

SEMINARIO PATOLOGÍA ULTRAESTRUCTURAL: INTRODUCCIÓN

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**XXV Congreso de la Sociedad Española de Anatomía Patológica y División Española de la
International Academy of Pathology**

18 mayo 2011 - Zaragoza



***Ernst Ruska and Max Knoll built
the first electron microscope in 1931***



Ernst Ruska, Premio Nobel 1986

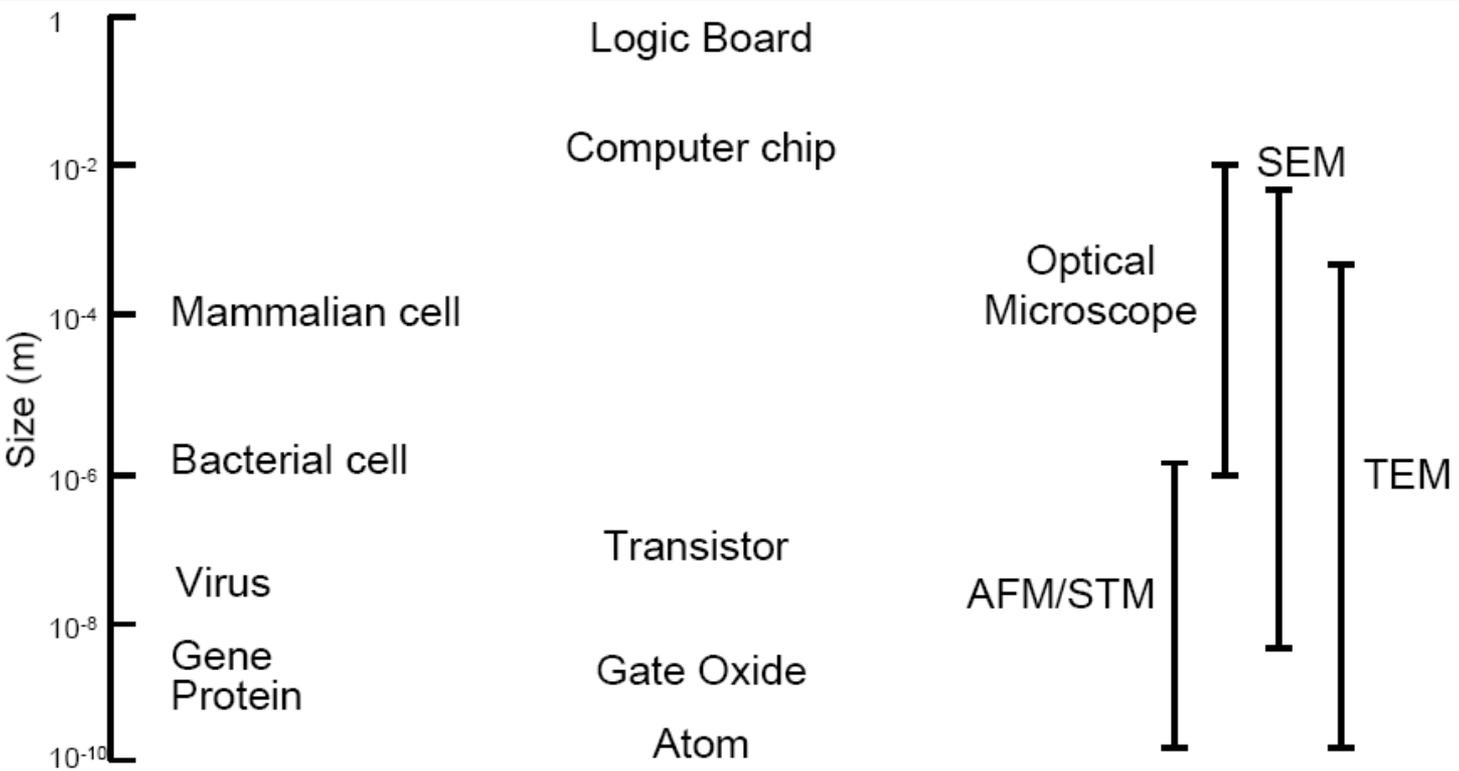
La Microscopía Electrónica cerró el hueco (1nm-1 μ m) entre la Difracción de Rayos X y la Microscopía Óptica.

DIMENSIONES BIOLÓGICAS Y ELECTRÓNICAS

Biología

Componente electrónico

Herramienta



David Muller 2008

AFM vs SEM vs TEM

Type	Applications
<i>Atomic Force Microscopy</i>	<ul style="list-style-type: none">• <i>Topographic Imaging on wafers</i>• <i>Accurate height measurements on flat surfaces (~ 0.5 nm vertical)</i>• <i>Lateral Resolution 10-20 nm</i>• <i>In-situ – no vacuum required</i>
<i>Scanning Electron Microscopy</i>	<ul style="list-style-type: none">• <i>Imaging of complex structures at 1-20 nm resolution</i>• <i>X-ray mapping at 100-500 nm</i>• <i>In-vacuum</i>• <i>Clark: High spatial resolution</i>• <i>Snee/Bard: best x-ray mapping, OIM</i>
<i>Transmission Electron Microscopy</i>	<ul style="list-style-type: none">• <i>1 nm (polymers) → atomic resolution of crystals in thin samples</i>• <i>X-ray mapping at 1 nm</i>• <i>EELS at < 1 nm</i>• <i>Requires sample thinning (except for nanoparticles)</i>

**Microscopía
electrónica y
conocimiento
científico**

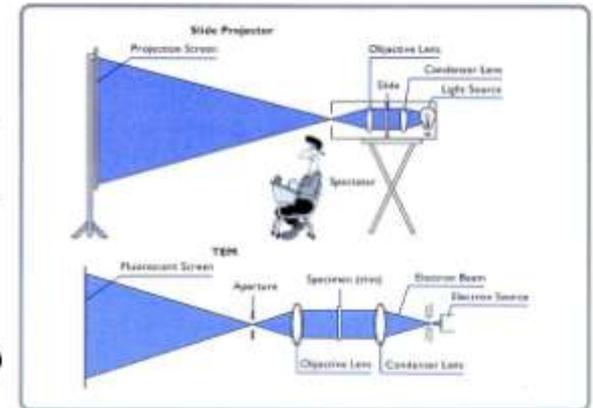
Microscopía electrónica

- Límite de observación microscopio óptico: ~ 400-700 nm (luz visible)
- Máxima resolución (d) limitada por la longitud de onda, λ :

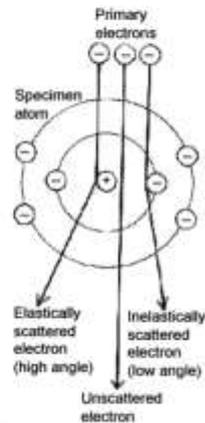
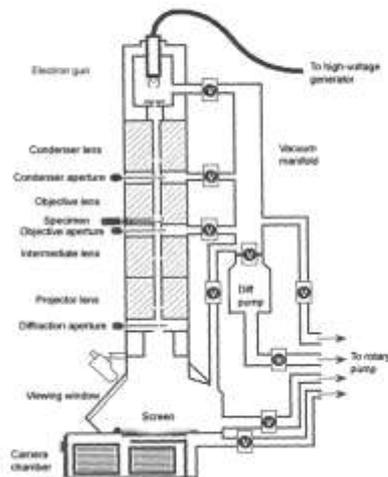
$$d = \frac{\lambda}{2n \sin \theta}$$
- Microscopía electrónica: $\lambda[e^-] \ll \lambda [luz] \rightarrow$ resolución atómica (~ 0.1 nm)
- 2 tipos generales: TEM y SEM

TEM (Transmission Electron Microscope)

- La imagen se obtiene a partir de los e^- que atraviesan la muestra.
- La muestra debe prepararse en láminas muy finas (grosor < cientos de nm).
- Haz de e^- muy energético (~100 keV)



TEM



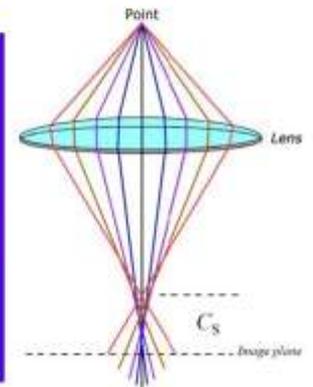
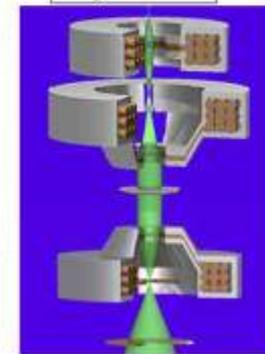
Aberration-Uncorrected Microscope

FEI TecnaiF20



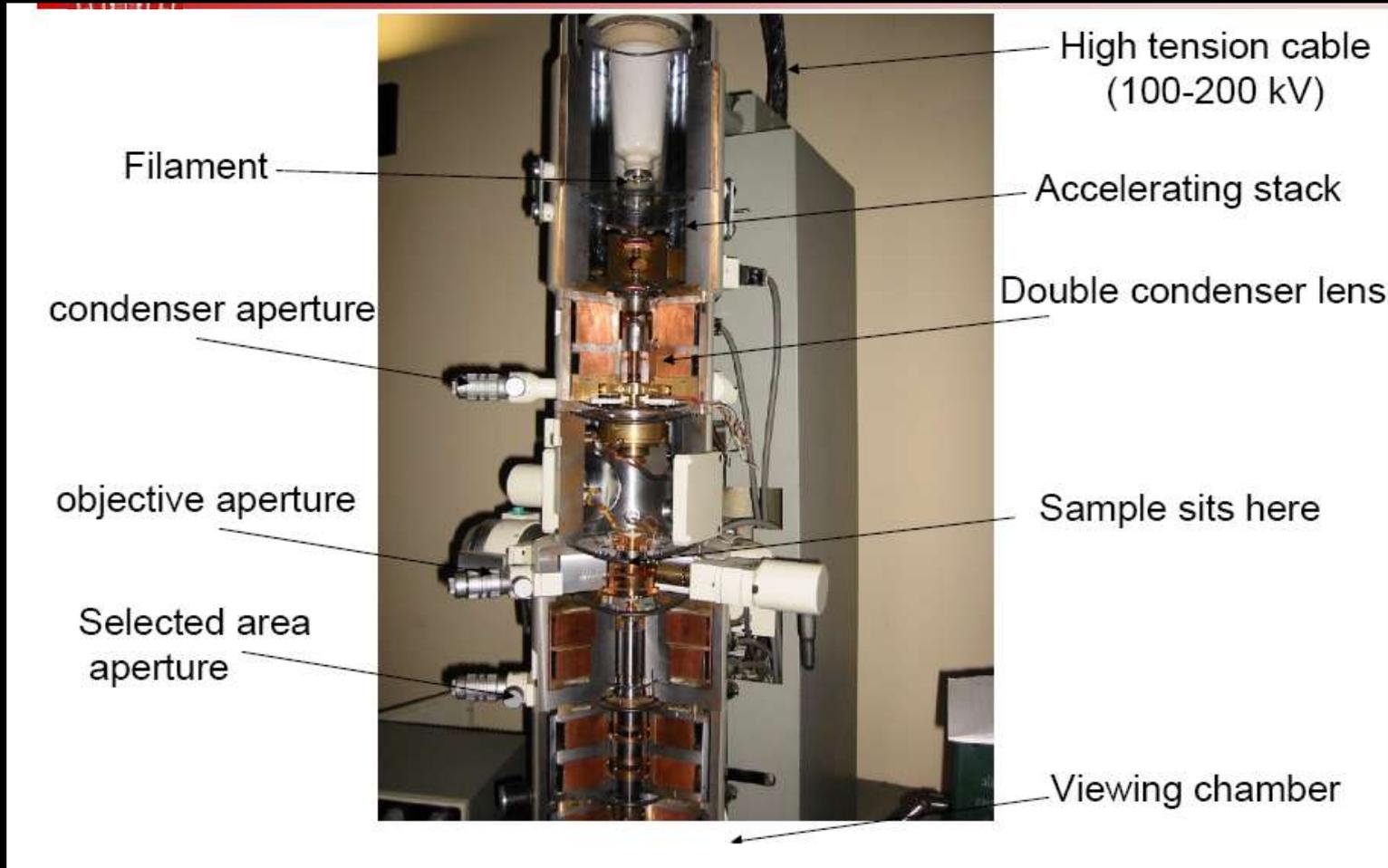
- C_s 1.2mm, C_c 1.2mm
- Point resolution 0.24 nm
- Information limit 0.14 nm
- Energy Dispersive X-ray Spectrometer
- Energy Filtered System
- Electron Tomography

