lesion may be questioned. Trace metal content of synovial fluid has also been reported as being elevated in rheumatoid arthritis (*12*).

The prompt recovery of the cartilage following removal of load in the viscous media suggests that the tissue selectively excluded the long-chain polymers while allowing water and electrolytes to enter the tissue; that is, the tissue acts as a rapid ultrafilter. This may provide a mechanism for concentrating synovial mucin at the surface of the cartilage, thereby increasing the effectiveness of synovial fluid as a lubricant. It has been suggested (*13*) that the ground substance in vascular and certain other connective tissues may regulate ultrafiltration.

In an attempt to see whether the various ions caused swelling or shrinkage of the ground substance, paraffin-embedded sections were made of dog rib and rabbit ear cartilage that had been immersed for 2 hours in distilled water, isotonic Na^{+}, Ba^{++}, La^{3+}, and 3.4-percent NaCl. They were subsequently fixed in the same solutions to which formalin was added. No histologic changes in cells or matrix were detected in hematoxylin-eosin, periodic acid-Schiff, reticulum, or Rinehart preparations, with the exception of the chondrocytes which appeared less crenated following immersion in lanthanum than in the other solutions.

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References and Notes

1. S. M. Elmore, L. Sokoloff, G. Norris, P. Carmeci, *J. Appl. Physiol.* **18**, 393 (1963).
2. C. W. McCutchen, *Wear* **5**, 1 (1962).
3. Mr. George Norris designed a new flexible chamber for the compressometer to isolate the specimen and immersion solution from the temperature-regulating water bath.
4. J. M. Wells, *J. Am. Pharm. Assoc.* **5**, 99 (1944).
5. I am much indebted to Dr. Maxwell Schubert for the cobalt salt and for valuable suggestions; and to Mr. James F. Scott for technical assistance. The significance of ultrafiltration by cartilage as a possible factor in synovial lubrication was recognized during discussions with Mr. Frank C. Linn.
6. I. Kagawa and H. P. Gregor, *J. Polymer Sci.* **23**, 477 (1957).
7. Nutritional Biochemicals Corp., Cleveland; molecular weight, 5 to 40 $\times$ 10^6.
8. The curves in Fig. 1 are not typical in this respect.
9. D. Hamerman and M. Schubert, *Am. J. Med.* **33**, 555 (1962).
10. J. H. Bryant, I. G. Leder, D. Stetten, *Arch. Biochem. Biophys.* **76**, 122 (1958).
11. F. R. Partington and G. C. Wood, *Biochim. Biophys. Acta* **69**, 485 (1963).
12. W. Niedermeier, E. E. Creitz, H. L. Holley, *Arthritis Rheumat.* **5**, 439 (1962).
13. H. S. Bennett, *J. Histochem. Cytochem.* **11**, 14 (1963).

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Tanning in the Adult Fly: A New Function of Neurosecretion in the Brain

Abstract. *Tanning in the newly emerged fly is induced by a hormone secreted by neurosecretory cells situated in the pars intercerebralis of the brain. The same hormone is contained in the compound ganglion of the thorax, in concentrations six times as high as in the brain. This hormone is believed to act directly on the effector organ, and not through the secretion of ecdyson or a corpus allatum hormone; its release is effected through nervous impulses reaching the brain by way of the ventral nervous system a few minutes after the fly has emerged from the puparium. The hormone appears to be different from both the prothoracotropic and the gonadotropic hormones.*

In a previous communication (*1*) we described an endocrine mechanism which triggers off "tanning," the darkening and hardening of the adult fly after its emergence from the puparium. When the head is ligatured immediately after emergence the rest of the body never tans, but it can be induced to tan by injection of "active" blood from a 30- to 60-minute old fly. Simultaneous with the appearance of our report, a series of papers came out by Cottrell (*2*) dealing with exactly the same topic and with similar conclusions, but there were considerable differences in approach and details of technique. At the time of our earlier communication we had not succeeded in localizing the source of this hormone, but we concluded that it was not ecdyson, and did not originate from the corpus allatum or the brain. Similarly, Cottrell's experiments failed to identify the site of origin of this hormone. We now present evidence that, contrary to our earlier conclusions, the hormone is, indeed, a product of neurosecretion in the brain. Extracts from whole brains of newly emerged flies, which have inactive blood, or of 1-hour old flies, which have active blood, induced tanning in ligatured newly emerged flies, but extracts from the brains of flies aged 1 day or more were entirely inactive. There was little or no difference between the activity of brain extracts from newly emerged flies and 1-hour old flies. Extracts from the compound ganglion in the thorax were about six times more active than the brain extract from the same fly. The compound thoracic ganglion from older flies was entirely inactive. Extracts from the entire ganglionic system, including the brain, of feeding larvae, prepupae, untanned pupae, or 1-day old pupae, showed little or no activity, while extracts from the brain or thoracic ganglion from pre-emergent flies taken from the puparia were already active.

