can birds (fig. 1A). We then used the African and German birds of the above experiment for a cross-breeding experiment in aviaries in 1978 and 1979. We successfully handraised 33 hybrids and investigated them for juvenile moult, wing length, and body weight in exactly the same way as their parents. The F_1-hybrids showed an intermediate time course of juvenile moult (fig. 1B) which differed significantly from that of the parental African population in onset, duration and termination ($p < 0.001$, $p < 0.02$, and $p < 0.01$ respectively, Mann-Whitney U-test) and from the parental German population in onset and duration ($p < 0.001$, and $p < 0.01$). Statistically significant intermediate features in the hybrids were also found in wing length and body weight (fig. 2; all comparisons $p < 0.001$). In conclusion, the F_1-hybrids showed consistent intermediate expression of physiological and morphological features associated with migration, all of which have now been shown to be under strong direct genetic control. Consequently individual populations of blackcaps and probably also a series of other migratory bird species with similar migratory adaptations are equipped with innate, genetically fixed time-programs for juvenile moult and genetically fixed morphological prerequisites for migration.

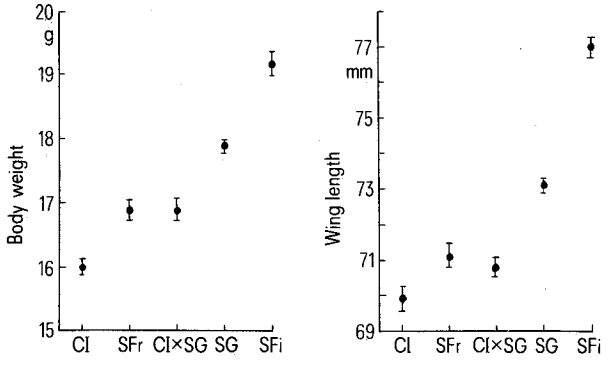

Figure 2. Premigratory body weight and wing length (mean values with SE) of 4 populations of blackcaps and of F_1-hybrids (CI × SG) (abbreviations as in fig. 1).

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Serotonin-containing cells in the ascidian endostyle

D. A. Sakharov and Natalia Salimova[1]

Koltzov Institute of Developmental Biology of the USSR Academy of Sciences, 117334 Moscow (USSR), 1 June 1981

Summary. In the ascidian endostyle, all fluorescence due to serotonin is localized in the peripheral, iodine-binding area of the endostylar epithelium, which is homologous to the vertebrate thyroid. The amine appears to be stored in granule-containing cells which may correspond to the vertebrate calcitocytes.

In a survey of the relative amounts of serotonin (5-hydroxytryptamine, 5-HT) in different tissues of an ascidian, the highest concentration was found in the endostyle or subpharyngeal ciliary groove[2]. An explanation for this finding was suggested by Gorbunova who investigated the ascidian endostyle by electron microscopy. Gorbunova described a previously-unknown cell type in the peripheral iodine-binding region of the endostylar epithelium and suggested that it corresponds to the parafollicular cells of the thyroid tissues[3]. The parafollicular cells (C-cells, calcitocytes) are known to be able to synthesize 5-HT[4]. Gorbunova speculated, therefore, that calcitocyte-like granule-containing cells of the endostyle might be responsible for the high content of 5-HT in the organ[3].

The hypothesis that calcitocytes of protochordates occur within the pharyngeal, i.e. endodermal, epithelium is obviously in disagreement with the assumption that the vertebrate calcitocytes are of neuroectodermal origin. This view, however, has received some support from recent findings of calcitonin-like immunoreactivity in ascidian endodermal epithelia[5,6]. In the present study we investigated the localization of 5-HT in the endostyle in order to test another prediction of the hypothesis.

Material and methods. 14 ascidians of 3 species, *Styela rustica*, *S. clava* and *Molgula retortiformis*, were used in this study. Animals were obtained from the Sea of Japan and the White Sea[7] and kept in aquaria before use. Cryostat sections or stretch preparations of the pharyngeal tissue were prepared for examination by fluorescence microscopy, using the formaldehyde[7] and glyoxylic acid[9] techniques for the localization of biogenic amines. The specificity of the fluorescence was checked using controls and microspectrofluorimetry, as described previously[10].

Results and discussion. In all 3 species investigated, specific fluorescence due to biogenic monoamines was obtained in stretch preparations of the pharynx treated with formaldehyde or glyoxylic acid. This is illustrated by figure 1 in which a row of brightly fluorescent cells can be seen against a dull background of autofluorescent structures. There were 2 such rows of fluorescent cells, 1 on each side of endostyle. The colour of the cells was yellow in both formaldehyde and glyoxylic acid preparations. The cells were typically smooth, ovoid or flask-like in shape. Within a row the cells were usually arranged in small clusters distributed at irregular intervals. Investigation of transverse sections of the endostyle confirmed that all specific fluores-

