Melanophore-Stimulating Substances in Amphibia
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Work begun on this project last summer revealed that grafts of the hypothalamus from embryos and albino larvae of the salamander, *Ambystoma tigrinum melanostictum* caused melanin dispersion to occur in the melanophores of larval albino hosts of the same species. This summer a similar study has been extended to the adult. Various areas of the brain of hypophysectomized adult salamanders have been transplanted to albino larvae to determine the specific source of this melanophore-stimulating substance reported earlier.

Adult salamanders were caught near Colter Bay Village, Grand Teton National Park, Wyoming, in late June and early July. Embryos, up to the tailbud stage, were collected from a small pond approximately 5 miles from the summit of Togwotee Pass, Teton National Forest, Wyoming, from June 16 to June 29.

The procedure used in these experiments involved three steps. Adult salamanders were hypophysectomized to remove the source of the known pigment granule dispersing substances, intermedin from the pars intermedia and adrenocorticotropin from the pars distalis. Second, albino larvae that were to be used as hosts for tissue grafts were prepared by removing the epithelial hypophysis at the tail bud stage. And third, one to two weeks after the above operations the adult salamander was sacrificed and parts of the brain were transplanted adjacent to the punctate melanophores and in the subcutaneous tissue of the albino larvae and observed daily for periods up to four weeks. A total of 282 grafts from the adults were transplanted to albino larvae and studied this summer.

Two types of controls were prepared. Relatively small segments of two pars intermedia were transplanted into eight albino larvae to serve as "positive" controls. The melanophores of these hosts showed maximum melanin dispersion and gave a black appearance to each larva for a period of 4 weeks. Twenty-eight "negative" controls were prepared by grafting tissues from the roof of the cerebral hemispheres (4), habenula (8), cerebellum (4), and medulla (12). Only 3 of these grafts caused melanin dispersion the first day and by the third day after transplantation none of the grafts were causing melanin dispersion in the host's pigment cells.

Forty of 42 grafts from the median part of the ventral hypothalamus posterior to the optic chiasma evoked pigment responses in the hosts that lasted at least 12 days whereas only thirteen of the 41 grafts from the lateral wall of the ventral hypothalamus in the same area evoked pigment responses that lasted 12 days.
Tissues from the superior colliculus (8), subcommissural organ (3), dorsal hypothalamus (20), and ventral thalamus (6) gave no response or only weak responses that generally lasted one to three days and in only 3 instances as long as 5 days. On the other hand grafts from the preoptic nucleus area (4) in general evoked melanin dispersion in the albino larvae that lasted 4 to 5 days and in 5 instances grafts from the anterior preoptic nucleus evoked strong responses that lasted from 7 to 14 days.

To summarize the work for this summer it has been shown that adult brain tissue from the tiger salamander will evoke melanin dispersion in melanophores of albino larvae and that essentially 100% of the grafts from the median ventral hypothalamus give a strong and sustained response. On the other hand between 30 and 40 percent of the grafts from the lateral half of the ventral hypothalamus and 10-15% of the grafts from the preoptic nucleus area give a similar response. Grafts from other areas of the brain either do not evoke a pigment response or cause only a weak response for 1-3 days.

This substance has not been identified but it can be said with confidence that it is not due to diffusion or by contamination of intermedin from the pars intermedia or by ACTH from the pars distalis. Because of the recent work of Novales ('63) where he used cultured melanophores to test the effect of various substances, it is very doubtful whether serotonin could be the cause of the melanin-dispersing activity reported here. Other substances which should be studied for their effect upon Ambystoma albino larvae are acetylcholine and the corticotrophic releasing factor that has been identified in the mammalian hypothalamus.

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