled by a blood-borne substance. Previous investigations of hardening have centered on the control of deposition of inorganic materials after the molt.

The dwarf crayfish, *Cambarellus shufeldti*, undergoes the typical tanning process after molting. A golden brown pigment is produced in the transparent exoskeleton. The experiments described herein were undertaken to determine whether the tanning process in the dwarf crayfish is under endocrine control.

Specimens were collected near the town of Pearl River, Louisiana, in September 1963 when the population was undergoing its fall molt and in February 1964 during the spring molt. In the laboratory, specimens were kept individually in white enamelled pans under constant illumination, 1320 lumen/m^2, at 24° to 26°C. The color of the pleura, the downwardly directed lateral processes of the abdominal exoskeleton, was recorded daily with colored pencils. In this way any change in the amount of pigment deposited from day to day would be apparent. The pleura were chosen because they are thin and the recording of their color is not complicated by the presence of other colored tissues.

Both eyestalks were removed from each of a number of crayfish that were actively preparing for the molt. Seventy-five crayfish without eyestalks survived long enough to molt at least once. The average length of time required for molting to occur in these 75 crayfish was 6.5 days after the operation. After the molt, the newly formed exoskeleton of each crayfish was a very pale yellow, almost colorless. Gradually a small amount of golden brown pigment, less than in intact specimens, formed in each exoskeleton. Thirty of the 75 crayfish that had molted after eyestalk removal were kept as controls. Of these 30, 6 molted a second time. The average period between molts was 10.5 days. The remaining 24 control specimens died before they could molt a second time. The period of survival of the 24 averaged 6.6 days after the first molt without eyestalks. When the six specimens without eyestalks that molted twice during the experiment molted for the second time, the amount of pigment that had formed in their exoskeletons was less than the quantity that had been present just prior to the first molt.

To determine conclusively whether a substance in the eyestalk accelerates tanning, the 45 additional crayfish that molted after their eyestalks had been removed were injected with eyestalk extract on the day they molted and every second day thereafter. The eyestalk extract was boiled and centrifuged, and the supernatant was used for the injection. Boiling the extract eliminated the possibility that an enzymatic rather than endocrine process was involved. The dose per crayfish was one eyestalk extracted in 0.02 ml of physiological saline. Only 1 of 45 injected crayfish, as opposed to the 6 out of 30 controls, molted a second time. Presumably, this difference was due to the molt-inhibiting hormone present in the eyestalk extracts. The average length of survival of the injected crayfish that molted only once was 5.7 days after molting. As a result of the injections of eyestalk extract these crayfish produced the golden brown pigment in their exoskeletons at a faster rate than did the uninjected group, that is, they showed more tanning. Hence the conclusion that the eyestalk contains a substance that hastens tanning.

Rather than postulate a "tanning hormone" to account for these observations, we suggest that the molt-inhibiting hormone also controls tanning. However, the fact that a specimen without eyestalks will deposit some pigment in its exoskeleton after molting suggests an additional, extra-eyestalk source of the substance that promotes tanning. Stephens (*4*) has already found that the supraesophageal ganglia and circumesophageal connectives of a crayfish contain a substance that inhibits molting.

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References and Notes

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5. This investigation was supported in whole by USPHS research grant B-838 from the National Institute of Neurological Diseases and Blindness.

24 April 1964