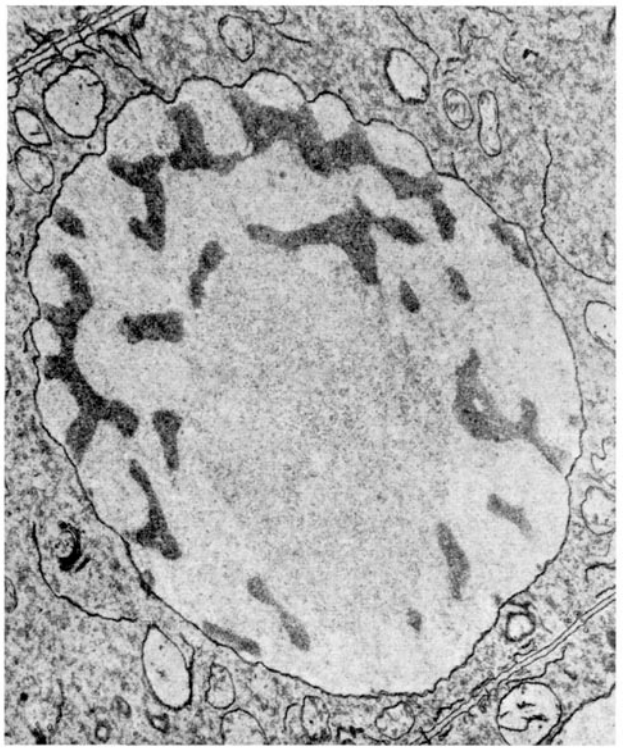

Nucleus showing the scalloping of the nuclear membrane in the upper region. In the invaginations the membrane can be observed contacting the chromatin network.

in the even regions the membrane is limited on its inner aspects by nuclear sap.

This pattern cannot be explained by a random distribution of the invaginations, nor is it apparently a result of deficient fixation.

In fact, this observation indicates that the nuclear membrane has a surface slightly greater than is barely necessary to envelop the nuclear mass. Since the chromosome has a negative charge[6], it is reasonable to suggest that the nuclear sap may have a positive charge. Thus, when, in a nuclear region bordering on the membrane, pieces of chromatin (negatively charged) alternate with nuclear sap (positively charged)[6], it is possible that scalloping of the adjacent membrane (positively charged) may be induced.

Résumé. Il existe un certain rapport entre la chromatine et des petites invaginations de la membrane nucléaire. Nous estimons que la charge positive de la membrane et la charge négative des chromosomes peut être la cause inductrice.

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Sección de Citología, Instituto de Biología Celular, C.S.I.C., Madrid (Spain), May 28, 1965.

[6] E. D. P. DE ROBERTIS, W. W. NOWINSKI, and F. A. SÁEZ, *Citología General* (Edit. 'El Ateneo', Buenos Aires, Argentina 1960).

Histochemical Demonstration of Carbonic Anhydrase Activity in Mast Cells

In connection with other investigations on carbonic anhydrase (CAH) activity, we observed a positive histochemical reaction of CAH in the mast cells. Since the presence of this kind of enzymic activity in mast cells has not been previously reported in the literature, a separate study on this subject was performed.

Small pieces of the root of the tongue and the eye of Wistar strain rats were fixed for 12 h at + 4°C in non-neutralized α-hydroxyadipaldehyde in 0.88 *M* saccharose, washed in 0.88 *M* saccharose at + 4°C for at least 6 h, and cut into 20–25 μ thick frozen sections with a cryostat microtome into a cold 0.25 *M* saccharose solution. The sections were incubated as freely floating at + 22°C on the surface of the modified Häusler's medium (containing sodium bicarbonate 0.157 *M*, sodium sulphate 0.014 *M*, sulphuric acid 0.0053 *M*, and cobaltous sulphate 0.0175 *M*). After incubation periods of 15–120 min, the sections were rinsed in physiological saline solution, and the precipitated cobalt carbonate was visualized as its sulphide, using 0.4% ammonium sulphide solution. Kidney sections were used as positive controls, and incubation of sections in a medium containing sodium acetazoleamide (Diamox Parenteral® Lederle) 10^{-4} *M* was used as negative control.

Black cobalt sulphide precipitate revealed the areas of CAH activity in the sections. The positive reaction in the mast cells appeared as coarse granules after an incubation of 45 min. The behaviour of all the mast cells seen in the root of the tongue, in the sclero-corneal junction, and around the papilla nervi optici of the eye was similar in this respect. The nuclei of the sections fixed in hydroxyadipaldehyde did not stain, as was the case with the use of some other fixation methods, e.g. formaldehyde, glutaraldehyde or various alcohols, as previously mentioned[1]. In the negative controls, incubated with a specific inhibitor, sodium acetazoleamide, no staining whatsoever occurred.

An intense positive reaction was observed in the capillary endothelium and in the erythrocytes. The reaction was clearly weaker in the germinal (basal) epithelium cell layer of the tongue. The reaction was also demonstrable in the striated muscle. The histochemical distribution of the CAH activity in the eye structures has been described elsewhere[1].

The Table gives the incubation time required for the appearance of the first visible CAH reaction, and suggests an estimate of the order of the relative magnitude of the enzymic activity in the various structures mentioned.