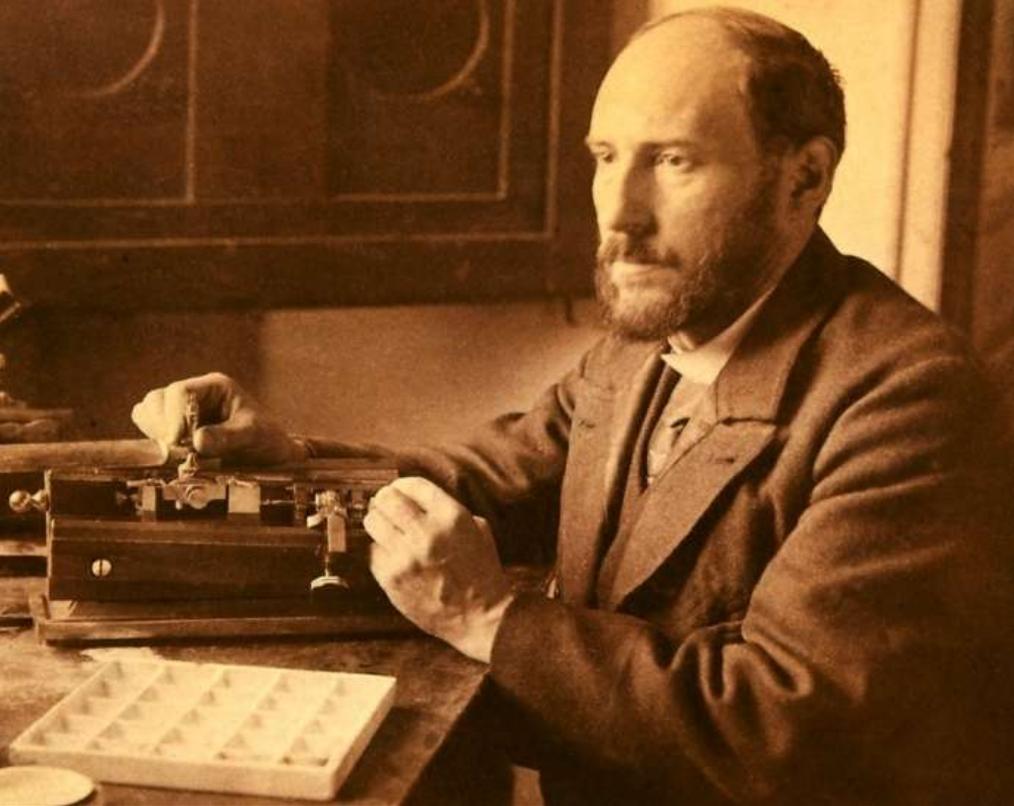
Magnetic Lens



C_s : Spherical coefficient

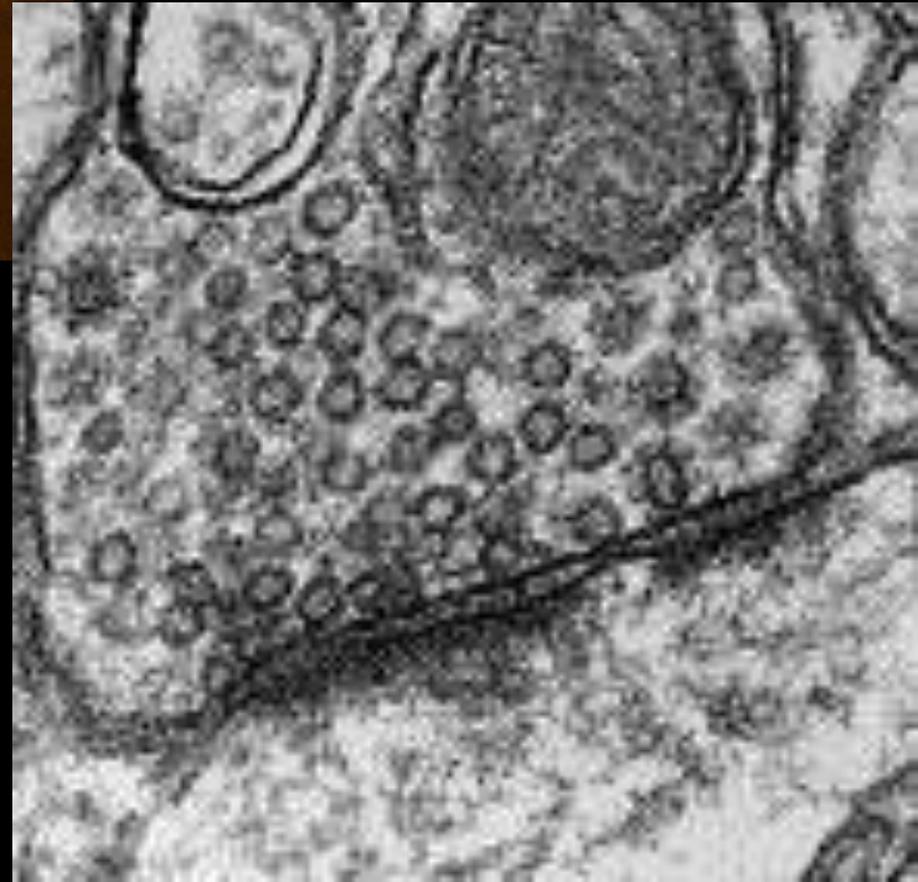
INTERIOR DE UN MICROSCOPIO ELECTRÓNICO TRANSMISIÓN





Sinapsis (ME)

Comprobación ultraestructural de la Teoría Neuronal

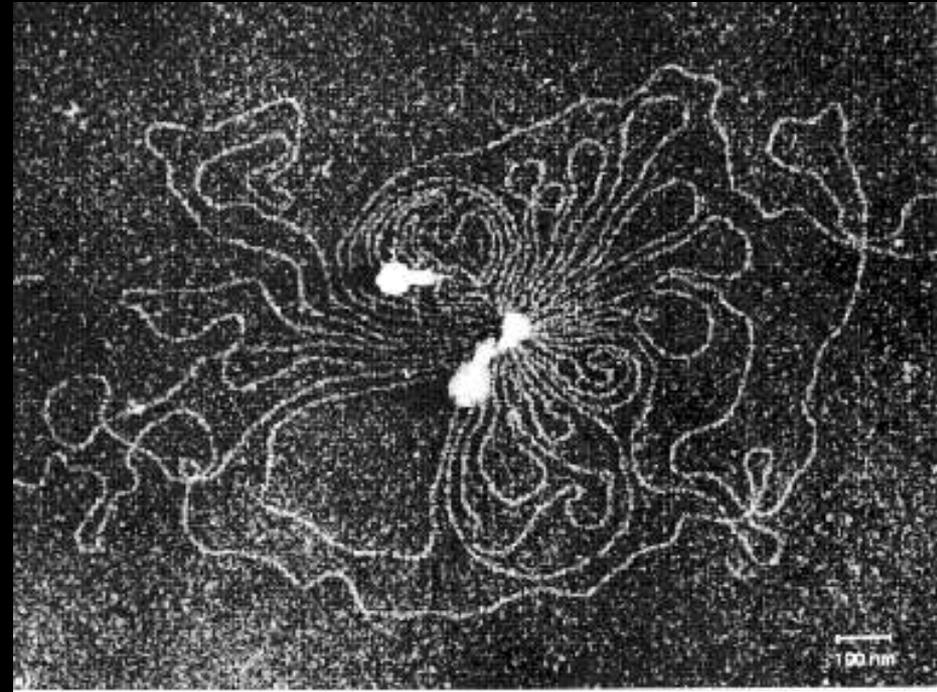


Santiago Ramón y Cajal

(Teoría Neuronal)

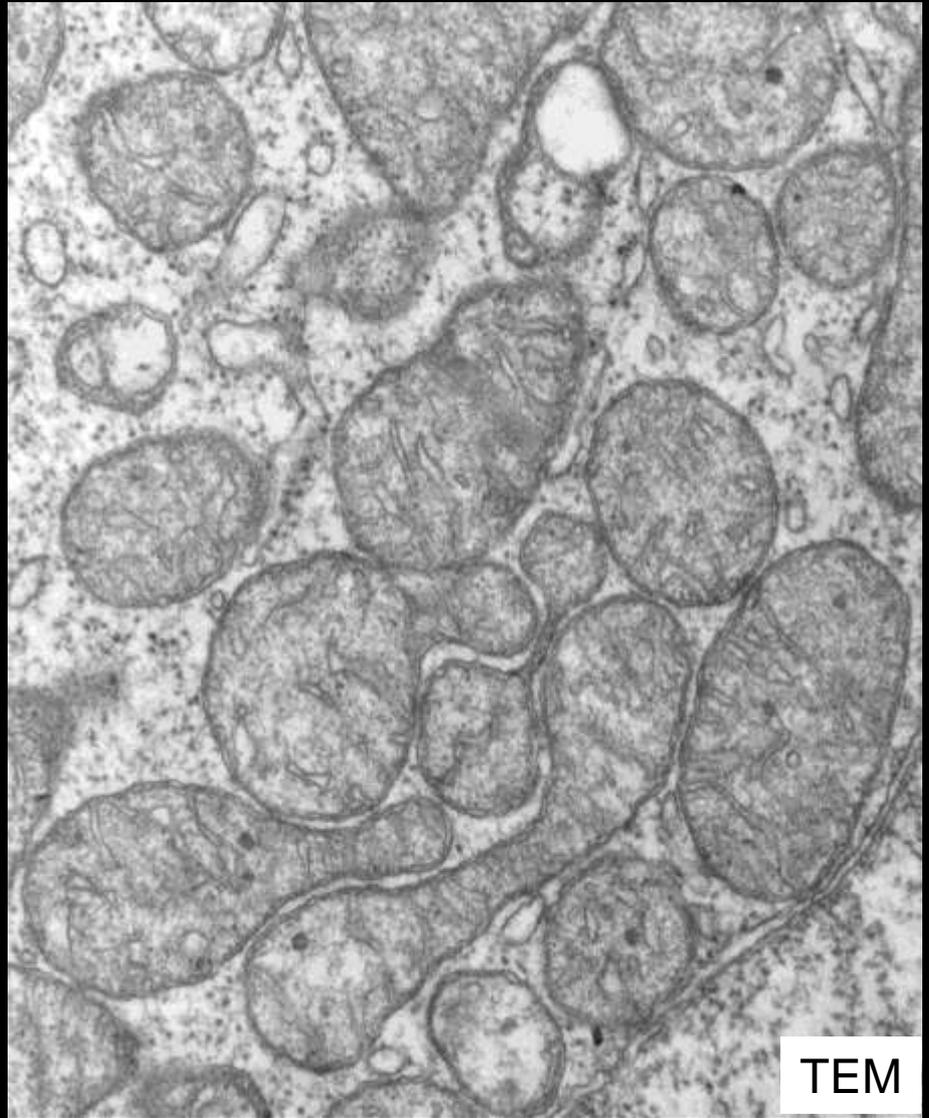
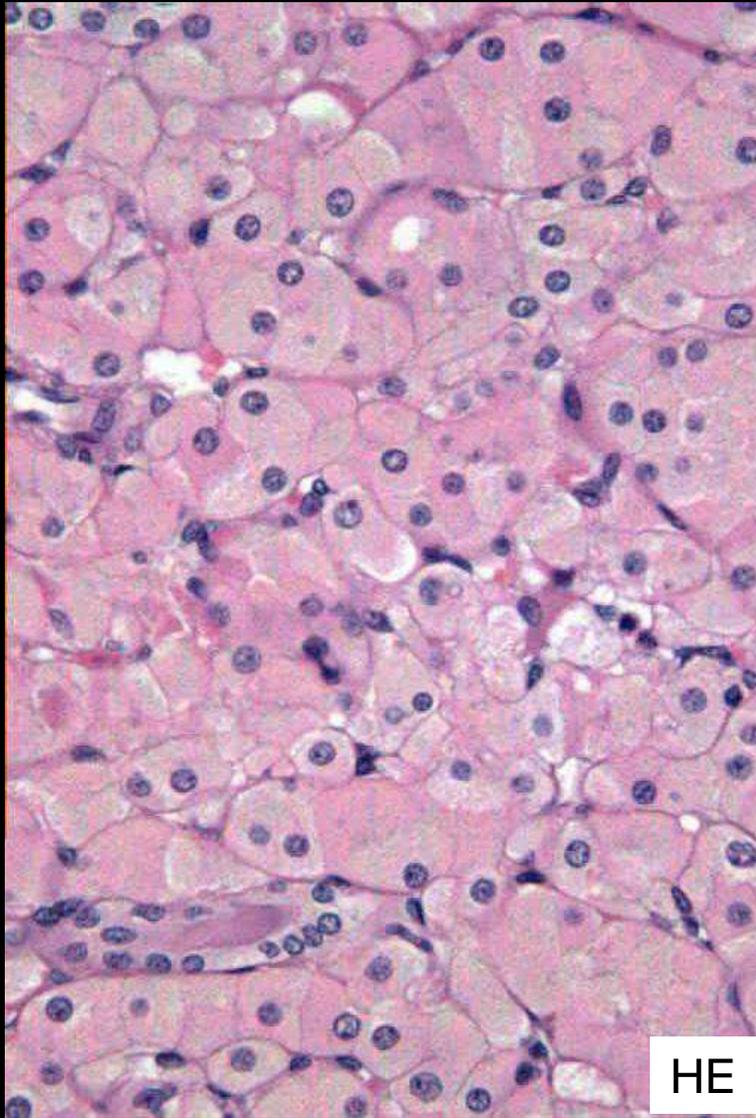


