A Melanophore-Stimulating Factor in Embryonic Skin. (25350)

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Preliminary experiments indicate presence of a factor in skin of *Ambystoma maculatum* tail-bud embryos capable of causing pigment dispersion in melanophores. Pigment-concentrating effect of hypophysectomy on amphibian melanophores has been known since the work of Smith(1). It has been reported, however(2-4), that extirpation of the epithelial anlage of the hypophysis is reflected by melanophores only after an initial period in which pigment cells appear morphologically identical to those in unoperated animals. In *A. maculatum, A. opacum* and *Siredon mexicanum* melanophores in hypophysectomized animals are maintained in stellate condition from their initial appearance, at Harrison stage 37, until embryos reach stage 41-42. A similar situation prevails in several anuran species(5), although they differ in length of time during which pigment cells remain stellate. Since for a period in development of the pigment pattern melanophores appear to be temporarily independent of pituitary for maintaining their stellate condition, we are faced with alternative hypotheses. Either (a) hormonal stimulation is unnecessary at first but required later for melanophores to maintain a dispersed arrangement of pigment granules, or (b) appropriate hormonal stimulation in tail-bud embryos comes from a source outside the pituitary gland. In either case the system operates only temporarily, between stage 37 and stage 41-42. The rudimentary condition of the circulatory system at these early stages suggested that, if a hormone were involved, its source might be tissues immediately adjacent to the integumentary pigment cells. The experiments reported here were undertaken to test the possibility that flank somatopleure in tail-bud embryos might contain temporarily a melanophore-stimulating principle, which might be the basis for the pituitary independence of melanophores in the initial pigment pattern of amphibian embryos.

Materials and methods. Flank ectoderm, with adhering hypomeric mesoderm cells, was stripped bilaterally from 46 *A. maculatum* embryos at stage 35. An homogenate was prepared from the pooled tissue in 1 ml of Holtfreter's Standard Solution. Two homogenates were prepared from 32 embryos of the black strain of *S. mexicanum* at stage 41-42, one from flank skin and one from tails of the same animals. Similar homogenates were also prepared from adult skin, heart, liver and brain of *Rana pipiens*. For testing the activity of homogenates, vesicles were prepared by removing from each *A. maculatum* embryo at stage 20 a rectangular piece of belly ectoderm. A piece of trunk neural crest from the same embryo was combined with the ectoderm, which rolls up to form a vesicle enclosing the neural crest tissue. As the vesicle, kept in Standard Solution, enlarges and becomes transparent, melanophores appear on the inner surface of the belly ectoderm fully punctate. In testing for melanophore-stimulating activity, vesicles and also hypophysectomized larvae were immersed in the crude homogenates and observed at 30-minute intervals. *Observations*: In the *A. maculatum* homogenate (stage 35 skin) melanophores in the vesicles and in hypophysectomized larvae began to disperse pigment after one hour and thirty minutes. The pigment cells appeared larger and a few cytoplasmic processes were visible. In the larvae this effect was restricted to the epidermal melanophores. At the end of three hours the vesicle melanophores had changed from their initial punctate condition to being stellate (from 1 on the Hogben scale to 3). After 12 hours pigment cells were graded 4 on the Hogben scale. Dermal melanophores in hypophysectomized larvae had dispersed pigment at the end of 3 hours, and after 12 hours both dermal and epidermal melanophores were at 3 on the Hogben scale. Homogenates prepared from *S. mexicanum* (stage 41-42 skin and tail) and from *R. pipi-*

ens (adult tissues) showed no pigment dispersing activity even after 12 hours.

Discussion. These results support the hypothesis that appropriate hormonal stimulation of initial melanophores comes temporarily from tissues outside the pituitary gland. The alternative hypothesis, that newly differentiated melanophores do not need hormones for maintaining dispersed pigment but later develop this need, is not excluded. It seems unlikely, however, that such an early autonomy is characteristic of melanophore differentiation because, if that were the case, hypophysectomized larvae should continue to show a small number of stellate melanophores representing the cells newly differentiated from melanoblasts in all parts of the pigment pattern(4) instead of appearing uniformly punctate over most of the body surface.

The evidence clearly indicates that something was extracted from embryonic flank skin at stage 35 which was capable of causing punctate melanophores to become stellate, both in epidermal vesicles and in hypophysectomized larvae. It might be thought that the active principle is really pituitary hormone which diffused through the embryo from the site of production in the hypophysis. While small but physiologically active amounts of MSH have been detected in pituitary anlagen even prior to cytological differentiation(5-8), in *A. maculatum* embryos Drager and Blount (8) were able to demonstrate pigment-dispersing activity in heads of embryos no earlier than stage 39. Furthermore, Kleinholz in *Rana pipiens* found no MSH activity in skin of embryos at any stage, even though activity was found in heads of some of the same embryos. Lastly, in our own experiments with homogenates of stage 41-42 embryonic skin, at an age when the pituitary may be supposed to be active, no pigment-dispersing activity was evident. The latter observation, together with the failure of homogenates of *R. pipiens* adult tissues to show melanophore-stimulating activity, is a check indicating that the active principle in the early embryonic skin is not an artifact released by the process of making the homogenates. Because of restrictions on availability of material, the sources of our embryonic homogenates differ in species as well as age. Preliminary interpretation from the results seems nonetheless justified because the patterns of melanophore development, including temporary pituitary independence, appear essentially the same in the species involved.

