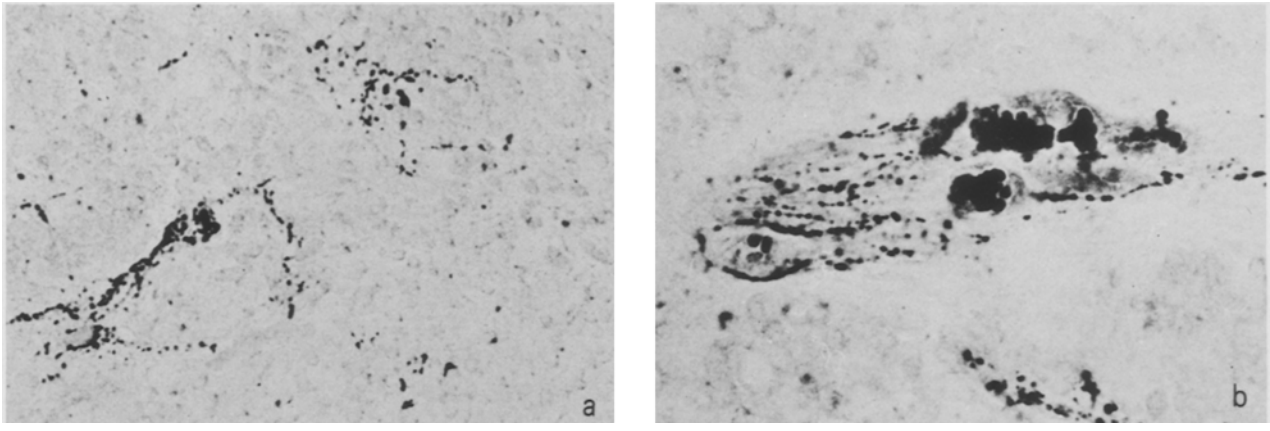

Cat pineal gland. PAP-procedure. Delicate varicose VIP immunoreactive nerves in the pineal parechyma (a) and in the wall of small blood vessels (b). Unspecific staining over red blood cells × 360.

the body. In the brain they are mainly located in cerebral cortical areas, hippocampus and hypothalamus[11]. VIP has a synaptic vesicular localization and can be released by a calcium-dependent, potassium-evoked mechanism[12]. Among known effects of VIP are relaxation of smooth muscle, dilatation of blood vessels and stimulation of intestinal and pancreatic secretion[13–15]. Its neuronal localization and strong biological actions make it tempting to assume that VIP exerts a neurotransmitter function.

The distribution of VIP immunoreactive nerves in the pineal gland suggests that they participate in the regulation of local blood flow. In addition, they probably influence glandular secretory activity.

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The control of melanoblast differentiation in the periodic albino mutant of *Xenopus*

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Summary. Studies on the incidence of melanophores in older ventral trunk tissues and in isolated regions of periodic albino embryos of *Xenopus* suggest that melanin granule formation in mutant melanoblasts depends on an environmental contribution which arises at stage 43 in the endodermal tissues.

Periodic albinism is a mutation (a^p/a^p) of *Xenopus laevis* which affects melanin synthesis. Mutant oocytes and embryos completely lack melanin. However during larval stages the pigmented eye epithelium and melanoblasts undergo a limited elaboration of melanin granules. Such granules later degenerate and post-metamorphic animals possess a typical albino phenotype[1]. Embryonic transplant and explant studies[2–4] have shown that the developmental action of the mutant gene is intrinsic in the neural crest derivatives and does not affect the ability of environmental tissues to support melanoblast differentiation. The mutant gene appears to act at the level of melanosome assembly[5] and does not affect either the migration of melanoblasts or the numbers of these cells colonising the tissues (unpublished results). The present work attempts to clarify the circumstances surrounding the transitory ability of mutant melanoblasts to overcome their deficiency and synthesize melanin during larval stages. Melanin synthesis in mutant melanoblasts commences at stage 43 compared with stage 33/34 in wild-type cells[1] (staging according to Nieuwkoop and Faber[6]). This delay in differentiation of mutant melanoblasts may reflect changes in intrinsic melanoblast prop-

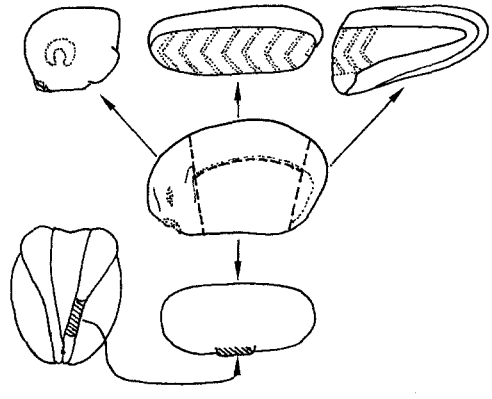
erties which control the timing of melanin synthesis. Alternatively, it is possible that mutant melanoblasts have no intrinsic ability to synthesize melanin and the differentiation of these cells at stage 43 is dependent on environmental factors arising at this time. If such is the case, it should be possible to induce an earlier differentiation of mutant melanoblasts by exposing them to older tissues. In the present study the influence of environmental factors on melanin synthesis in mutant melanoblasts was investigated by examining the development of these cells in older environmental tissues and in isolated tissues of various regions of the embryo. Both mutant and wild-type tissues were used as hosts for mutant melanoblasts so that, in the event of environmental influences becoming apparent, it would be possible to determine whether or not such influences were mutation-dependent.

