The ratio of P_i content in erythrocytes to the content in plasma

Conservating medium	Weeks of storage at 4 °C			
	0	1	3	6
ACD	1.2	3.9	2.1	1.7
ACD-A	0.3	1.2	3.8	1.9
ACD-A-P persantin (1×10^{-4} *M*)	0.3	1.0	5.9	3.7
ACD-A-P persantin (4×10^{-4} *M*)	0.5	1.4	6.4	6.1

In conclusion, it may be stated that during the conservation of blood at 4 °C in the medium ACD-adenosine-persantin: 1. The concentration of 2,3-DPG sinks more slowly and the content of P_i in red cells increases more slowly than in ACD and ACD-adenosine. 2. The red cell membrane in the presence of these 2 compounds reduces the P_i transport to the plasma.

Zusammenfassung. Persantin verzögert den Konzentrationsanstieg des anorganischen Phosphats in Erythrozyten und Plasma bei der Lagerung von Blutkonserven gegenüber der alleinigen Zugabe von ACD oder ACD-Adenosin.

B. Zachara[12]

Department of Physiological Chemistry, AM, Pl. 9 may No. 1, P–90 647 Łódź (Poland), 20 February 1973.

[12] The author is very much indebted to Dr. Barbara Lewandowska for revision of the English text.

Immunohistochemical Demonstration of Corticotrophic Cells Concentrated in the Rostral Zone of the Pars Intermedia of the Mouse Hypophysis

Cells whose fine morphology makes them comparable to the corticotrophic cells of the pars distalis (PD), have been described in the pars intermedia (PI) of the rat and mouse hypophysis[1–4]. These cells are particularly abundant in the rostral zone of the PI. This is especially clear in the mouse, where the rostral zone of the intermediate lobe comprises essentially ACTH type cells which are obviously stimulated after adrenalectomy[1]. The mouse is therefore particularly suitable for an immunohistochemical study intended to reveal the corticotrophic nature of these cells in the PI and to distinguish them clearly from the MSH-producing cells.

Technique. 10 mouse hypophyses were fixed either with Elfmann's fluid or with Stieve's solution. The 'indirect' reaction was performed on 5 µm sections with anti-ACTH or anti-MSH antisera obtained from the rabbit using a synthetic ACTH (β 1–24 corticotropine: Synacthen, Ciba) or synthetic α or β MSH (Ciba), and with sheep antirabbit γ globulin coupled with fluoresceine isothiocyanate.

The specificity of the antibodies used was evaluated by immunofluorescence inhibition tests[5]. As for the antigenic affinity of the anti β 1–24 corticotropine antibodies, it should be noted that doses of synthetic α or β MSH 40 times higher than the minimum inhibiting dose of β 1–24 corticotropine do not inhibit the reaction.

The specificity of the reaction, on the other hand, is verified by successive application to the sections of: 1. rabbit anti ACTH or anti MSH antibodies, 2. unlabelled sheep anti-rabbit γ globulins and 3. fluoresceine isothiocyanate labelled sheep anti-rabbit γ globulins. No reaction is detected under these conditions.

Results. As we have already shown[1], the rostral zone of the mouse PI reveals a characteristic appearance by light and electron microscopy (Figure 1). The MSH-producing cells are immediately adjacent to cells which are small and clear, with extremely intricate prolongations and contain dense marginal secretory granules, between 160 and 230 nm in diameter. These cells are in direct relation – without intervening basement membrane – with the nervous tissue of the pituitary stalk. Some of these cells are detached from the epithelium and located either singly or in small clusters in the stalk and in the neural lobe. Cells of the same type, which sometimes contain finer granules, are encountered in the remainder of the PI, at the periphery of the lobe, i.e. underneath the lining epithelium of the hypophysial cleft and along the neural lobe.