Severo Ochoa y Albrecht K Kleinschmidt (1967)



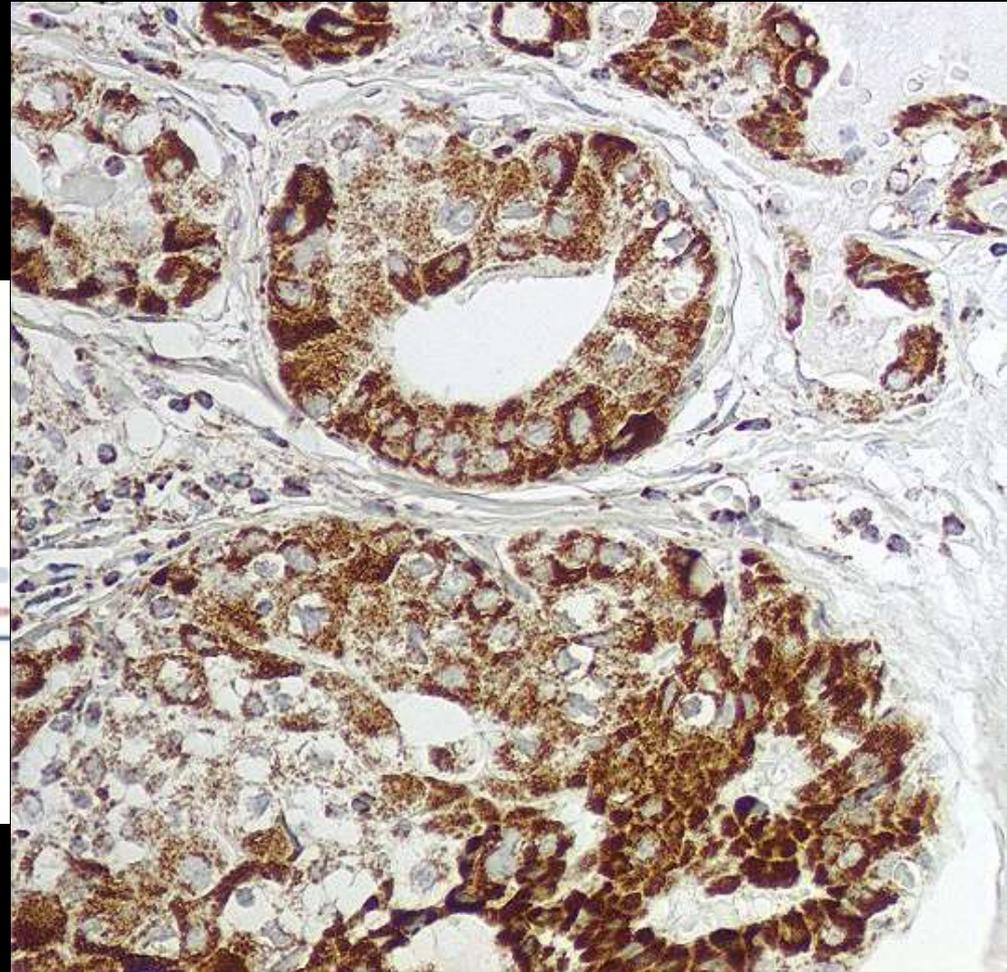
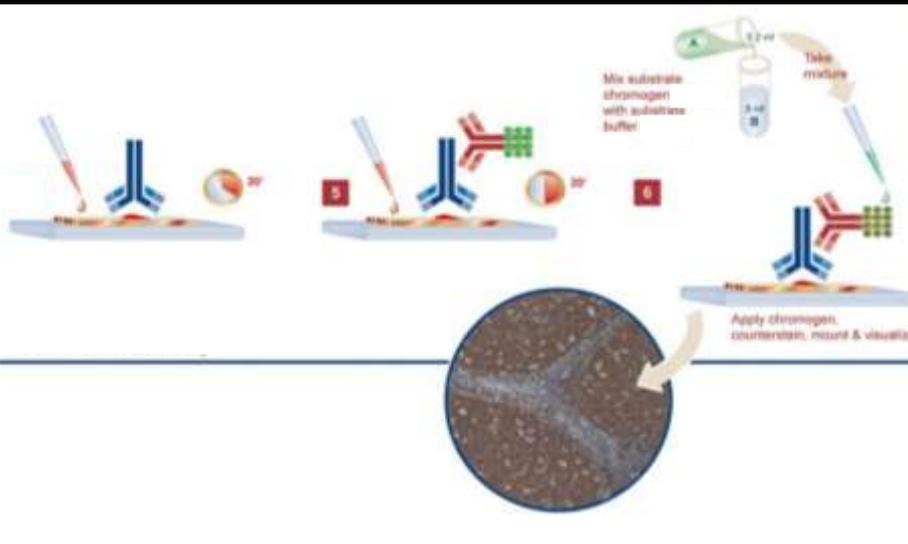
Sombreado en oro de ADN

- Microscopía óptica y Microscopía electrónica de transmisión (TEM) **dominios técnicos complementarios**.
- La TEM es muy efectiva en evaluar células y estructuras subcelulares, y la microscopía óptica estructuras tisulares, núcleos y citoplasma.
- Durante largo tiempo ambas técnicas han sido **complementarias y fundamentales** en el diagnóstico histopatológico



Riqueza mitocondrial de las células oncocíticas

- La inmunohistoquímica **cambió el paradigma del diagnóstico histopatológico** pareciendo superfluo, en ocasiones, el estudio de microscopía electrónica



Inmunohistoquímica: anticuerpo mitocondriales
(anticuerpo contra la proteína del gen SHDA en células
de carcinoma oxifílico de tiroides)

Microscopía electrónica y diagnóstico

Diagnóstico Ultraestructural de las Ciliopatías

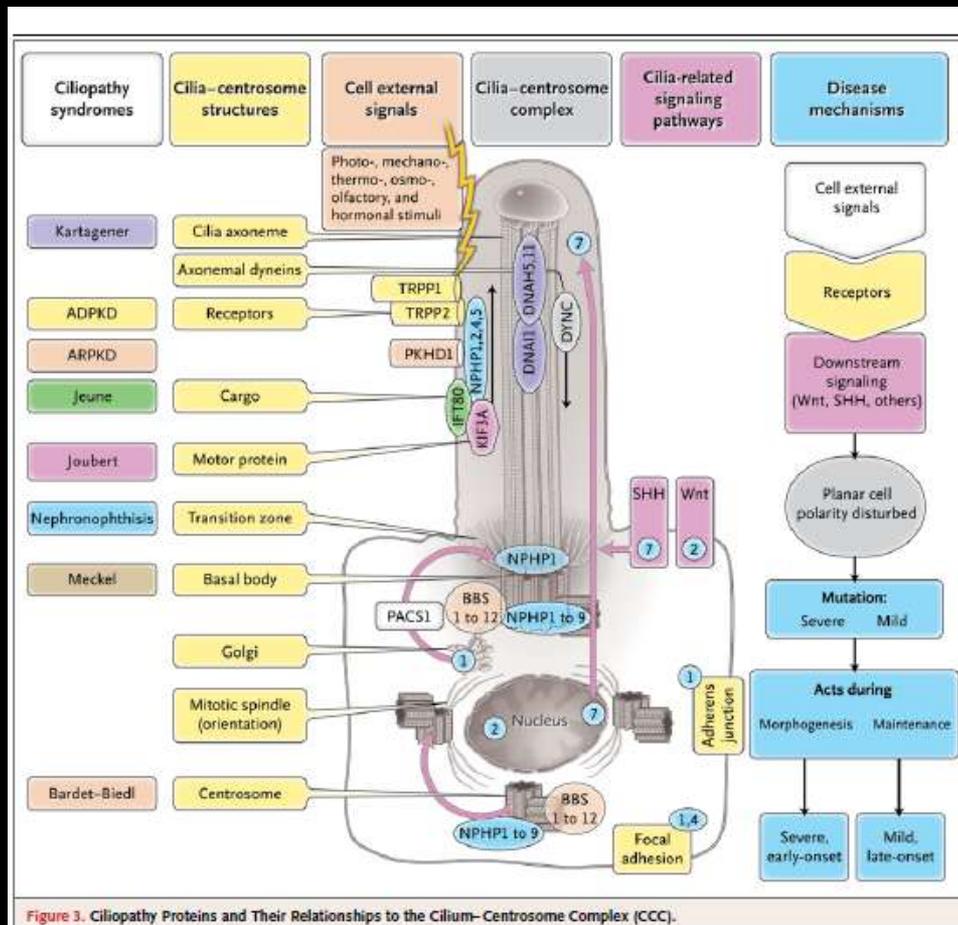
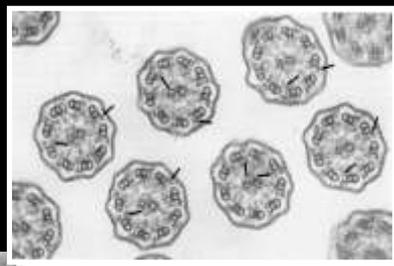
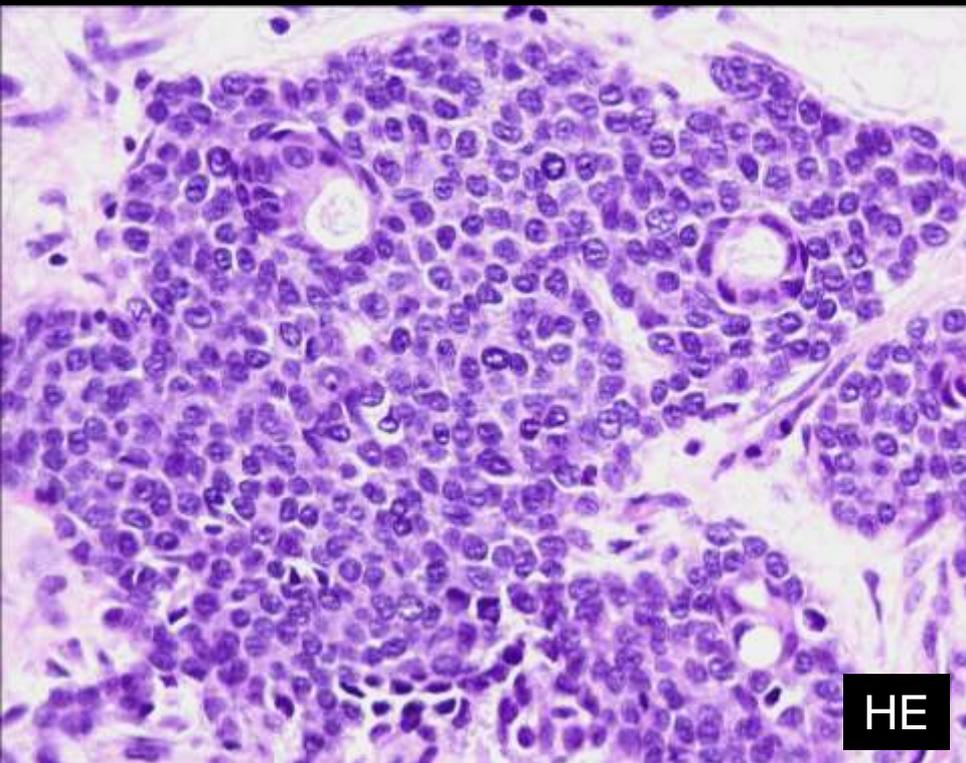