The hemolymph (blood) of newly emerged flies was already considerably active 2 to 3 minutes after emergence, as shown by a full tanning reaction in flies ligatured at that time. Fifteen minutes later the activity was so great that blood taken at that stage could be diluted up to 30 times with Ringer's saline and still cause full tanning when injected into ligatured flies. Evidently, at the height of activity in the blood, there is enough hormone present in one fly to induce tanning in over a hundred flies. Since the activity is so much greater in the thoracic compound ganglion than in the brain, it is assumed that most of the activity in the blood is derived from the thoracic ganglion. The disappearance of activity from the ganglion within a day indicates its release into the blood. This release must be triggered by an action of the brain, because it did not occur in the fly ligatured at the cervix.

The removal, at the time of emergence, of the median neurosecretory cells of the pars intercerebralis of the brain, by the technique of Thomsen (*3*), had the same effect as decapitation: tanning no longer took place. Extracts of brains from which the neurosecretory cells had been removed were entirely inactive. The activity of extracts made from the cells was about equal to that of an intact brain, although the section of the brain removed in the preparation of the extracts was probably less than one hundredth of the weight of the brain. However, implantation of intact cells had no effect at all, indicating that the activity is not released readily from the isolated cells. Corresponding experiments with the lateral neurosecretory cells indicated that they are probably not involved in this reaction.

In view of the much greater hormonal activity of extracts from the thoracic compound ganglion, and the fact that neurosecretory regions had never been described from this organ, we proceeded to search for such structures. Two groups of large cells which stain prominently with methylene blue

were discovered; they were similar in size to the median neurosecretory cells, although of a somewhat different appearance. One group was situated near the anterior rim of the ganglionic mass, and the other where the abdominal nerve leaves the ganglion. The secretory function of these cell groups has not yet been verified by histological or experimental means.

In the normal fly, release of activity is most probably triggered by nervous stimuli from the periphery, reaching the head by way of the ventral nervous system. If the ventral nerves were cut in the neck of the newly emerged fly, tanning no longer occurred. This was due to the severance of the nerve connections between body and head and not to a wound effect or interference with blood circulation. Tanning was not at all hindered by a sham operation performed in exactly the same way except for the final step of severing the nerves. We therefore conclude that the hormone is released by a nervous impulse from the periphery at the time when the fly has freed itself from the puparium and has dug its way out of the medium in which the puparium was confined. [In normal flies the process of tanning is delayed until the fly has freed itself from all confinement (*4*).]

Further experiments indicated that the hormone from the median neurosecretory cells acts directly at the sites of tanning, and not through another hormone system. When active blood was injected into flies with ligatured heads, and the abdomen was ligatured immediately (within seconds) afterwards, the isolated abdomen tanned. No tanning occurred if inactive blood or Ringer's saline was injected. Although the abdomen was isolated from the endocrine organs in the thorax in the ring gland (*5*), it is possible that the active blood already contained a second hormone released by the brain hormone. In a similar experiment, in which we used extracts from neurosecretory cells instead of active blood, we again found that the isolated abdomen tanned. Thus it seems improbable that a second hormone takes part in the tanning process, there being no evidence that the abdomen contains a gland that secretes the tanning hormone in response to the hormone from the brain.

Results of chemical analysis of the hormone indicate that it is a protein or large polypeptide; this is in agreement with the results obtained by Cottrell (*2*). The hormone was inactivated when active blood was treated with alcohol, acetone, or trichloroacetic acid. Upon heating at 95°C for 3 minutes or at 80°C for 5 or 10 minutes the supernatant fluid retained considerable activity, varying from about 25 percent to 50 percent of the original activity in different experiments. Ammonium sulfate at half-saturation precipitated the entire active fraction which could then be fully recovered by redissolving the precipitate in Ringer's saline. The hormone was non-dialyzable (*6*).

