intact C^2H_3-units possibly by nucleophilic attack of an appropriate enolate and precludes a possible mechanism via cyclopropane or methylene intermediates.
The incorporation of the different labeled atoms in cyclosporin A is schematically shown in fig. 1.

1 Acknowledgments. We thank Prof. Dr D. Arigoni (Federal Institute of Technology, Zürich) for helpful discussions, Miss S. Bogner and Messrs S. Mangin, R. Masero and R. Reimann for their excellent technical assistance.
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0014-4754/83/080873-04$1.50+0.20/0

Immunocytological study of the distribution of C-cells calcitonin in the thyroid gland of the normal adult gerbil

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Summary. The distribution of C-cells in the thyroid gland of the gerbil *(Meriones inguiculatus)* was studied on serial sections in the cranio-caudal direction. C-cells were visualized by immunofluorescence with an anti-calcitonin immune serum. A very heterogeneous distribution was found. C-cells first appear in the posterio-interior position. Then they progressively occupy the whole posterior part and finally are confined to the posterio-exterior position. No C-cells at all were detected in the upper and lower poles. According to the species, there is a wide variation in the relative distribution of C-cells in the thyroid gland. Our results add to the information available about the distribution of C-cells and its bearing on phylogenic evolution.

The parafollicular C-cells have been ascribed as the site for synthesis, storage and release of the hypocalcemic factor calcitonin[2,3]. Their distribution has been described in some vertebrate species, including man[4-8]. There is no such description for the thyroid gland of the gerbil *(Meriones inguiculatus)*, an animal more and more frequently used for research[9-15].
The purpose of this paper is to illustrate the distribution of C-cells in the thyroid of the adult Gerbil.
Material and methods. Sheep antiserum to synthetic human calcitonin (Ciba-Geigy) was obtained by repeated intradermal injections of this antigen coupled to ovalbumine (Sigma) with glutaraldehyde and emulsified with Freund's complete adjuvant.
Adult gerbils were sacrificed at 4 weeks: their thyroids were excised, fixed in aqueous Bouin's solution, dehydrated and embedded in paraffin. The thyroid gland was along its entire length in a plane perpendicular to the long axis, and sections (6 µm thick) were taken every 3 mm.
Deparaffined sections were used for an indirect immunofluorescence technique. The reaction was performed with sheep anti-human calcitonin immune serum (dilution 1:10) and fluorescent conjugate of goat anti-sheep gammaglobulin (Institut Pasteur). The staining period was 2 h at room temperature. Evans Blue (0.01%) was used as a counter stain. The tissue sections were examined with a Leitz fluorescence microscope with a vapour mercury lamp for epi-illumination, BG12 exciter filter and K530 barrier filter.
The immunofluorescent reaction detected a moderate number of cells within the thyroid. No fluorescence was present when the conjugate was used alone, or when the specific antibody was saturated by synthetic human calcitonin. Thus, the specificity of these antisera and cross-reaction between human and gerbil thyrocalcitonin can be assumed.
Results. The C-cells were irregularly distributed through 1 section and between 2 other sections. Depending on the follicule, one or several fluorescent cells were visualized around it (fig. 1). The localization was referred to the

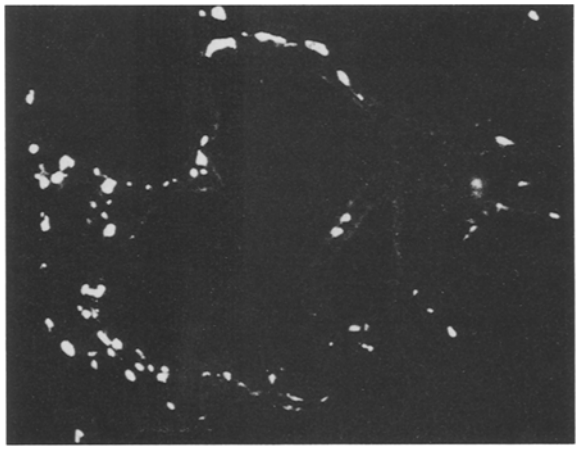

Figure 1. C-cell distribution in gerbil thyroid gland is visualized by immunofluorenscence with antihuman calcitonin sera. Note their parafollicular position.

position of the section along the long axis of the thyroid. The distribution of C-cells was symmetrical between the 2 lobes, but differed according to the level of the section (fig. 2).

The C-cells were confined to the posterior part of the lobe, and never appeared in the anterior situation. No cell was present in the upper pole (fig. 2, A). Sections were examined in the cranio-caudal direction. Initially C-cells were detected in the posterio interior position (fig. 2, B), and progressively others appeared in the outer situation, to occupy centrally the whole posterior part (fig. 2, D and C). Then they progressively disappeared from their inner situation, to be confined to the posterio exterior position (fig. 2, G). Then they disappeared at the lower pole (fig. 2, H).