Figure 3. Ciliopathy Proteins and Their Relationships to the Cilium-Centrosome Complex (CCC).

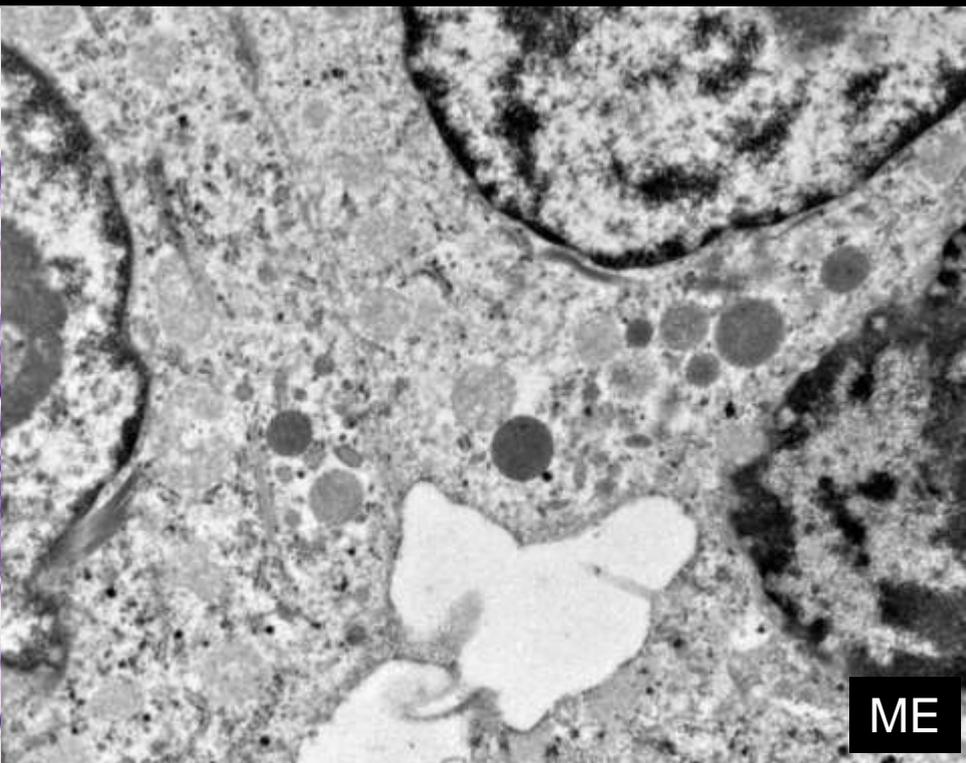
SURGICAL PATHOLOGY



Lauren Ackerman
(1905-1993)



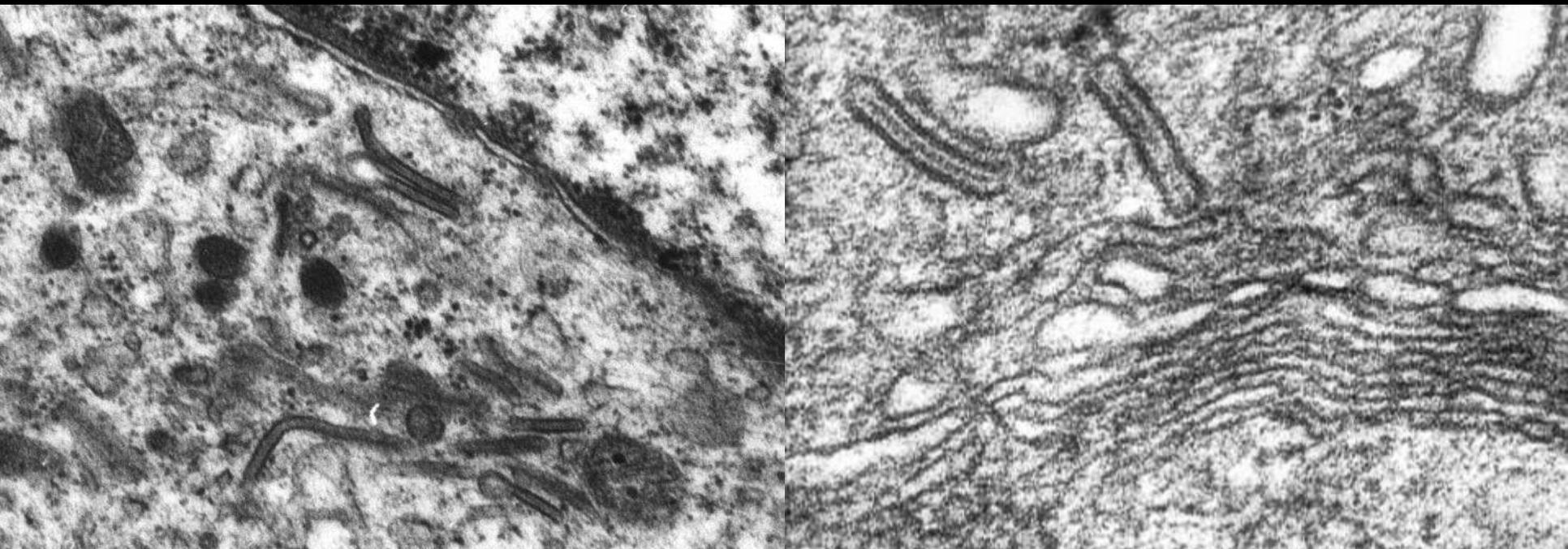
HE



ME

Carcinoma de páncreas de células acinares con gránulos de secreción

(Cortesía Dr. Juan Rosai)



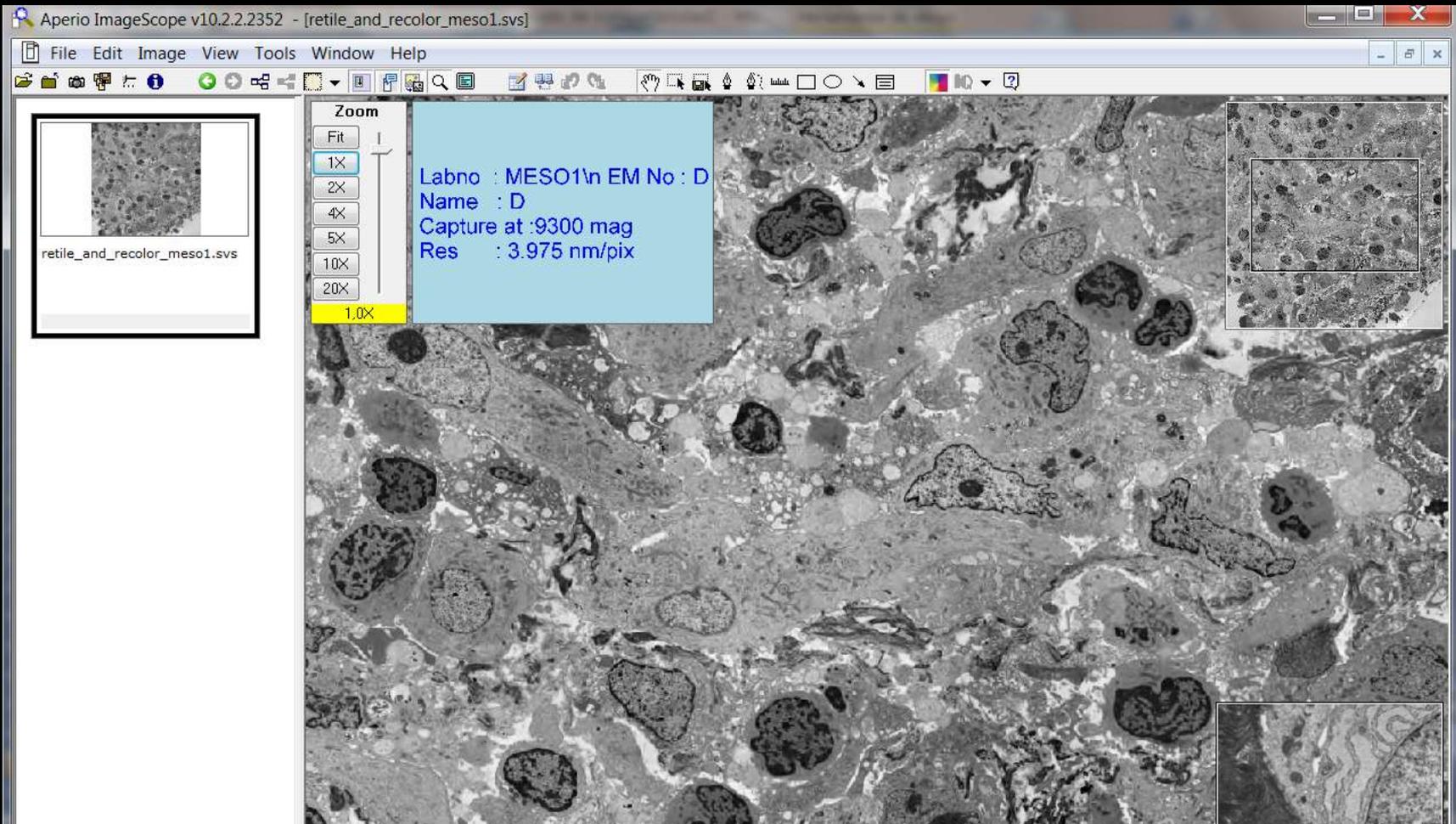
MICROSCOPIA ELECTRÓNICA:

Gránulos de Birbeck.

Granulomatosis de Células de Langerhans

- La microscopía electrónica tiene **especial valor** en determinados campos como la **Patología Renal, Neuromuscular**, Piel, Corazón etc...
- Es **eficiente** en evaluar células aisladas, **estructuras subcelulares**, organelas y citoesqueleto pero su utilización es limitada para grupos celulares y áreas tisulares extensas.

