peripheral subcapsular tumor regions, spots of high labelling are seen. It deserves special attention that the tumor does not grow exclusively at its periphery invading the normal tissue, though apparently very actively just here, but a considerable proliferative compartment is found in the interior of the tumor mass. This part may be of minor importance for the increment of tumor size because of rapid cell loss. However, this proliferating compartment could be critical for metastatic spread via blood vessels.

This map gives an impression of the heterogeneity of human tumor growth. It stresses the need for direct studies of human tumors as the rules of proliferation in small animal tumors are obviously not without limitations applicable to voluminous masses of human tumors. The new method of tumor perfusion with radioactive DNA precursors might be helpful for understanding the strategy of human tumor growth.

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## The control of onset of melanoblast differentiation in *Xenopus* larvae[1]

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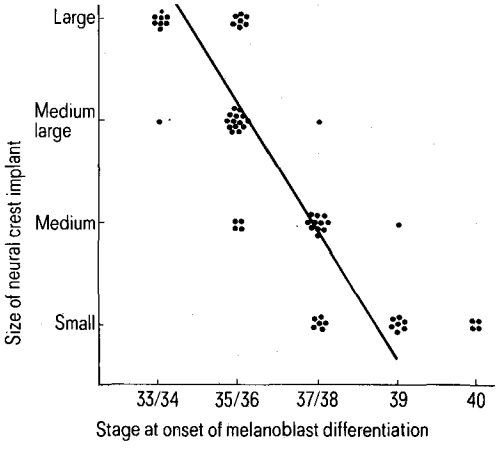
*Summary.* Studies on the time of first appearance of melanophores in uniformly-sized vesicles of hypomeric tissues containing different masses of neural crest tissues suggest that regional differences in the time of first appearance of trunk melanophores in *Xenopus* are regulated by regional variation in the population density of melanoblasts.

*Xenopus* larvae show regional differences in pigmentation and in the time of first appearance of melanophores. Trunk melanophores first appear at stage 33/34 (staging according to Nieuwkoop and Faber[3]), taking the form of a dense band of pigmentation in the hypomeric mesoderm immediately ventral to the somites. At stage 35/36 a 2nd band of melanophores forms on the dorsal surface of the neural tube followed by the appearance at stage 37/38 of melanophores sparsely distributed along the dorsal ridges of the somites. During this time and until stage 41 the hypomeric band of melanophores spreads ventrally and less densely in the lateral mesoderm. In general the most ventral tissues of the hypomere do not become pigmented. The present study describes an investigation of the mechanism determining the time of first appearance of melanophores. Previous studies[4,5] by the present author have demonstrated that various tissues of the trunk have similar capacities for supporting melanoblast differentiation, such capacities arising several stages prior to those at which melanoblasts normally differentiate. Hence a regional variation in the time of first appearance of melanophores cannot be explained by a successive development of local environmental factors favourable for melanoblast differentiation. Regional variation in the time of onset of melanoblast differentiation does however appear to be related to the final distribution of trunk melanophores. Heavily pigmented tissues, e.g. upper hypomeric mesoderm and neural tube were observed to exhibit melanophores at earlier stages than tissues such as the more ventral hypomere and somites which are only sparsely pigmented. MacMillan[5] has shown that the distribution of trunk melanophores is determined by a hierarchy of melanoblast-tissue affinities which influence melanoblast migration. Melanoblast-tissue affinity is reflected by the numbers of melanoblasts colonising tissues. Hence the timing of melanoblast differentiation in different regions of the trunk may depend upon regional differences in the population density of melanoblasts. To test this hypothesis the effect of different melanoblast population densities on the time of onset of melanoblast differentiation was investigated by recording the time at which melanophores first appeared in uniformly-sized vesicles of hypomeric tissues containing different masses of implanted neural crest tissues. Melanoblasts migrate from implant into vesicular tissues; the resulting population density of melanophores in vesicular tissues was considered to be directly related to the initial size of the neural crest implant.

*Methods.* Eggs of *Xenopus laevis* were obtained by standard methods[6] and allowed to develop until stage 22. A portion of neural crest was excised from the anterior trunk region of a stage 22 embryo and divided into 4 unequally-sized portions; the latter, arbitrarily defined as small, medium, medium-large and large, were cultured separately in vesicles derived from similar-sized sheets of lateral hypomeric epidermis and subadjacent mesoderm also obtained from stage 22 embryos. The techniques used in preparing and




Curve relating size of neural crest implant to time of onset of melanoblast differentiation. Dots correspond to individual vesicles.

culturing vesicles have been described elsewhere[5]. 17 vesicles containing small, 16 containing medium, 16 containing medium-large and 15 containing large neural crest implants were examined at regular intervals. The time of first appearance of melanophores and the total number of melanophores in each vesicle were recorded. Other stage 22 embryos were allowed to develop normally for use as controls.