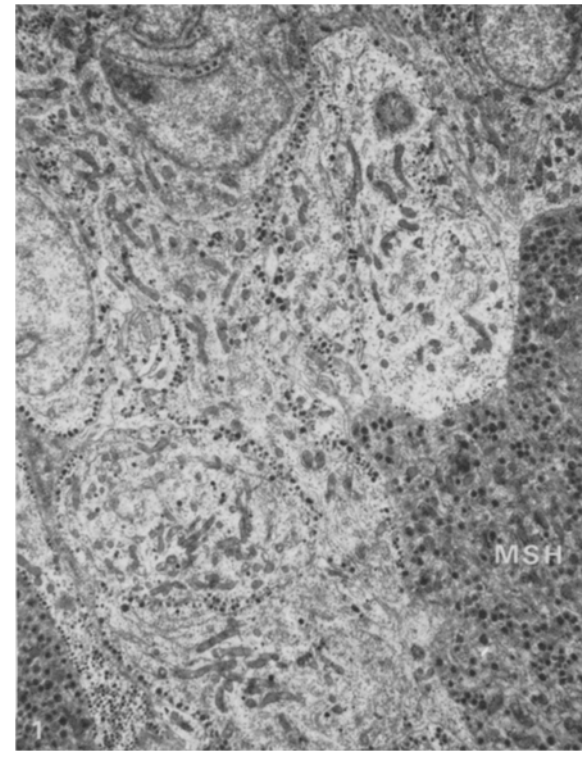

Fig. 1. Appearance, under the electron microscope, of a portion of the rostral zone of the pars intermedia showing the replacement of MSH cells by small corticotrophic-type cells containing dense marginal granules. ×4,500.

[1] M. E. Stoeckel, H.-D. Dellmann, A. Porte and C. Gertner, Z. Zellforsch. *122*, 310 (1971).
[2] G. C. Moriarty and N. S. Halmi, Z. Zellforsch. *132*, 1 (1972).
[3] D. V. Naik, Z. Zellforsch. *133*, 415 (1972).
[4] M. E. Stoeckel, H.-D. Dellmann, A. Porte, M. J. Klein and F. Stutinsky, Z. Zellforsch. *136*, 97 (1973).
[5] M. P. Dubois, Z. Zellforsch. *125*, 200 (1972).

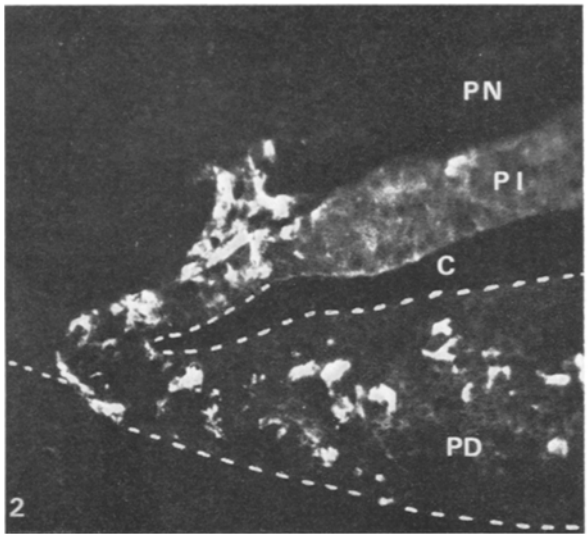

Fig. 2. Positive reaction of the rostral zone of the pars intermedia (PI) with anti β 1–24 corticotropine antibodies. Note the infiltration by positive epithelial cells of the pars nervosa (PN). The remainder of the pars intermedia does not react, except a few peripheral cells. Numerous positive cells in the pars distalis (PD). C, hypophysial cleft. $\times 210$.

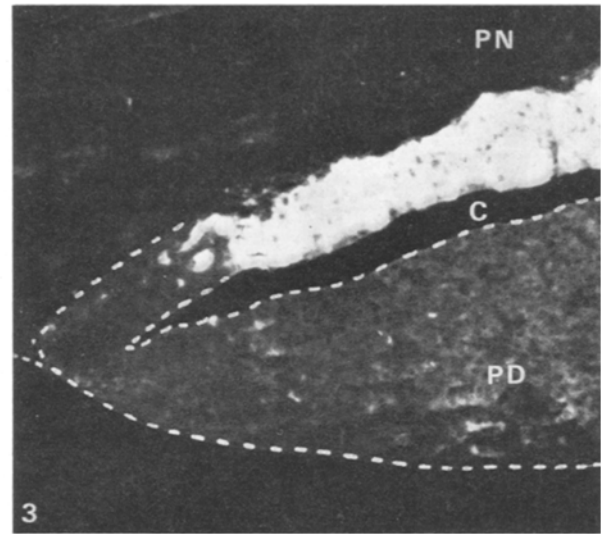

Fig. 3. Negative reaction of the rostral zone of the pars intermedia with anti β MSH antibodies. Massive positive reaction of the remainder of the pars intermedia. Absence of reaction in the pars distalis. $\times 210$.