- Una solución a la utilización limitada de la Microscopía Electrónica en Patología Quirúrgica: la microscopía electrónica virtual con áreas escaneadas.
- Se capturan varios cientos de imágenes que son luego manejadas usando un software tipo *Aperio*. Permite trabajar con bajo aumento, con buena localización y orientación de la estructura tisular



Mesotelioma

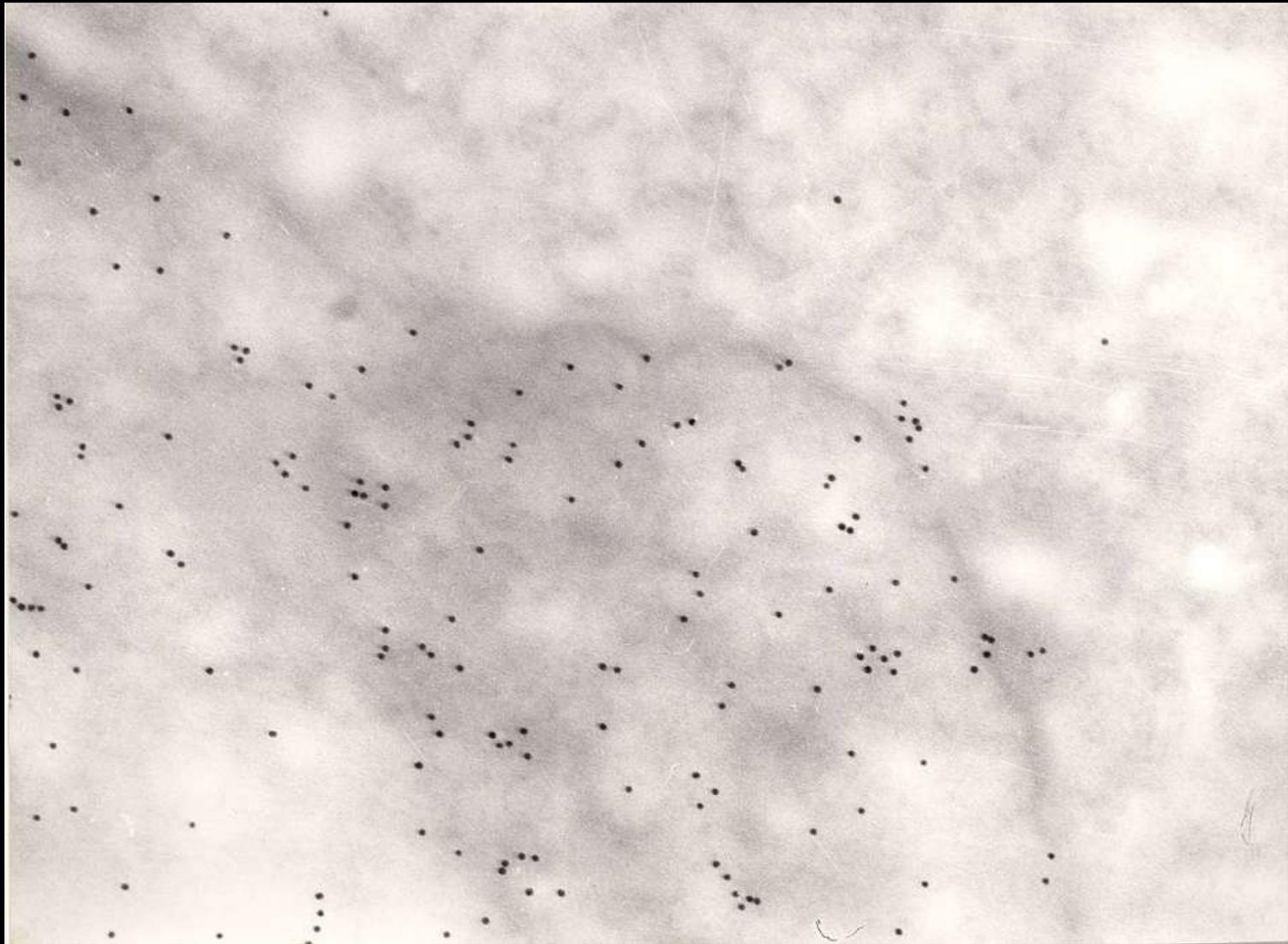
<http://www.keepandshare.com/doc/2758239/retile-and-recolor-meso1-svs-april-20-2011-12-24-am-87-4-meg?da=y>
Cortesia de John K. Chan. Hong Kong

- Es muy importante un **procesamiento rápido** de las muestras (2 a 3 horas) para que sea útil para el diagnóstico, metodología que no es habitual, pero posible.

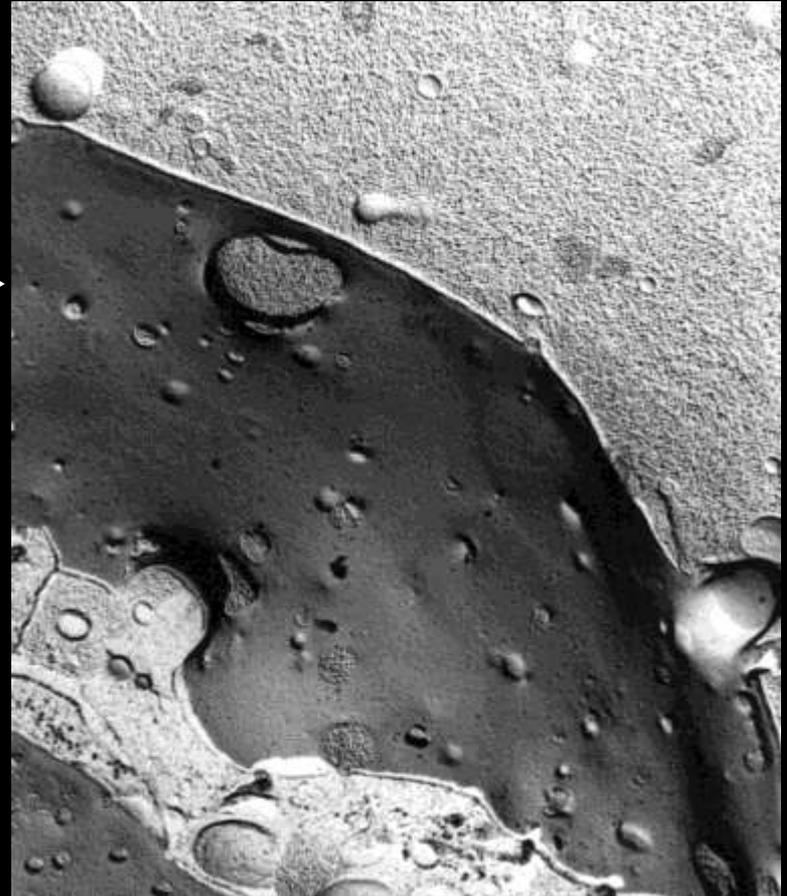
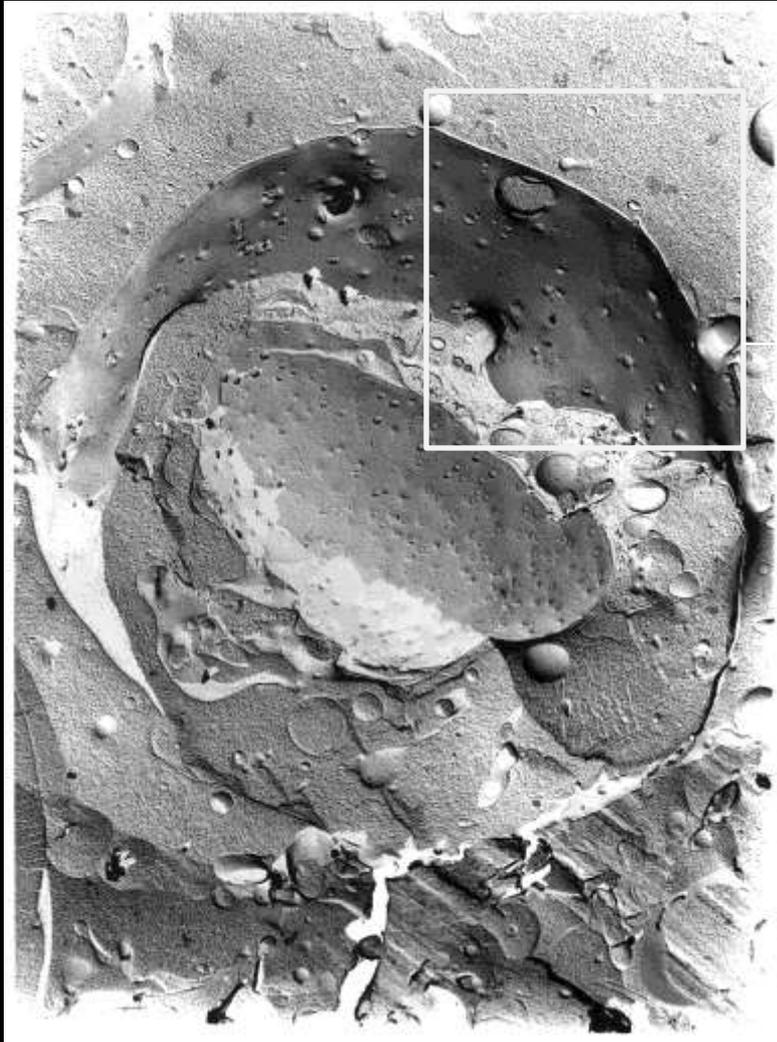
En la valoración de la Microscopía Electrónica en el diagnóstico podemos diferenciar:

- 1. Casos donde la ME es de **gran ayuda** para el diagnóstico
- 2.- Casos donde la ME es una **ayuda complementaria** para el diagnóstico.
- 3 . Casos donde la ME **no influye** en el diagnóstico

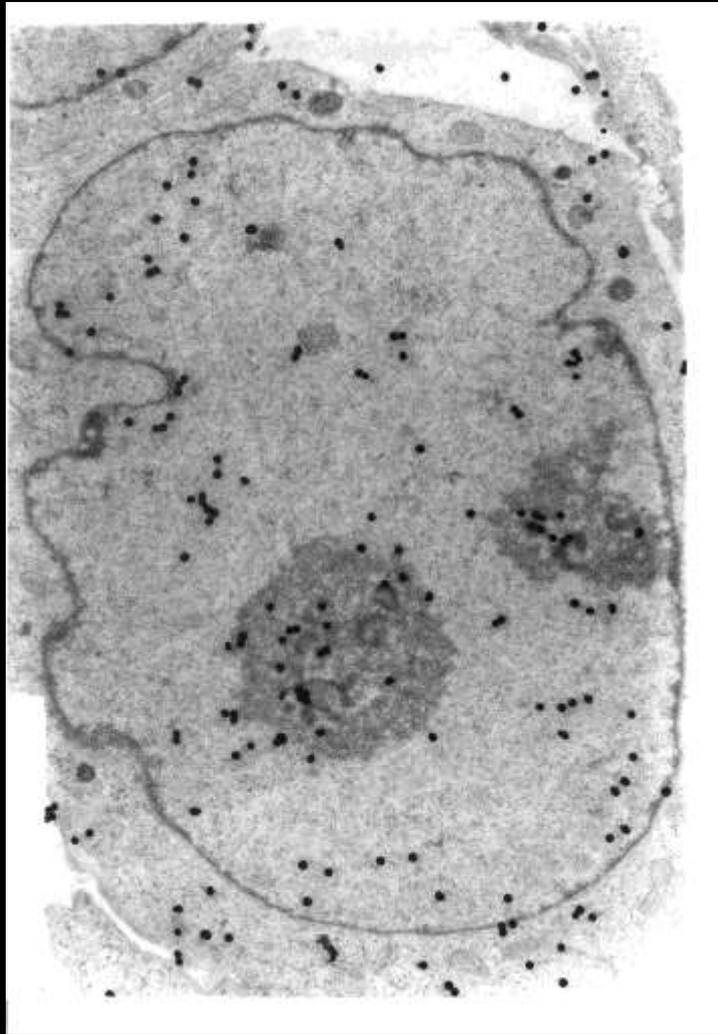
**METODOLOGÍAS USADAS
EN COMBINACIÓN CON
MICROSCOPIA
ELECTRÓNICA**



Inmunomarcage con **protimosina-alfa** en un núcleo de una Célula de Reed Sternberg