With Hematoxylin-Eosin-Safran, the C-cell-rich areas appeared as hypercellular foci. Grimelius stain was negative. Sections from other tissues (including the parathyroid) which were studied did not contain any C-cells outside the thyroid gland.

Discussion. The distribution of C-cells in the thyroid was studied by Wolfe et al., Leroyer-Alizon et al.[6] in the human, by Das and Das[4] in the Rhesus Monkey, and by Blahser[7] in the horse, pig, deer, mole and rat. Generally, except in the pig, heterogeneity was noted in the C-cells' distribution. They were reported in the central region of the lobe (monkey, rat), in the posterio-lateral part (mole) and in the upper and middle lateral part (human). Except in the pig, polar regions are devoid of C-cells. Distribution as sharply delimited as we observed has never been reported previously. The concentration of C-cells in a distinct part of the thyroid seems to constitute an intra-thyroid 'C-cells gland' (fig. 3). Grimelius' method, used to detect argyrophilic cells, was consistently negative; in contrast, argyrophilic granules were detected in human medullary carcinoma. No explanation could be provided for these negative results.

It is known that C-cells migrate from the neural crest[16]. In fish, amphibians, reptiles and birds, they are present in a distinct organ known as the ultimobranchial body[17]. In the mammalian embryo the ultimobranchial body fuses with the thyroid gland, and C-cells migrate through the thyroid[8].

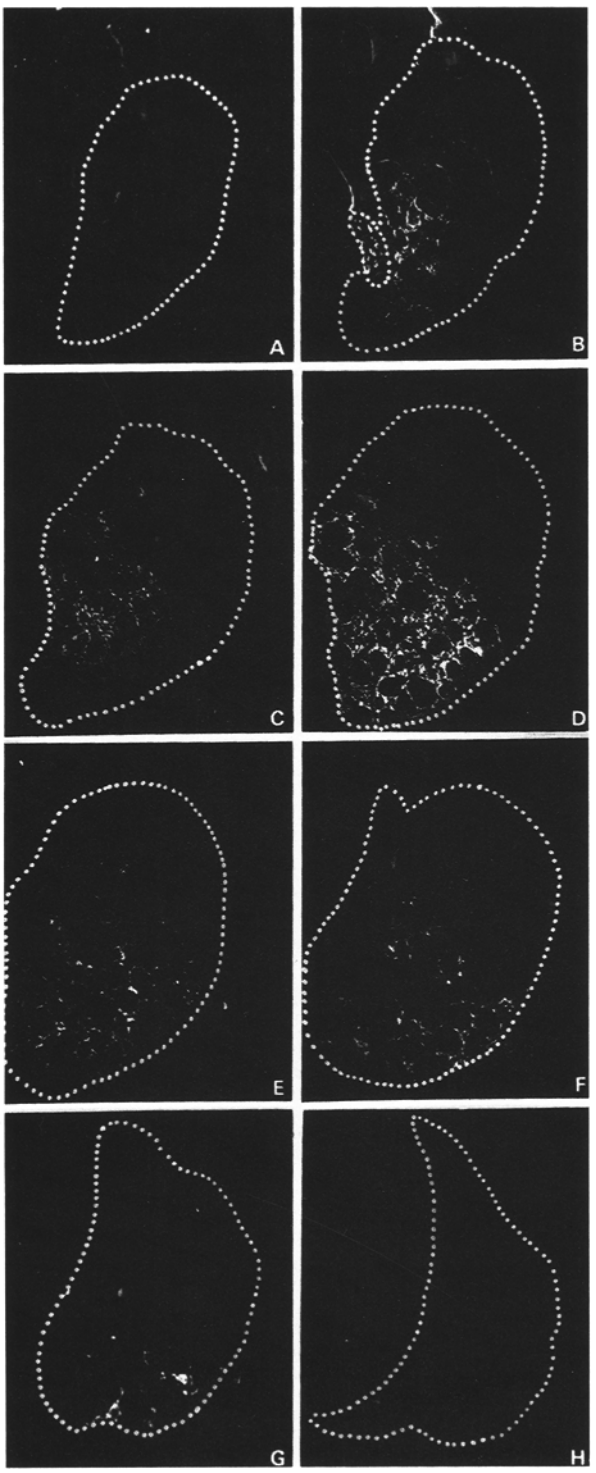

Figure 2. C-cell distribution in gerbil thyroid gland. C-cells are first detected in the inner part (B), then they occupy the whole posterior part (D), and finally are confined to the outer part (G). No C-cells are detected in the pole (A, H).