Methods. Eggs of mutant and wild-type *Xenopus laevis* were obtained by standard methods[7]. Similar-sized portions of neural fold were excized from stage 15 mutant embryos and transplanted to stage 33/34 mutant or wild-type ventral trunk tissues. To ensure absence of incipient melanoblasts, ventral trunk tissues were previously removed from embryos at stage 22 (prior to neural crest migration) and reared as isolates until they reached an age equivalent to stage 33/34 (figure). The techniques used in preparing and culturing embryos and isolates have been described elsewhere[8]. Other embryos of stage 15 (neural crest controls) and stage 33/34 (ventral trunk controls) were allowed to develop normally for use in determining the developmental ages of ventral trunk tissues and neural crest derivatives, respectively. The incidence of melanophores in 20 mutant and 12 wild-type isolates bearing neural crest grafts was recorded when ventral trunk controls had reached stage 43. The embryos from which the ventral trunk tissues had been taken also provided isolates of head, tail and dorsal trunk tissues (figure). The development of melanophores in these isolates was also examined and is reported here as the results appeared to have some bearing on the present investigation.

Results. When ventral trunk controls had reached stage 43 mutant and wild-type ventral trunk isolates bearing mutant neural crest grafts were found to possess 31.45 ± 11.56 (mean $\pm$ SE) and 34.08 ± 7.11 melanophores, respectively. At this time mutant ventral trunk controls also exhibited melanophores, whereas mutant neural crest controls, now at stage 39, completely lacked these cells. Wild-type isolates of head, tail and dorsal trunk exhibited melanophores in the same distribution as wild-type control larvae. In contrast, similar isolates from mutant embryos completely lacked melanophores although these were present in the equivalent regions of mutant control larvae.

Discussion. Stage 43 ventral trunk tissues were found to be capable of eliciting differentiation of stage 39 mutant melanoblasts which would not otherwise have formed melanin granules at this time. This result suggests that melanoblasts in mutant larvae synthesize melanin granules in response to an environmental contribution which arises in ventral trunk tissues at stage 43. The time of differentiation and numbers of melanophores were similar in wild-type and mutant ventral trunk isolates indicating that the factor (s) which enables mutant melanoblasts to differentiate is present in both wild-type and mutant tissues and hence does not result from mutant gene action. Mutant melanoblasts in head, tail or dorsal trunk isolates completely failed to differentiate suggesting that the tissues of these regions are unable to provide suitable conditions for mutant melanoblast differentiation. However, melanoblasts differentiated in the head, tail and dorsal trunk tissues of intact mutant larvae suggesting that the factor(s) present in ventral trunk tissues which elicits mutant melanoblast differentiation must, under normal conditions, circulate to other regions of the larva. The ventral trunk isolates used in the experiments were composed of epidermis, lateral plate mesoderm and endoderm and exhibited on average 30 melanophores per isolate. In a previous study[4], in which mutant neural crest was cultured in vesicles composed of lateral plate mesoderm and epidermis, only about 5 melanophores differentiated in most vesicles and in some vesicles melanoblasts failed to differentiate at all. The higher incidence of melanophores in the present study can be attributed to the presence of endoderm and suggests that this tissue is the main source of the ventral trunk factor. It seems unlikely that this factor, arising as it does at stage 43, has any role in normal wild-type melanoblast differentiation as these cells begin to differentiate at stage 33/34. Furthermore, the time of onset of differentiation and ability to synthesize melanin granules in these cells can be shown to be attributable to intrinsic melanoblast properties[9,10].

The nature of ventral trunk factor and its role in mutant melanoblast differentiation is not clear. It is possible that it is a substance capable of substituting for the defective gene product. Studies so far suggest that the periodic albino defect lies in proteins involved in pre-melanosome assembly[5,11] or in inducers of melanogenesis[3]. Observations that melanosomes synthesized during larval stages in the mutant are both abnormal in structure and unstable[3] are consistent with the concept of substitute molecules forming part of their assembly.



Experimental procedure. Isolates of ventral trunk tissues were prepared form stage 22 embryos by means of 3 straight cuts through the embryo (indicated by interrupted lines). Such isolates were allowed to develop until stage 33/34, at which stage they received grafts of neural fold from stage 15 donors. Head, tail and dorsal trunk isolates, resulting from the same incisions, were allowed to develop without further interference.

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