Crio fractura y Ultraestructura Celular

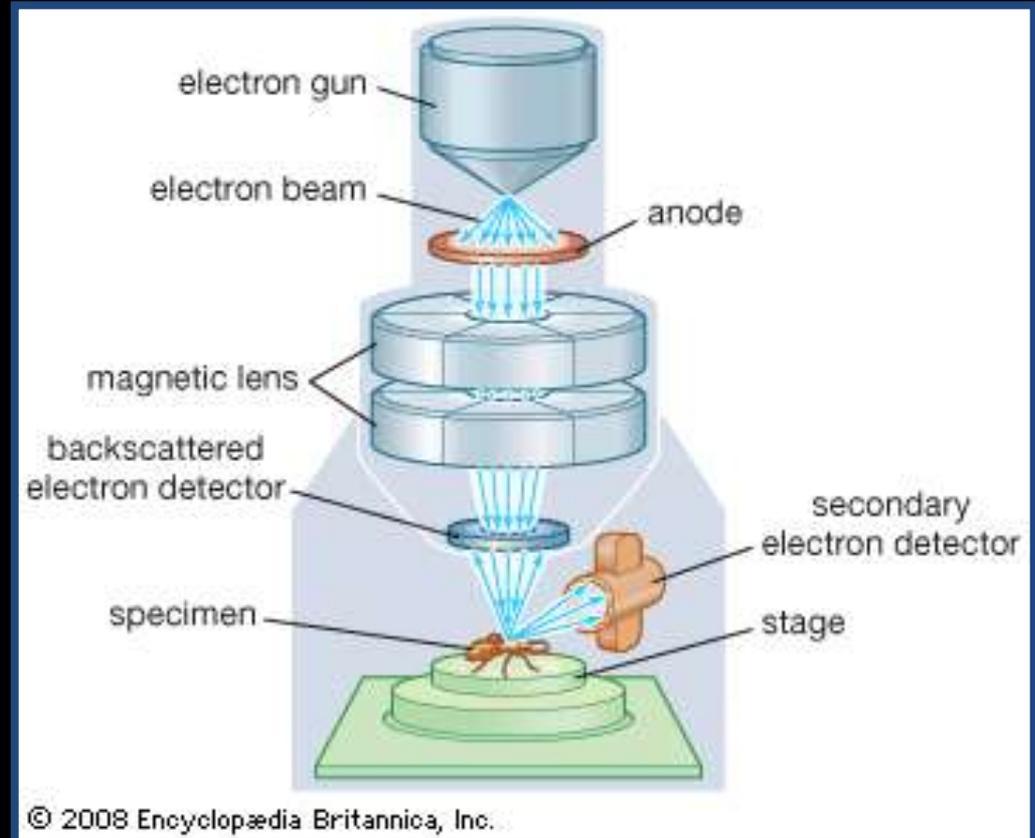


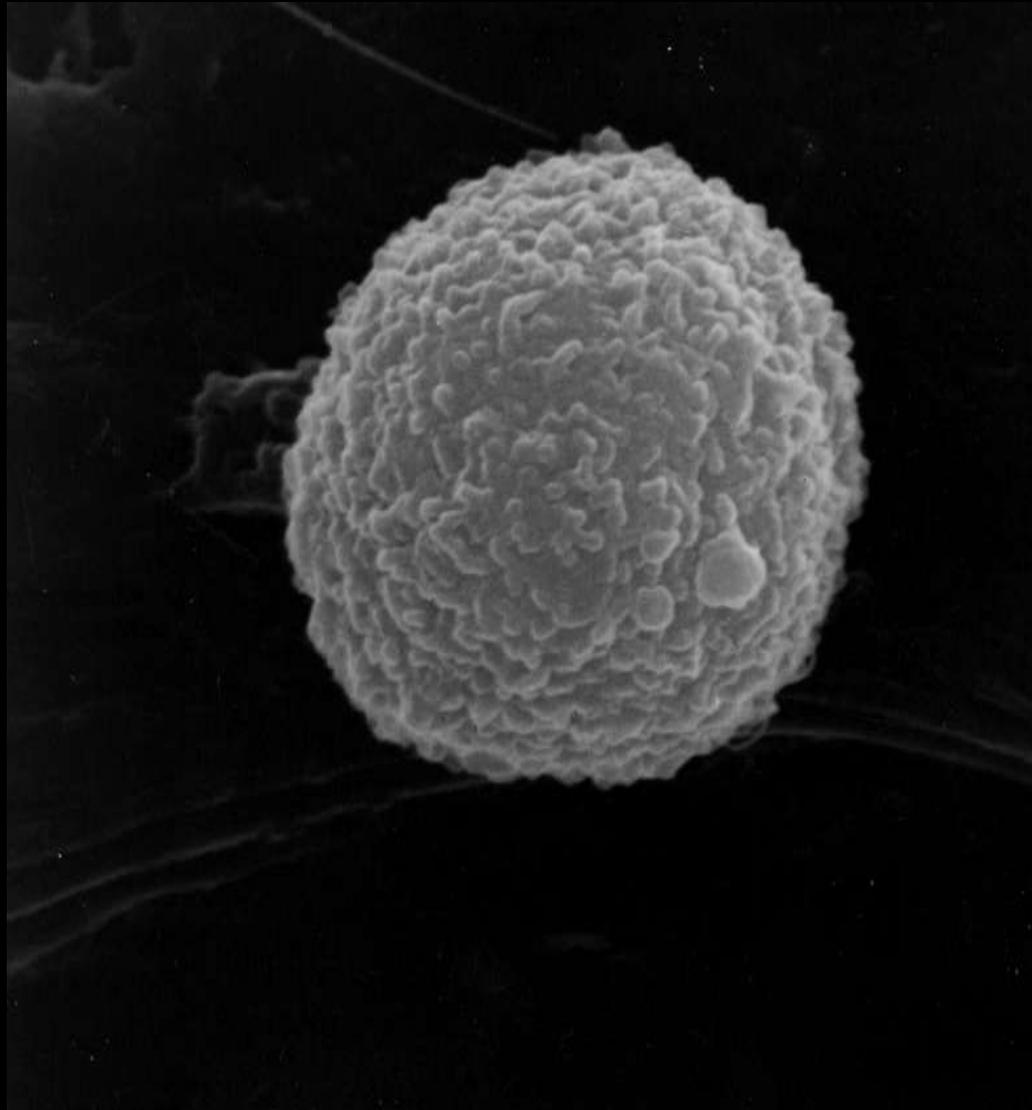
Autoradiografía y Ultraestructura Celular

OTROS TIPOS DE MICROSCOPIO ELECTRÓNICO

SEM (Scanning Electron Microscope)

- La imagen se obtiene a partir de los e^- rebotados y emitidos.
- Imagen de superficie: fácil obtención de 3D
- e^- menos energéticos ($< \sim 30$ keV)
- Menor coste que TEM



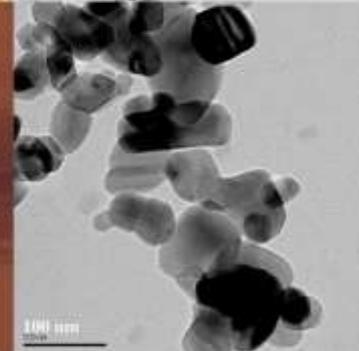
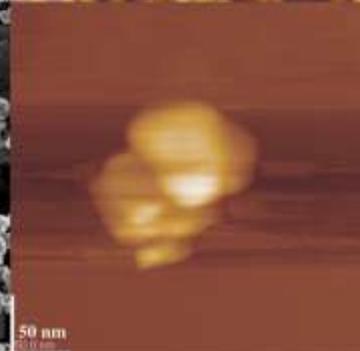
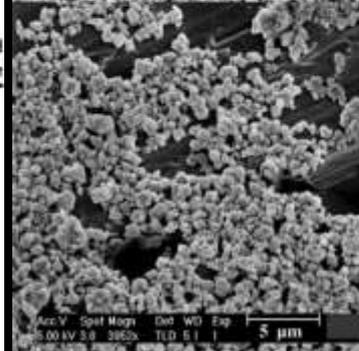
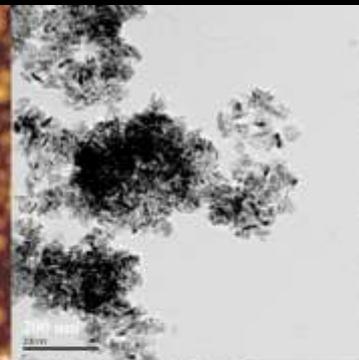
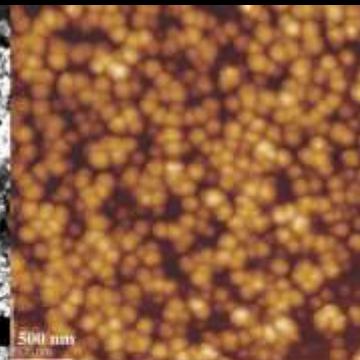
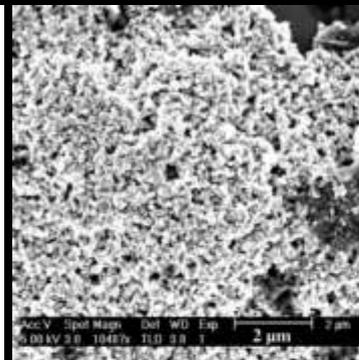
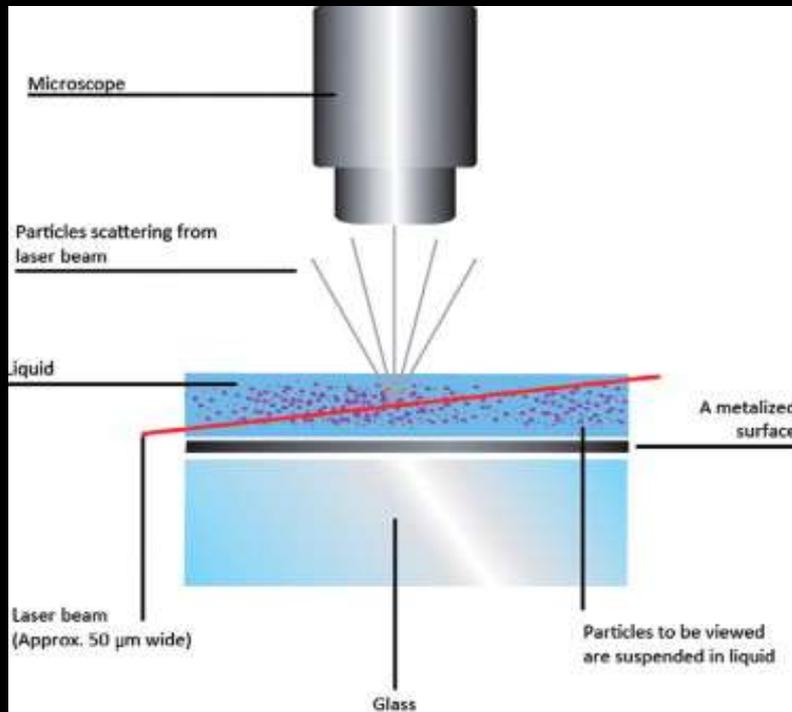


Célula linfoide B con moderado número de *microvillis* (SEM)

Microscopía de Nanopartículas

Con un microscopio electrónico de alta resolución se puede llegar a visualizar una sola partícula.