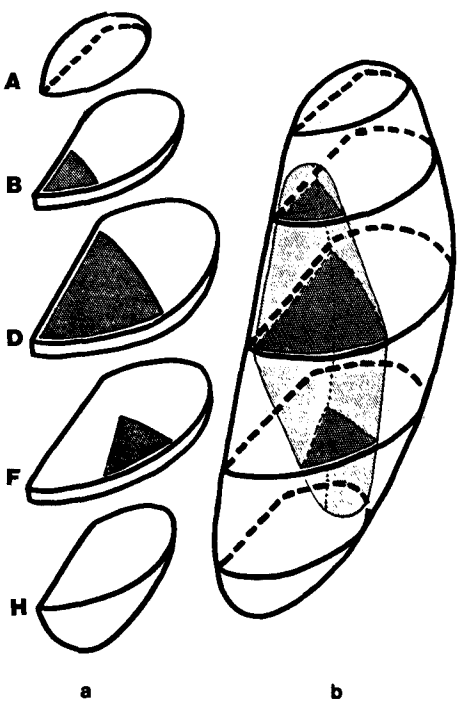

Figure 3. Schematic space distribution of C-cells within the right lobe of gerbil thyroid gland (inner part at left, outer part at right). Dark area represent C-cell-rich region. *a* Repartition of C-cells according to the level in the thyroid gland. *b* Volume filled by the area containing C-cells in the thyroid gland.

Thus, we have added some information to that available about the phylogenic dependent relationship between the thyroid gland pharyngeal IV regions (parathyroid IV, thymus IV) and the neural crest.

1 Acknowledgments. The authors are greatly indebted to Mrs M-Ch. Pejot and to Mr D. Cox for advice.
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0014-4754/83/080876-03$1.50 + 0.20/0

Selective staining of β-esterase of *Drosophila*

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Summary. It is shown that the potent protease inhibitor phenylmethylsulphonylfluoride (PMSF) strongly inhibits the activity of all *Drosophila* esterases but β-esterase. We suggest the application of PMSF for the selective staining of β-esterase in *Drosophila* tissues.

Understanding of the molecular mechanisms of cellular differentiation needs the analysis of cell population heterogeneity in the process of individual development[1]. Determination of the ratio of different forms of the same enzyme at the level of individual cells is one of the most promising methods in this field. A large variety of histochemical methods, as well as microelectrophoresis, have been successfully used for this purpose.

As reported in a number of papers[2-4], the investigation of esterases in *Drosophila* permits us to obtain valuable information about differentiation processes and regulation of genetical expression during the development of this classical object of genetics. However, the investigation of esterases was limited by the ability of many of these enzymes to interact with the same substrates. This allows the determination of esterases only as a group, and important information about the distribution of a particular esterase isozyme in different tissues or in a single cell cannot be obtained. The same is true for the determination of qualitative changes of particular esterases during differentiation and development.

In the present paper we describe a novel approach which permits selective elucidation of *Drosophila* β-esterase. The procedure is based on the inhibitory action of PMSF on all of *Drosophila* esterases except β-esterase.

Materials and methods. Experiments were made with organs of *Drosophila virilis* of the strain $Ce^{-}S^{+}$ which is characterized by high activity of the organ-specific S-esterase in the tissues of the bulbus ejaculatorius. Microelectrophoresis was done as described earlier[5]. Single organs were used to prepare the samples under investigation. Tissues were homogenized in a 0.5% aqueous solution of Triton × 100 (Merck) and the extract obtained was loaded on to a polyacrylamide gel prepared in a glass capillary (0.5 mm ID). The electrophoresis system was Tris-EDTA-borate (Serva) at 220 V, 2 mA. To stain the esterase isozymes after electrophoresis we employed the enzymatic reaction with α-naphthylpropionate and β-naphthylacetate[3].

Histochemical staining of various *Drosophila* tissues was done as described earlier[5]. Three types of incubation mixtures were used: a) containing α-naphthylpropinate; b) containing β-naphthylacetate and c) the mixture of both substrates.

The solution of 0.1 M PMSF (Calbiochem) in dimethylsulfoxide (Serva) was added to the incubation mixtures during histochemical procedures to a final concentration of 1 mM. The same concentration of PMSF was used to extract tissues with 0.5% Triton × 100. During the staining of isozymes after microelectrophoresis the inhibitor was not added to the reaction mixture.

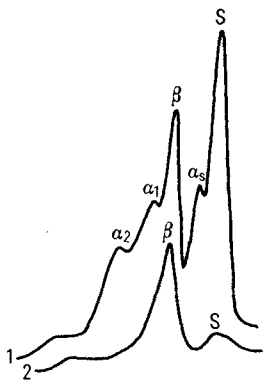

Figure 1. Scanogram of the stained gel after microelectrophoresis of: 1, 0.5% Triton × 100 extract of a single male fly; 2, the same but with the addition of PMSF to the extraction solution.