Tests with anti-ACTH and anti-MSH antibodies reveal a basic difference between the rostral zone and the remainder of the PI: The cells of the rostral zone react with the anti β 1–24 corticotropine antibody as intensely as the corticotrophic cells of the PD (Figure 2). The epithelial cells enclosed in the nervous tissue are as fluorescent as those composing the epithelial mass. The reaction of the remainder of the PI is diffuse and varies according to the individuals, but is always weak in comparison with the rostral zone; a few peripherally located cells are, however, intensely positive.

Anti α and anti β MSH antibodies are fixed massively in the PI, except in the rostral zone which remains remarkably negative, as does the whole PD (Figure 3).

Discussion. 1. The elective and massive reaction of the rostral zone of the PI to anti β 1–24 ACTH antibodies, and its non-reactivity towards anti α and anti β MSH antibodies, confirm the corticotrophic nature of these cells, which have dense marginal granules, and are the principal constituent of this part of the mouse PI. A precise correlation exists between the immunohistochemical data and the electron microscope observations.

2. The corticotrophic activity of the neuro-intermediate lobe[6,7] cannot be explained entirely by the presence of this ACTH-cell system in the PI. Immunohistochemical techniques combined with electron microscopy in the rat[2] do not in fact exclude a possible synthesis (or a reversible fixing[4]) of ACTH by the MSH-producing cells. According to our results in the mouse, where the weakness of crossed immunohistochemical reactions between MSH and ACTH cells and the higher concentration of corticotrophic cells in the rostral portion of the PI permit extremely precise observations, the ACTH cells of the PI possibly play a predominant role.

3. In the mouse and in the rat (and probably also in the cat[8]), the corticotrophic cells of the PI are in close topographical relation with the terminal portal vessels and with the various types of nerve fiber of the stalk and of the proximal portion of the neural lobe. Synaptic contacts occur at this level between the ACTH cells and the mainly aminergic nerve terminals[1]. This lay-out of corticotrophic cells in the PI permits a better understanding of the particular control of the neuro-intermediate lobe ACTH liberation, which seems to constitute a specific response to neurotropic stresses[6,7].

Résumé. La nature corticotrope des cellules constituant la partie rostrale de la pars intermedia de l'hypophyse de souris est établie par la microscopie électronique et la cytoimmunofluorescence. Cette zone réagit positivement avec les anticorps anti-ACTH et négativement avec les anticorps anti-MSH.

M. E. STOECKEL[9], J. DOERR-SCHOTT[10], A. PORTE[9], H. D. DELLMANN[11] and M. P. DUBOIS[12]

Institut de Physiologie et de Chimie Biologique, Université Louis Pasteur, Section Sciences, rue Descartes, Esplanade, F–67000 Strasbourg (France); Laboratoire de Cytologie animale, Université Louis Pasteur, Section Sciences, 12, rue de l'Université, F–67000 Strasbourg; Department of Veterinary Anatomy, University of Missouri, Columbia (Missouri 65201, USA); and Station de Physiologie de la Reproduction, I.N.R.A., F–37380 Nouzilly (France), 18 April 1973.

[6] C. MIALHE-VOLOSS, Acta endocr. (Kbh) Suppl. *35*, 1 (1958).
[7] G. J. ROCHEFORT, J. ROSENBERGER and M. SAFFRAN, J. Physiol., Lond. *146*, 105 (1959).
[8] M. E. STOECKEL, H.-D. DELLMANN, A. PORTE and F. STUTINSKY, Bull. Ass. Anat., Paris *57*, 165 (1973).

[9] Institut de Physiologie et de Chimie biologique, Université Louis Pasteur, Section Sciences, Rue Descartes, Esplanade, 67000 Strasbourg, France.
[10] Laboratoire de Cytologie animale, Université Louis Pasteur, Section Sciences, 12, rue de l'Université, 67000 Strasbourg, France.
[11] Department of Veterinary Anatomy, University of Missouri, Columbia, Mo. 65201, USA.
[12] Station de Physiologie de la Reproduction, I.N.R.A. 37380 Nouzilly, France.