Número, tamaño y visualización de nanopartículas



MICROSCOPIA ELECTRÓNICA E INVESTIGACIÓN TRANSLACIONAL

INVESTIGACIÓN
BÁSICA



INVESTIGACIÓN
CLÍNICA



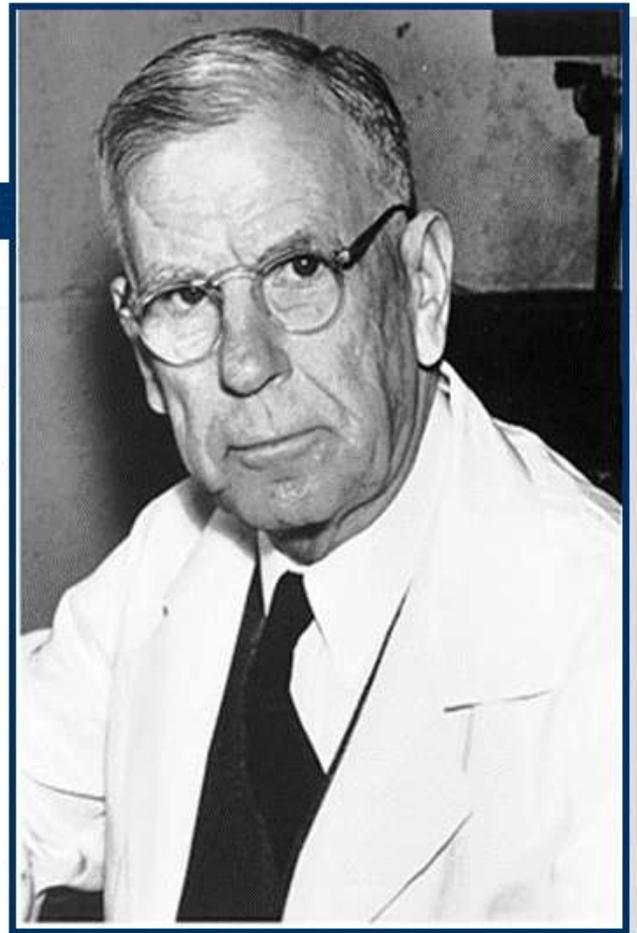
Facultad de Medicina Santiago

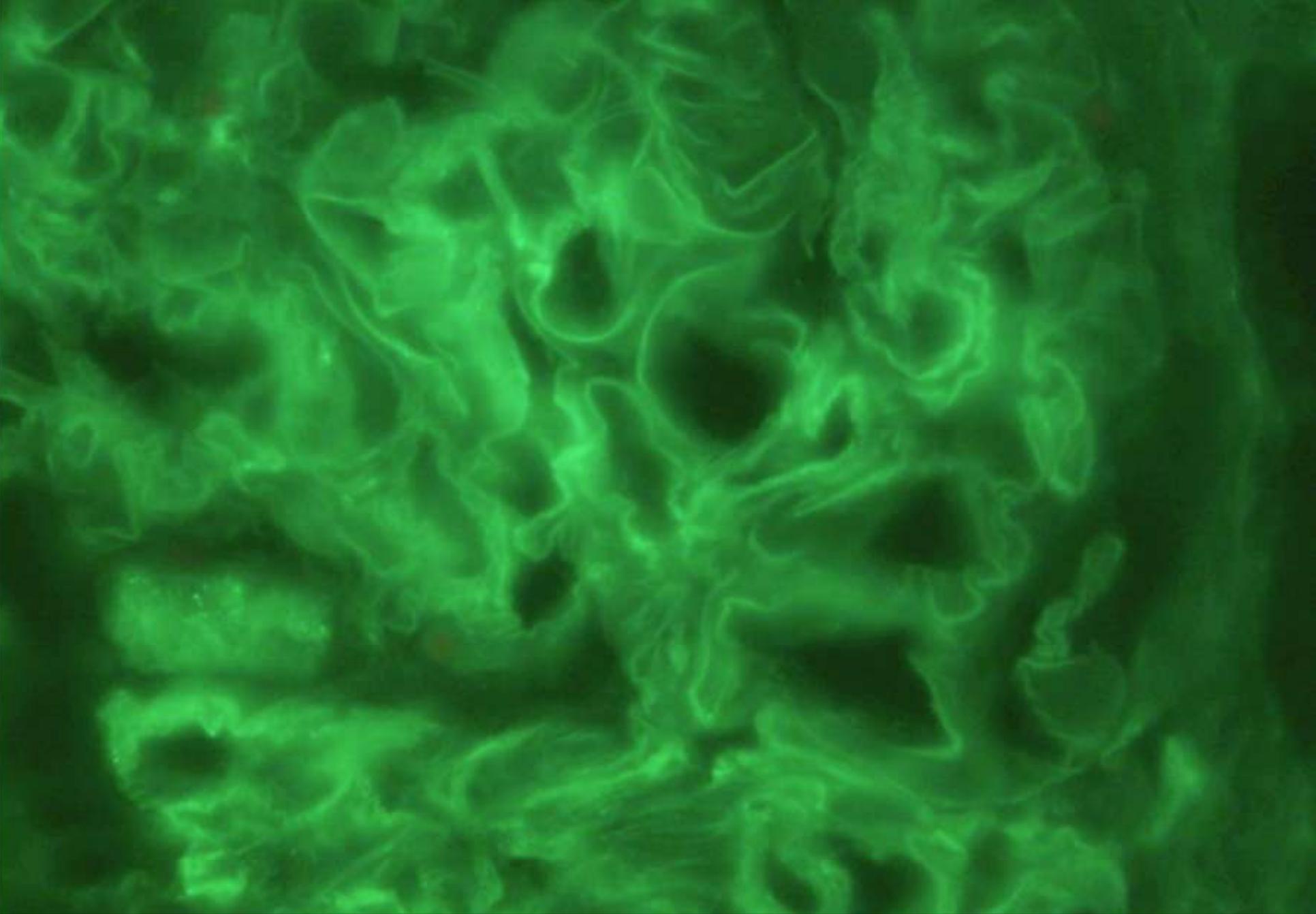


Hospital Clínico Universitario Santiago

- Ernest Goodpasture (a Harvard Medical School pathologist) reported the autopsy findings of a 18 year old male with a trace of albumin in his urine who died with extensive pulmonary hemorrhage shortly after developing influenza infection.
- Autopsy findings: alveolar hemorrhage, glomerulonephritis and vasculitis affecting spleen and gut.

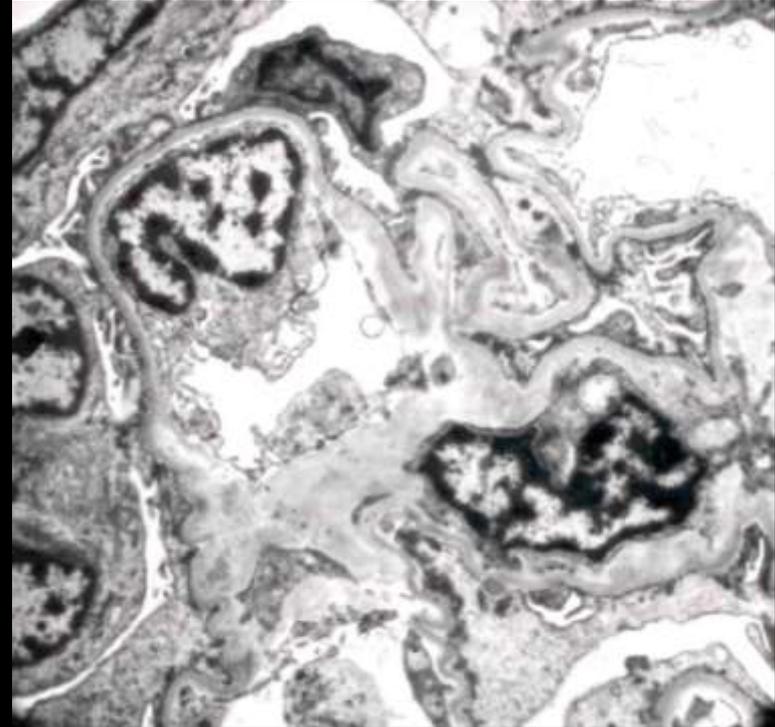
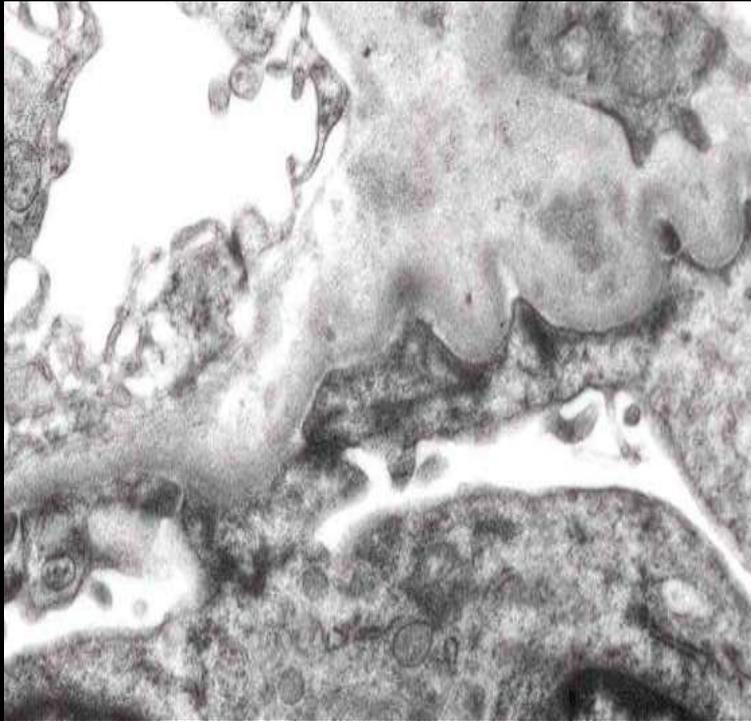
Goodpasture E.W. *The significance of certain pulmonary lesions in relation to the etiology of influenza* American Journal of Medical Science, 158:863-870 (1919)



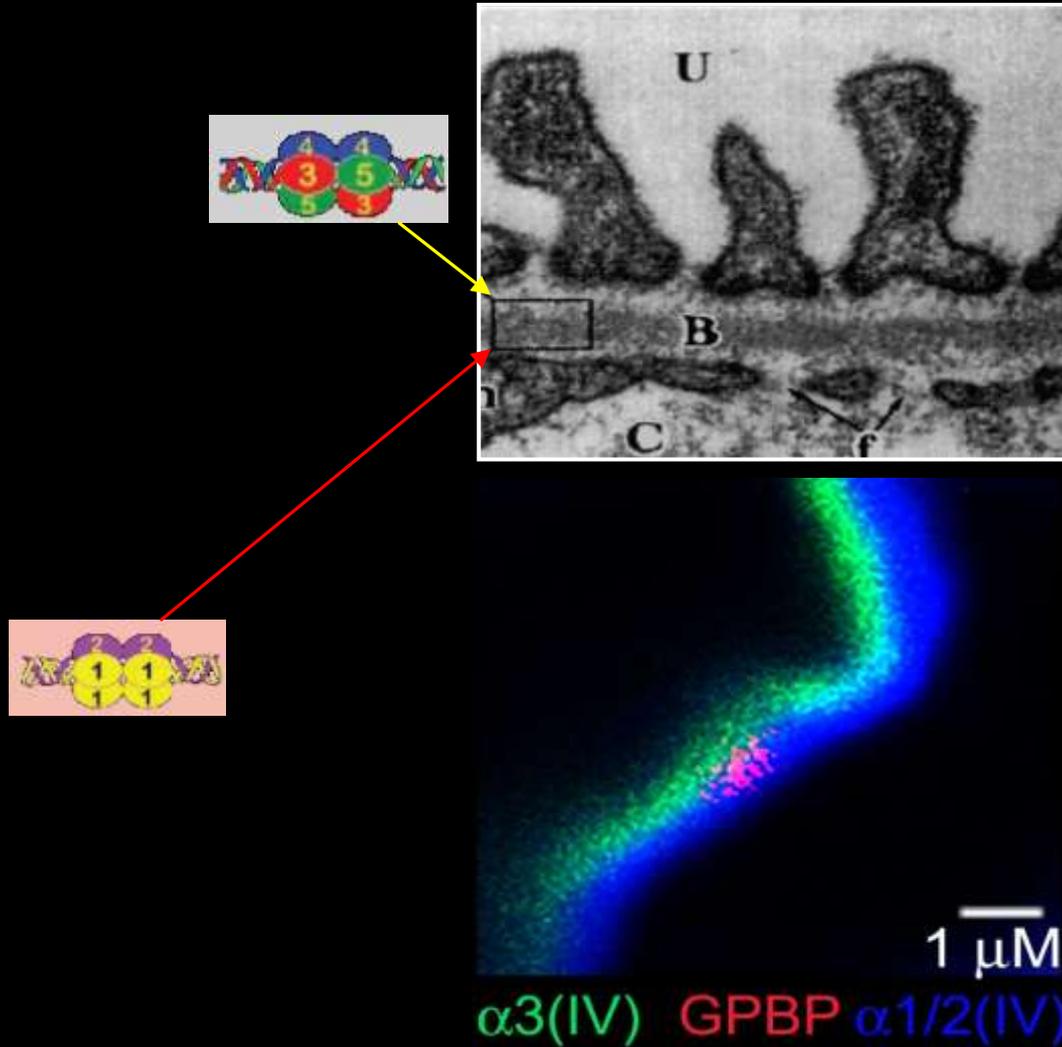


IGG – PATRÓN LINEAL (Goodpasture)

