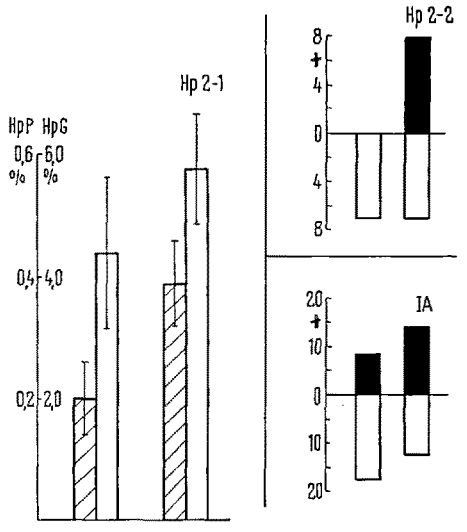


Relative Haptoglobinanteile (Typ 2-1) an der Proteinausscheidung (HpP, schraffierte Säulen) und an der α_2-Globulinfraktion im Harn (HpG, weisse Säulen) diabetischer Patienten (linker Teil des Diagramms). Häufigkeitsverteilung der Ausscheider (schwarze Säulen) und Nichtausscheider (weisse Säulen) von Hp (Typ 2-2) und Isoagglutininen (IA) im Harn (rechter Teil des Diagramms). Linke Säulen bzw. Säulenpaare aller Diagramme Patienten mit keinem oder leichtem, rechte mit mittlerem und schwerem Nierenschaden bei diabetischer Angiopathie.

Moleküls) nicht stattgefunden haben können[2], stützen die mitgeteilten Befunde die Hypothese, dass für den Mechanismus der Proteinurie die Porengrösse des glomerulären Filters offenbar doch die entscheidende Funktion besitzt.

Eine ausführliche Mitteilung dieser Ergebnisse, die zum Teil in Zusammenarbeit mit B. GIBB, R. GIEBELMANN und FRIEDELENE MARTIN gewonnen wurden, ist in Vorbereitung.

Summary. Haptoglobin and isoagglutinin were found in the urine of diabetic patients, and their renal excretion was higher in patients with diabetic angiopathy. It is concluded from these results (the biological activity of the two polypeptides in urine was intact) that the mechanism of proteinuria can perhaps be explained only by an 'extension of pores' of the renal 'glomerular filter'.

H. G. LIPPMANN, S. MARTIN
und M. SCHULZ

Institut für Diabetes «Gerhardt Katsch», Karlsburg bei Greifswald und Institut für Gerichtliche Medizin der Universität Greifswald (DDR), 14. Juni 1965.

[23] H. E. SCHULTZE und K. HEIDE, in K. F. BAUER, *Medizinische Grundlagenforschung*, Bd. 3 (Stuttgart 1960).
[24] S. FILITTI-WURMSER, Ann. Eugen. *18*, 183 (1953/54).
[25] K. JAHNKE und W. SCHOLTAN, Dtsch. Arch. klin. Med. *200*, 821 (1953).

Nuclear Membrane and Chromatin Network

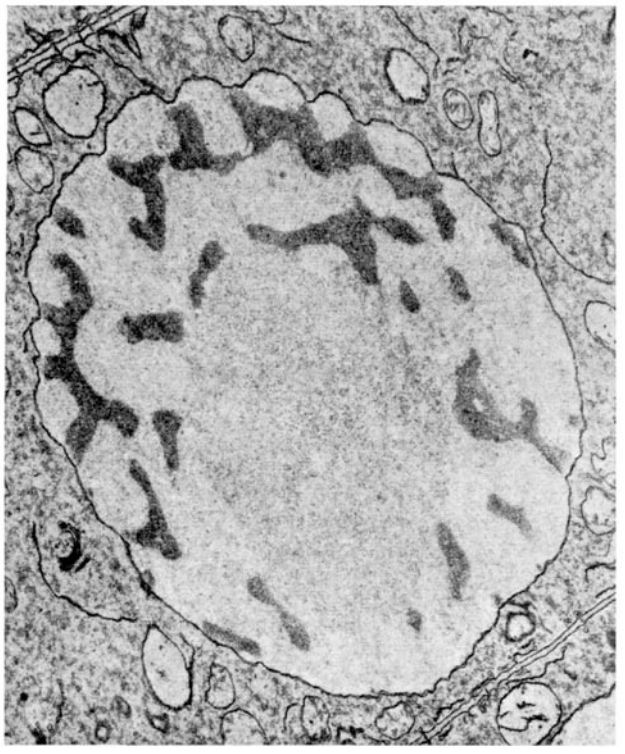
BARR et al.[1–3] were pioneers in the study which led to the discovery of a definite localization of the chromatin in relation to the nuclear membrane when they found that in the somatic cells of the cat the sex chromatin is located at the nuclear membrane. BURGOS[4] demonstrated the accumulation of Feulgen reactive material on the inner aspect of the nuclear membrane in unfertilized eggs of *Arbacia punctulata*, and GAY[5] studied the relations between chromatin and nuclear membrane outpocketings. However, the relation between chromatin network and the membrane in the interphase nucleus remains practically unexplored.

In this paper we study a certain relation often observed between chromatin and nuclear membrane in the interphase nucleus of meristematic cells.

Material and methods. Seeds of *Phalaris canariensis* were germinated at room temperature, using filter paper and tap water. The seedlings were grown for 2 or 3 days. At the end of this period the root-tips (2–3 mm) were removed and immediately fixed by the following method: $KMnO_4$ 2% in distilled water, for 2 h at 20–22°C. The fixed material was dehydrated in an acetone series and embedded in Durcupan ACM (Fluka). During the dehydration, the material was stained overnight in 2% uranyl acetate dissolved in 75% acetone for contrast enhancement. To obtain the ultra-thin sections an Ultratom LKB was employed. The sections were stained with lead hydroxide; the observations were made with a Siemens Elmiskop I, and the pictures taken on Sciencia Gevaert plates.

Results and discussion. The nucleus of the meristematic cells generally appears spherical in form as observed with the light microscope. From early interphase to the following prophase, this form is maintained. The restitution of the nuclear membrane at telophase can be observed with the electron microscope. The nuclear membrane, newly formed around the chromosomes, adopts the form of the chromatin mass and presents the same irregularities as the outline of the mass. In the course of interphase the nucleus becomes spheroidal and the nuclear membrane fits into the new shape of the content. If no association exists between the chromatin and the membrane, the latter should be observed in the sections as a circumference or an ellipse. However, even though the interphase nucleus adopts a *grosso modo* regular shape, the membrane shows a scalloped shape in certain regions of the nuclear surface. In the invaginations the membrane contacts the chromatin network. In the evaginations and

[1] M. L. BARR, L. F. BERTRAM, and H. A. LINDSAY, Anat. Rec. *107*, 283 (1950).
[2] M. A. GRAHAM and M. L. BARR, Anat. Rec. *112*, 709 (1952).
[3] M. A. GRAHAM, Anat. Rec. *119*, 469 (1954).
[4] M. BURGOS, Exp. Cell Res. *9*, 360 (1955).
[5] H. GAY, Cold Spring Harbor Symposia Quant. Biol. *21*, 257 (1956).



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.

M. C. RISUEÑO, G. GIMÉNEZ-MARTÍN, and J. F. LÓPEZ-SÁEZ

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).