*Results and discussion.* The numbers of melanophores in vesicles were found to be in direct proportion to the size of the neural crest implant (table); the stage at which melanophores first appeared in vesicles varied according to the size of the implant, the larger the implant, i.e. the higher the population density of melanoblasts, the earlier the appearance of melanophores (figure). Melanoblast populations of high density arising from large implants began to differentiate at stage 33/34 and, less frequently, stage 35/36, a time corresponding to the 1st appearance of melanophores in the upper hypomere of control larvae. Less dense populations of melanoblasts showed a delay in time of onset of differentiation in direct proportion to the population density. Thus melanoblast populations of medium-high density arising from medium-large implants began to differentiate at stage 35/36, a time corresponding to the appearance of melanophores on the dorsal surface of the neural tube in control larvae, while melanoblast populations of medium density arising from medium implants began to differentiate at stage 37/38, a time corresponding to the appearance of melanophores on the dorsal ridges of the somites in control larvae. The time of onset of melanoblast differentiation in populations of medium or low densities arising from medium or small implants, stages 37/38 through 40, corresponded to the period of ventral spreading of hypomeric melanophores in control larvae. The results therefore support the proposal than in *Xenopus* larvae regional differences in the time of first appearance of trunk melanophores are regulated by regional variation in the population density of melanoblasts.

The nature of the melanoblast interactions which facilitate melanoblast differentiation under conditions of high population density is not clear. There is some evidence that, in vitro, melanoblasts produce substances capable of facilitating differentiation of neighbouring melanoblasts[7,8]; the present results may be relatable to such a mechanism.

Numbers (mean ± SE) of melanophores in vesicles of hypomeric tissues containing neural crest implants of different sizes

| Developmental stage | Size of neural crest implant Small | Medium | Medium-large | Large |
|---|---|---|---|---|
| 33/34 | 0 | 0 | 0.3± 1.0 | 3.0± 3.1 |
| 35/36 | 0 | 0.9± 1.5 | 4.0± 2.1 | 11.6± 3.2 |
| 37/38 | 0.7± 1.1 | 4.8± 2.5 | 13.9± 6.1 | 21.7± 8.0 |
| 39 | 2.0± 1.7 | 9.9± 2.8 | 22.8± 8.2 | 38.4± 10.3 |
| 40 | 4.0± 2.5 | 12.9± 5.1 | 32.9± 9.7 | 55.3± 11.9 |
| 41 | 6.9± 3.6 | 20.8± 5.2 | 51.6± 11.3 | 71.3± 15.8 |

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## A histochemical demonstration of the $Na^+ + K^+$-ATPase activity in the thyroid and the effect of cyclic adenosine monophosphate (c-AMP)[1]

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*Summary.* With a suitable modification of the Farquhar and Palade technique the $Na^+ + K^+$-ATPase activity in guinea-pig thyroid is demonstrated. The addition of c-AMP ($5 \times 10^{-6}$ M or $1.5 \times 10^{-5}$ M) to the incubation media produced an apparent intensification of the $Na^+ + K^+$-ATPase activity in the thyroid.

Interest in research into the $Na^+ + K^+$-ATPase activity in the thyroid gland dates from 1958–1963, with findings made by Wolff et al.[2–4] that in normal functioning of iodide pump the $Na^+ + K^+$-ATPase activity of thyreocytes is necessarily involved. Since then many biochemical reports concerning $Na^+ + K^+$-ATPase activity in the thyroid have been published[5–8]. The $Na^+ + K^+$-ATPase activity in the guinea-pig thyroid has been recently shown by Fujita and Nanba[9].

This paper deals with the histochemical demonstration of $Na^+ + K^+$-ATPase activity in the thyreocytes and parafollicular cells of the guinea-pig thyroid. The effect of c-AMP on the $Na^+ + K^+$-ATPase activity will also be presented.

*Material and methods.* 8 male guinea-pigs weighing 600–800 g were used. After the animals had been killed, the thyroid glands were quickly removed and frozen. The cryostat slices of 10 µm thickness were incubated in freshly prepared media for 20 min at 35 °C. The composition of the media used in our experiments was similar to that described by Farquhar and Palade[10] and used later by Fujita and Nanba[9] for demonstration of the $Na^+ + K^+$-ATPase activity in the thyroid; but the concentration of $Mg^{2+}$ ions in our media was 1 mM only, and the $Na^+ + K^+$-ATPase activity in our experiments was defined as the difference of reaction deposits observed on slices incubated in a medium containing $Na^+$ 100 mM, $K^+$ 20 mM and $Mg^{2+}$ 1 mM and another which contained $Mg^{2+}$ 1 mM only. In the original Farquhar and Palade[10] technique, 5 mM $Mg^{2+}$ was used and ouabain was added to media with $Mg^{2+} + Na^+ + K^+$ for evaluation of $Na^+ + K^+$-ATPase activity. To avoid the necessity of formalin prefixation, the media used in our experiments contained 1.44% gelatine. The postincubation procedure in our experiments (lavages and ammonium sulfide treatment) was the same as in the Farquhar and Palade technique.

The investigation of the effects of c-AMP on the $Na^+ + K^+$-ATPase activity was performed by adding the c-AMP to both incubation media: the one containing $Mg^{2+}$ ions