The fact that the brain and blood of *S. bullata* contain a substance that is active only at the time of emergence of the fly suggests that the hormone responsible for tanning is different from the other brain hormones. It is very improbable that both the prothoracotropic and the gonadotropic (*7*) hormones would be so uniformly absent from the brain or blood of larvae, pupae, and older adults whose blood is devoid of tanning activity. Experiments with the cockroach, *Periplaneta americana,* led to somewhat similar results. It was reported earlier (*1*) that the blood of *P. americana* is active in promoting tanning of flies only at the time of the molt. On the other hand, extracts from corpora cardiaca, corpora allata, or brains were active not only at the time of the molt, but also in fully tanned nymphs and adults. The activity of corpora cardiaca actually exceeded that of the brain of the same roach, by two to four times, despite the fact that the brain is so much larger. These results corroborate the finding of median neurosecretory cells of the fly brain as the site of origin of the hormone, since the corpora cardiaca function as a storage organ for brain hormone, and a similar function is also known for the corpora allata. Cockroach blood is active only (in the tanning of the fly) at the time of the molt, but would be expected to contain prothoracotropic hormone at least in nymphal stages. Therefore, the hormone which promotes the tanning of the fly and, presumably, the roach can be considered different from the prothoracotropic hormone. This explanation is not necessarily contradicted by the finding of activity in extracts from brains, corpora allata, and corpora cardiaca in stages where the blood is inactive. We find, similarly, that the blood in the newly emerged fly is inactive, while extracts from brain or thoracic ganglion are active.

An attempt was made to identify the tanning hormone with the gonadotropic hormone from the brain of the fly. The median neurosecretory cells were removed immediately after emergence and the flies fed on liver and sugar. In agreement with Thomsen (*3*), no ovarian development occurred. Such treated flies were then injected with "active" blood (four injections within 2 days) and there was no effect on the ovaries. This suggested that the hormones are different, but when blood from liver-fed flies with ovarian development in progress was injected repeatedly into flies from which these cells had been removed immediately after emergence, further development of their ovaries did not occur. Apparently, previous workers investigating the hormonal control of egg development in flies have not done similar injection experiments with blood, yet have concluded that a gonadotropic factor is carried in the blood. Evidently, the concentration of such substances in the blood at any time is too small to produce a visible effect upon injection into a fly with the median neurosecretory cells removed. If the tanning hormone and the gonadotropic hormone were the same, the activity in "active" blood would be vastly greater than that in the blood at the time when ovarian development is in progress. Still, "active" blood had no effect on ovarian development in the experiments mentioned above. This makes it most improbable that the two hormones are identical, a conclusion which still remains to be proved (*8*).

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References and Notes

1. G. Fraenkel and C. Hsiao, *Science* **138**, 27 (1962).
2. C. B. Cottrell, *Trans. Roy. Entomol. Soc. London* **114**, 317 (1962); *J. Exptl. Biol.* **39**, 395, 413, 431, 449 (1962).
3. E. Thomsen, *ibid.* **29**, 137 (1952).
4. G. Fraenkel, *Proc. Zool. Soc. London* **87**, 894 (1935).
5. The ring gland is apparently still intact in the newly emerged fly, but later changes through degeneration of the lateral sections which secrete ecdyson.
6. The hormone responsible for tanning resembles in these respects the prothoracotropic hormone of the silkworm *Philosamia cynthia ricini,* which also possesses the properties of a protein [M. Ichikawa and H. Ishizaki, *Nature* **191**, 933 (1961); **198**, 308 (1963)].
7. The term "gonadotropic" hormone is used here in the sense given it in the latest communication from E. Thomsen's laboratory [A. O. Lea and E. Thomsen, in *Neurosecretion,* H. Heller and R. B. Clark, Eds., *Mem. Soc. Endocrinol.* (1962), No. 12, pp. 345–347] according to which ovarian development in the fly is activated by the corpus allatum by means of a secretion from the median neurosecretory cells, and not, as was formerly assumed (*3*), by the cells through the corpus allatum.
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