Doubts as to the nature of the histochemical reaction for CAH activity have often been expressed in the literature. The original method of KURATA[2] is considered as

[1] E. KORHONEN and L. K. KORHONEN, Acta ophtalmolog., in press.
[2] Y. KURATA, Stain. Technol. *28*, 231 (1953).

unspecific, because there is a quite remarkable precipitation of metal carbonate, independently of the enzymic catalysis[3–5]. This is caused by the spontaneous dehydration of bicarbonate in Kurata's medium[3]. The spontaneous dehydration of bicarbonate in Häusler's medium is very slow, and not observed during the usual incubation times. Various steps in the reaction chain in this medium have been described by HÄUSLER[4]. The following facts speak for the specificity of Häusler's histochemical method[6]: (1) The reaction may be demonstrated by purified enzyme preparations. (2) The reaction is completely inhibited by sodium acetazoleamide. (3) The reaction does not appear in sections inactivated by boiling. (4) The good correspondence between the activities demonstrated by histochemical method and by biochemical activity measurements after electrophoresis on 'Oxoid' film[6]. (5) The good correspondence between the histochemical observations of the specific localization of the CAH activity in brain and eye structures[6]. (6) The unspecific cation substitution in some tissues may be avoided by using chelating agents[7]. In the mast cells, CAH reaction is not due to the cation substitution, because no staining appears in the sections incubated without substrate (sodium bicarbonate) for 2–3 h, or incubated with acetazoleamide, or inactivated by boiling.

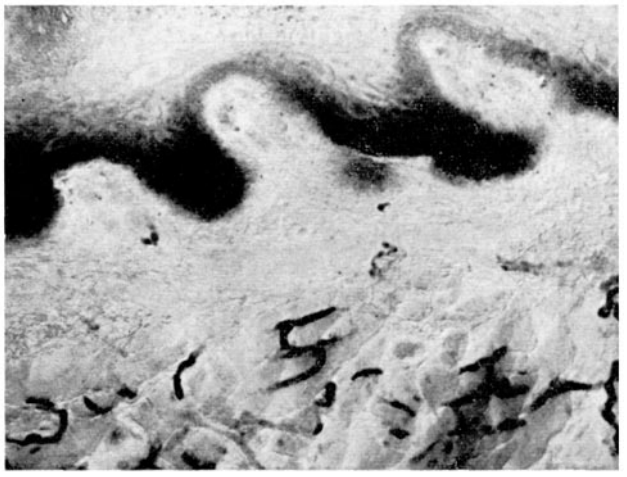

Fig. 1. Positive CAH reaction in the capillary endothelium and basal cells of the tongue epithelium after 45 min incubation (× 100).

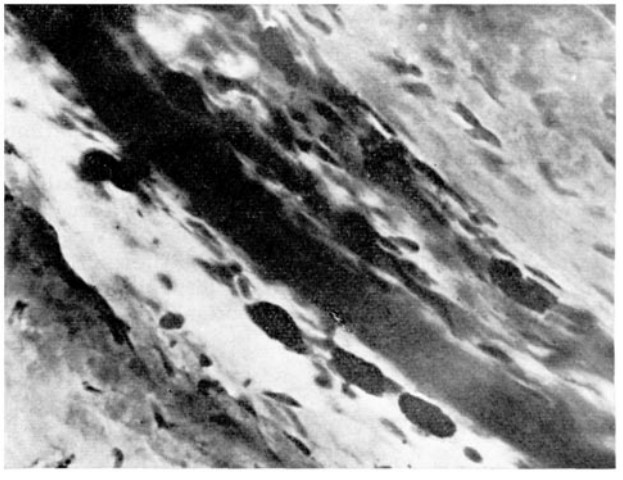

Fig. 2. Positive CAH reaction in the mast cells of the sclerocorneal junction of the eye after 75 min incubation (× 400).

Fig. 3. Positive CAH reaction in a single mast cell in the tongue after 60 min incubation (× 650).

Many kinds of enzymic activities have been demonstrated in the mast cells[8]. The function of the CAH activity in the mast cells is not known. Generally, the CAH activity is revealed in cells, where rapid interconversion of CO_2 and HCO_3^- is needed in connection with the metabolic processes yielding these ions, or with the secretory processes, which require an effective bicarbonate-carbonic acid buffer system[9]. The mast cells have an active metabolism, and obviously the CAH activity is associated with either of these processes. – Reports of the merocrine secretory process observed in the mast cells have also been presented in the literature[8].

	Incubation time required for the first visible CAH reaction (min)			
	15	30	45	60
Capillary endothelium	+	++	++	++
Proximal kidney tubules	–	+	++	++
Basal cells of the tongue epithelium	–	–	++	++
Mast cells	–		+	++
Striated muscle	–	–	–	++

Zusammenfassung. Carboanhydraseaktivität in Mastzellen der Ratte wurde mittels Modifikation der Methode von HÄUSLER[4] demonstriert. Kapillarendothel, Basalzellen des Zungenepithels und Muskelgewebe reagierten ebenfalls positiv.

L. K. KORHONEN and E. KORHONEN

Department of Anatomy, University of Turku (Finland), March 16, 1965.

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