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 (5×10^{-6} M or 1.5×10^{-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

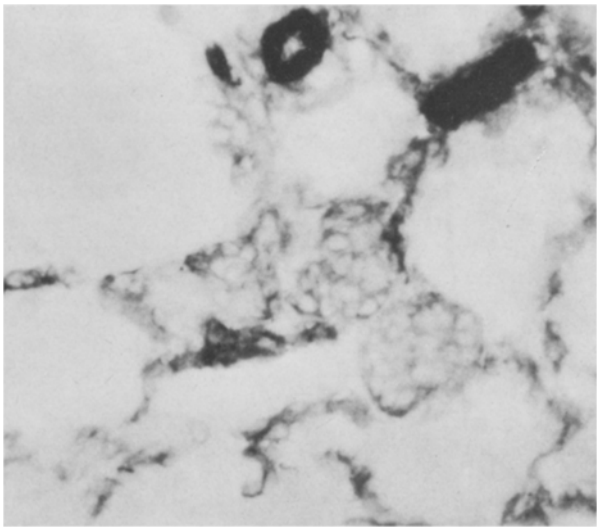

Fig. 1. Guinea-pig thyroid. The ATPase reaction after incubation in medium with $MgCl_2$ 1 mM. × 480.

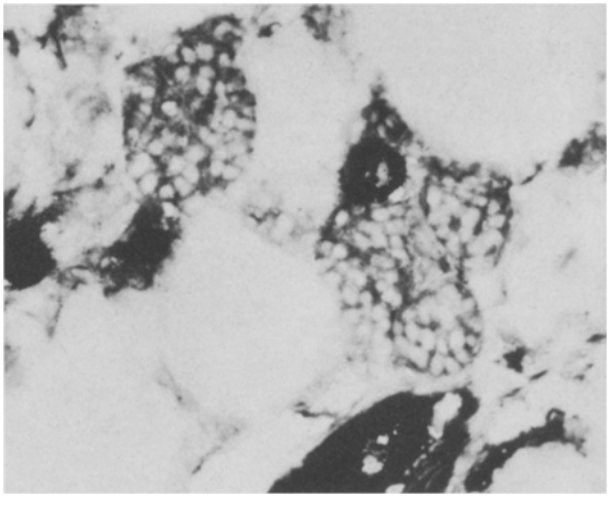

Fig. 2. Guinea-pig thyroid. The ATPase reaction after incubation in medium with $MgCl_2$ 1 mM + NaCl 100 mM + KCl 20 mM. A more intensive reaction product is seen than that in figure 1 with 1 mM of $MgCl_2$ only. × 480.

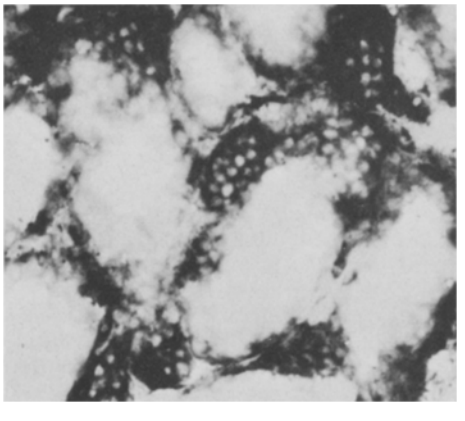

Fig. 3. Guinea-pig thyroid. The ATPase reaction was obtained in the same conditions as in figure 2, but c-AMP 5×10^{-6} M was added to the medium for incubation. A more intensive reaction is seen than in figure 2. × 480.

only, and the other containing $Mg^{2+} + Na^+ + K^+$. The c-AMP was applied in concentrations of 5×10^{-6} M or $1{,}5 \times 10^{-5}$ M. In the 2 corresponding media which served as controls, the 5'AMP was added to the concentration equimolar with that of c-AMP. This was used to avoid a possible difference in the quantity of reaction product (PBS) as a consequence of the hydrolysis of the degradation products of c-AMP by 5'-nucleotidase activity in experimental media.

Results and discussion. The results obtained in media with Mg^{2+} ions only (figure 1), and after incubation in media containing $Mg^{2+} + Na^+ + K^+$ (figure 2), show that the $Na^+ + K^+$-ATPase activity is present in thyreocyte membranes as well as in parafollicular cells and blood vessels endothelium. A comparison of experiments performed with media which contained the c-AMP with corresponding controls indicate: a) the failure of visible changes of Mg^{2+}-activated ATPase activity, and b) the apparent intensification of the reaction product in media with $Na^+ + K^+ + Mg^{2+}$ to which c-AMP was added (figure 3).

It seems that the modification of the original Farquhar and Palade technique, here employed, made possible the demonstration of the $Na^+ + K^+$-ATPase in a more precise manner. The authors of the technique themselves state in their original work[10] that in their experiments the distribution pattern of reaction product did not change in the presence of $(Na^+ + K^+)$ or $(Na^+ + K^+)$ and ouabain, and that the difference in the intensity of lead deposits was too small and not reproducible enough to reach a conclusion. Our opinion is that this weak sensitivity of the $Na^+ + K^+$-ATPase to ouabain inhibition in histochemical experiments may be the consequence of 2 causes: a) The binding of ouabain with its receptors requires a few minutes[11]. – In biochemical experiments this is performed in the preincubation time of warming. In histochemical experiments ouabain comes in contact with the tissue just during the incubation time. b) The ouabain sensitivity of $Na^+ + K^+$-ATPase is strongly influenced by the conformational state of the membrane[12] and this may be different in cryostat sections from that existing in preparations obtained with biochemical techniques. The technique applied in this experiment made it possible to show the $Na^+ + K^+$-ATPase activity in the parafollicular cells in the thyroid. As far as we know, the $Na^+ + K^+$-ATPase activity in parafollicular cells has not yet been described.

The effect of c-AMP added to corresponding incubation media was studied. The more intensive reaction visible after the addition of c-AMP in media with $Na^+ + K^+ + Mg^{2+}$ and the absence of visible difference after the addition of c-AMP in media with Mg^{2+} ions only, demonstrates that c-AMP has a positive influence on the $Na^+ + K^+$-ATPase activity in the thyroid.

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