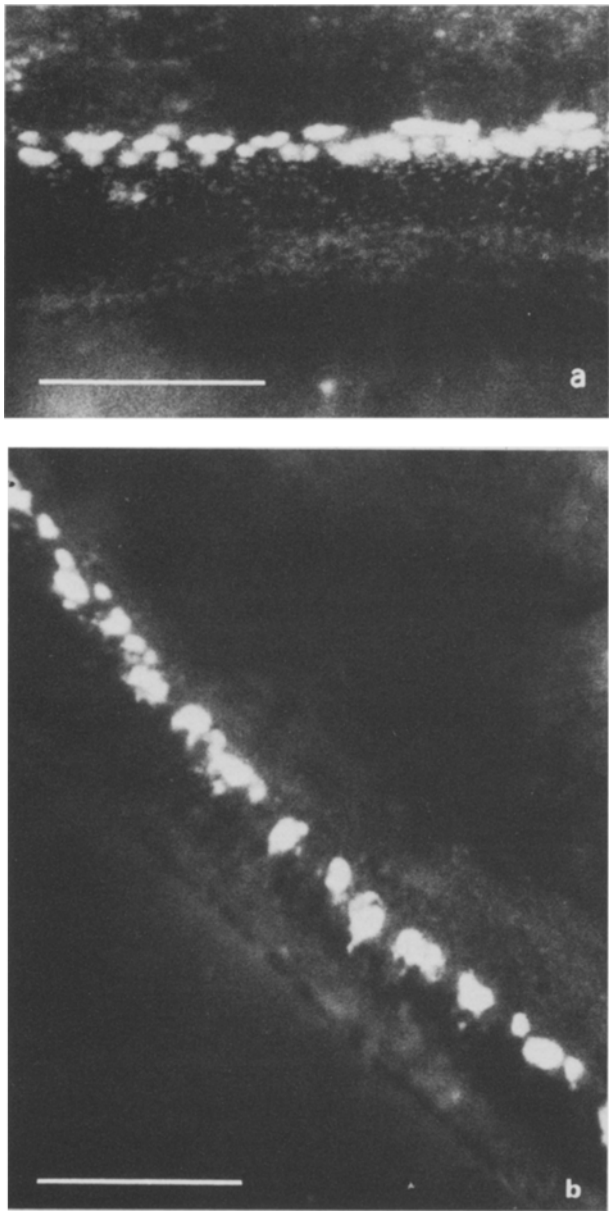

Figure 1. One of 2 paired rows of yellow fluorescent cells in stretch preparations of the pharyngeal wall of the ascidian *Styela rustica*. The cells lie in the peripheral region of the endostyle. *a* Aqueous formaldehyde method, bar: 100 µm. *b* Glyoxylic acid method, bar: 100 µm.

cence was confined to 2 symmetrical rows of yellow cells and showed them to be localized at the periphery of the endostylar epithelium. On each side these cells occupy a narrow and clearly defined part of the 7th cellular zone (in Barrington's nomenclature[11]) (fig. 2). Single yellow fluorescent and green fluorescent cells were also observed scattered in the pharyngeal epithelium, outside the endostyle. We could find no monoamine-containing neurons in the pharynx.

Microspectrofluorimetric analysis of the glyoxylic acid-induced yellow fluorophore in the cells of the 7th zone of the endostyle revealed an emission peak maximum at 525 nm which corresponded to that of 5-HT fluorophore in a model system[12].

The finding of yellow fluorescent cells in the ascidian endostyle is consistent with the reports of a high 5-HT

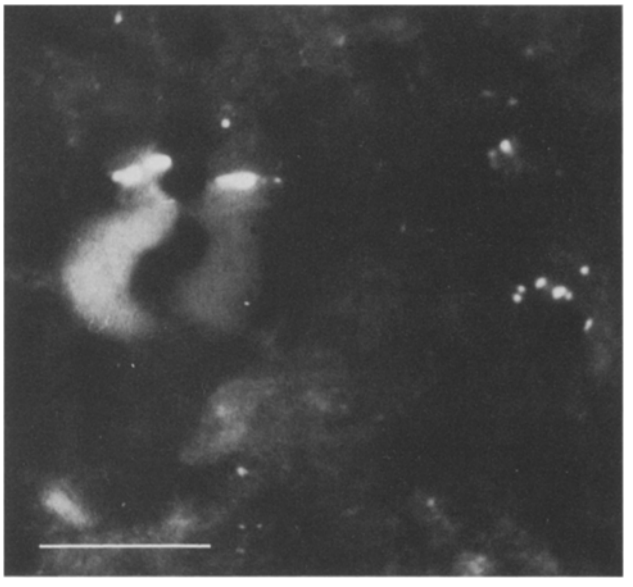

Figure 2. The endostylar area of the pharynx of *Styela clava* cut transversely. Glyoxylic acid method. Note in upper left corner of picture fluorescent cells occupying 2 symmetrical parts of the endostylar epithelium. Single fluorescent cells are visible in the pharynx to the right of the picture. Bar: 200 µm.

content in this organ. Both the color of the fluorescence and the results of microspectrofluorimetric analysis justify the conclusion that the fluorescence is due to the presence of 5-HT. The shape, position and distribution of 5-HT-containing cells indicate that they are granule-containing cells found at the periphery of the endostyle by electron microscopy[3-5]. It has been reported by Thorndyke and Probert that similarly localized cells cross-react positively with anti-calcitonin[5]. One may conclude that the predictions made by Gorbunova[3] were correct: specific cells of the 7th zone of the ascidian endostyle in fact contain both a calcitonin-like peptide and 5-HT, the characteristic substances of vertebrate calcitocytes.

The iodine-binding area of the ascidian endostyle (the 7th and, to a lesser extent, the 8th zones) is generally assumed to be homologous to the vertebrate thyroid. Our results confirm previous suggestions that the 7th zone is not homogeneous: besides cells which correspond to the follicular cells of the thyroid tissue, there occur 5-HT-containing cells which seem to correspond to the parafollicular cells.

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