All these observations lead to the conclusion that there is a tissue-specific and time-specific occurrence of a melanophore-stimulating hormone in the embryonic skin of *A. maculatum* just at the period when the first melanophores appear in stellate condition in all embryos. At the age when melanophores in hypophysectomized embryos become punctate, melanophore-stimulating activity in embryonic skin is no longer demonstrable. How widespread among amphibian species this phenomenon may be must be further investigated. Kleinholz's report(6,7), that *R. pipiens* embryos of all ages show no pigment dispersing factor in skin, may not be definitive since in the process of testing he treated his embryonic samples with acetone prior to desiccation. Lee and Lerner(9) have shown that alpha-MSH is soluble in acetone. There is no evidence at this time concerning the chemical nature of the factor demonstrated in these experiments. Any supposition that early embryonic skin produces MSH, rather than acetyl choline or other humoral agent affecting melanophores, must remain speculative until further tests are made.

Summary. Homogenates from flank skin of *Ambystoma maculatum* embryos at stage 35 show MSH-like activity when tested with punctate melanophores in epidermal vesicles and in hypophysectomized larvae. Homogenates from flank skin of older embryos (*Siredon mexicanum* at stage 41-42) and from adult tissues (*Rana pipiens*) show no such activity. The temporary occurrence of an MSH-like factor in early embryonic skin may explain the initial pituitary independence of the earliest melanophores differentiating in urodele pigment patterns.

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Further Characterization of Hemolytic Component of Fire Ant Venom, Mycological Aspects. (25351)

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Preliminary observations about the antibiotic character of venom were made by Caro *et al.*(3), who noted that of pustules produced by experimental stings, all, with one exception, were bacteriologically sterile. On one occasion non-hemolytic staphylococci were isolated, but organisms were not seen in tissue removed for biopsy. Using the paper disk method, Blum *et al.*(2) demonstrated the effectiveness of venom against *Micrococcus pyogenes, Streptococcus pyogenes, Escherichia coli, Lactobacillus casei* and a variety of molds which were not named. The present report is concerned with the antimycotic effects of crystalline, hemolytic component of fire ant venom isolated by Adrouny *et al.*(1). It is an attempt to add another means of biological characterization of the crystalline material and is not to be interpreted as a study on the antimycotic effects of this material for future clinical use.

TABLE I. Effect of the Crystalline Hemolytic Component of Fire Ant Venom on Fungal Growth.

Inhibition of growth	No inhibition of growth
Blastomyces dermatitidis	*Epidermophyton floccosum*
Candida albicans	*Microsporum Audouini*
Cladosporium Wernecki	" *canis*
Cryptococcus neoformans	*Monosporium apiospermum*
Geotrichum sp.	*Oospora* sp.
Hormodendrum compactum	*Sporotrichum Schenckii*
Hormodendrum Pedrosoi	
Microsporum gypseum	
Nocardia asteroides	
" *brasiliensis*	
Phialophora verrucosa	
Trichophyton mentagrophytes	
Trichophyton rubrum	
" *tonsurans*	
Trichosporon Beigelii	

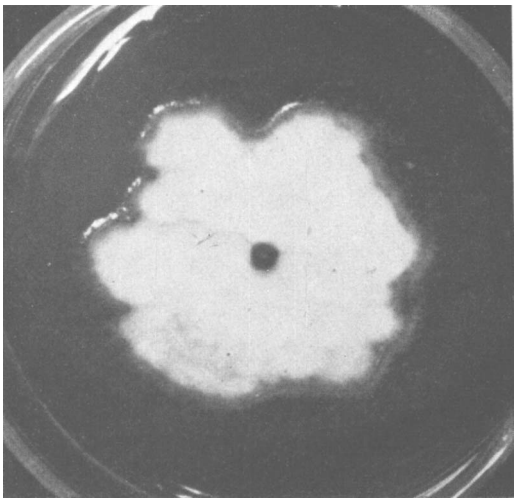

FIG. 1. Inhibition of *Trichophyton rubrum* by a crystal of hemolytic component of fire ant venom.

Methods. A series of fungi, mostly human pathogens, were streaked onto Petri dishes containing a medium consisting of 2% glucose, 1% yeast extract, 1% casamino acids and 2% agar in distilled water. All cultures were tested with a single lot of crude crystalline venom which had a melting point of 139-140°C and was insoluble in aqueous media. A few cultures were also tested with a recrystallized venom having a melting point of 144°-

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