Microscopía Electrónica (Goodpasture)

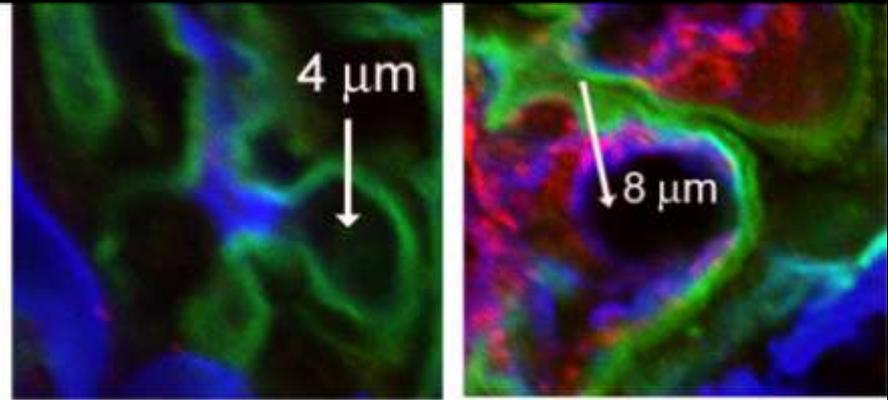


GPBP se asocia al colágeno de la membrana basal glomerular (MBG)



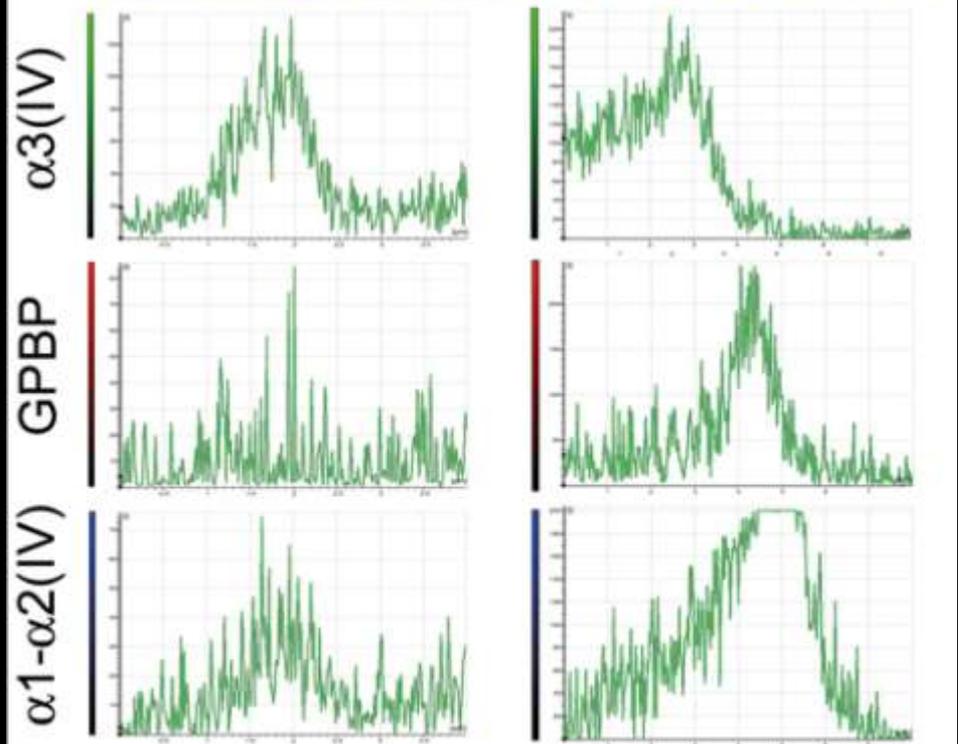
Control

Tg-hGPBP



La sobreexpresión de GPBP induce la disociación de los componentes $\alpha3.\alpha4.\alpha5(IV)$ y $\alpha1.\alpha2.\alpha1(IV)$ de la MBG

Revert F, et al. Am J Pathol. 2007; 171:1419-1430





PRINCIPE FELIPE
CENTRO DE INVESTIGACION



Dr. Juan Saus

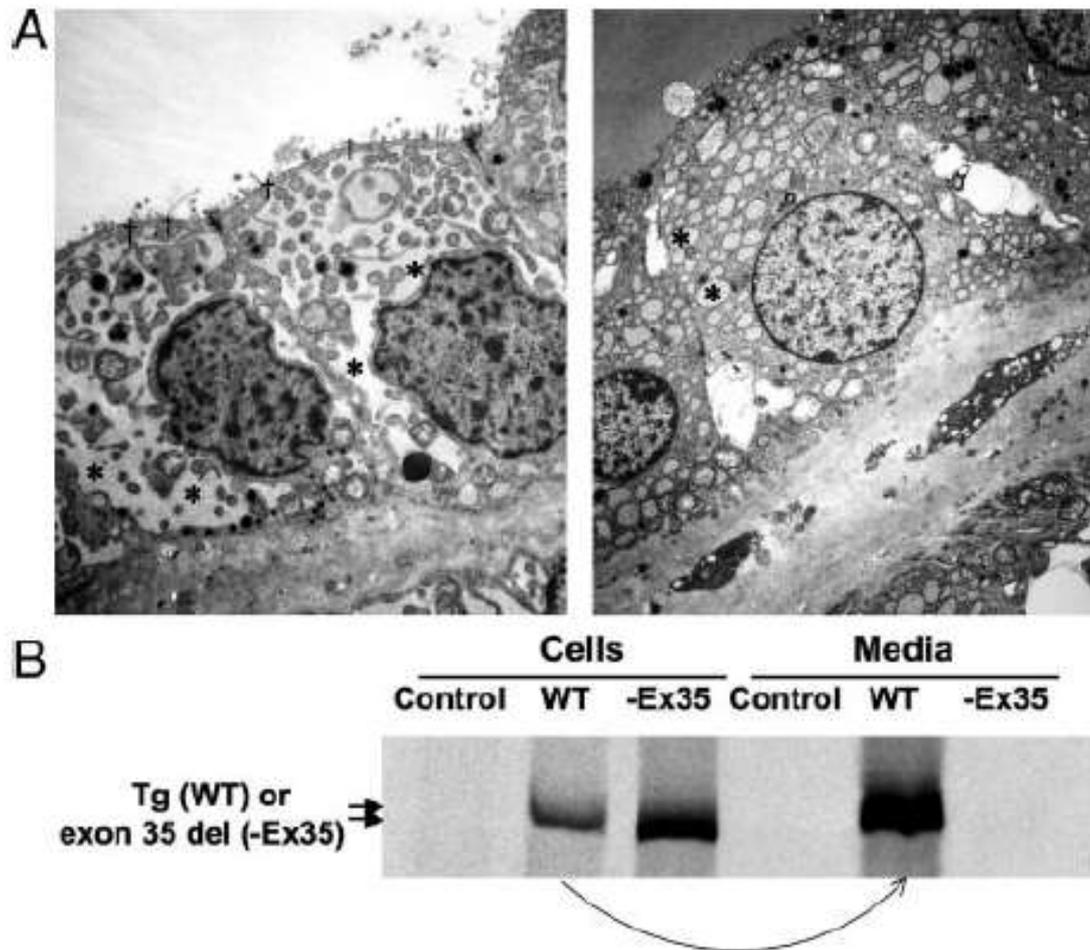


Bocio Tiróide

New Insights into Thyroglobulin Pathophysiology Revealed by the Study of a Family with Congenital Goiter

D. Peteiro-Gonzalez, J. Lee, J. Rodriguez-Fontan, I. Castro-Piedras, J. Cameselle-Teijeiro, A. Beiras, S. B. Bravo, C. V. Alvarez, D. M. Hardy, H. M. Targovnik, P. Arvan and J. Lado-Abeal

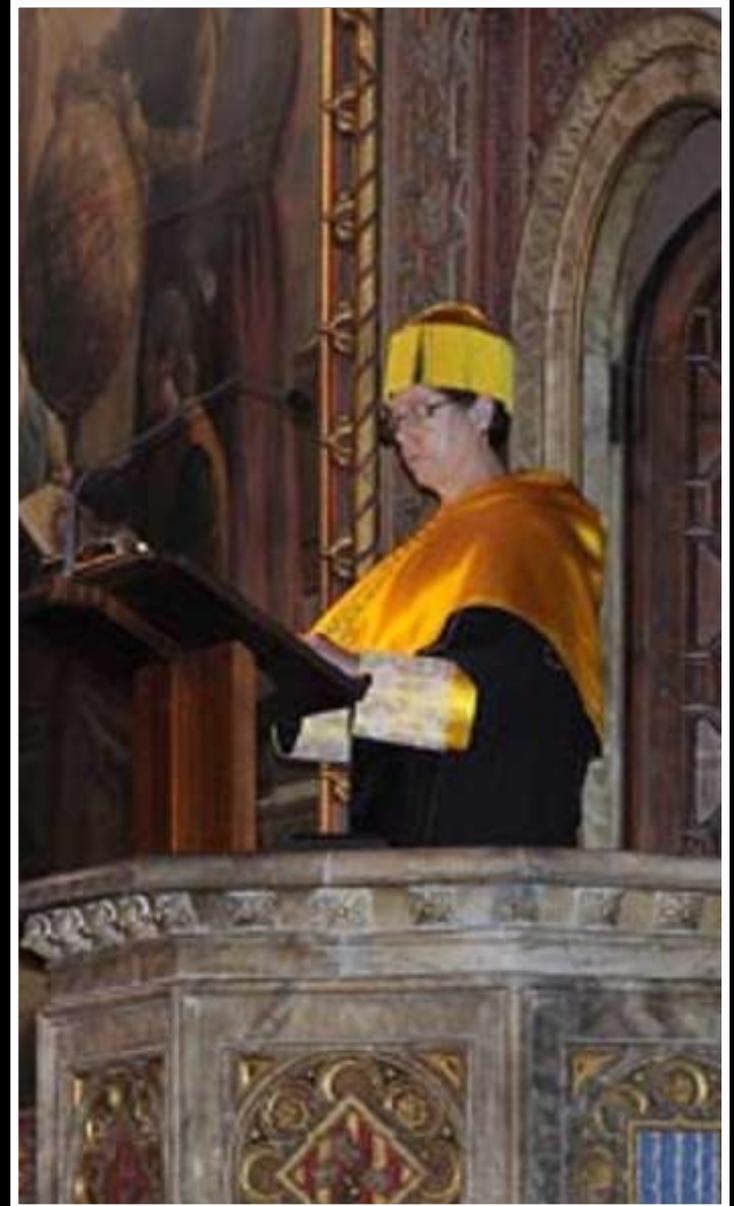
Endocr. Rev. 2010 31: 396

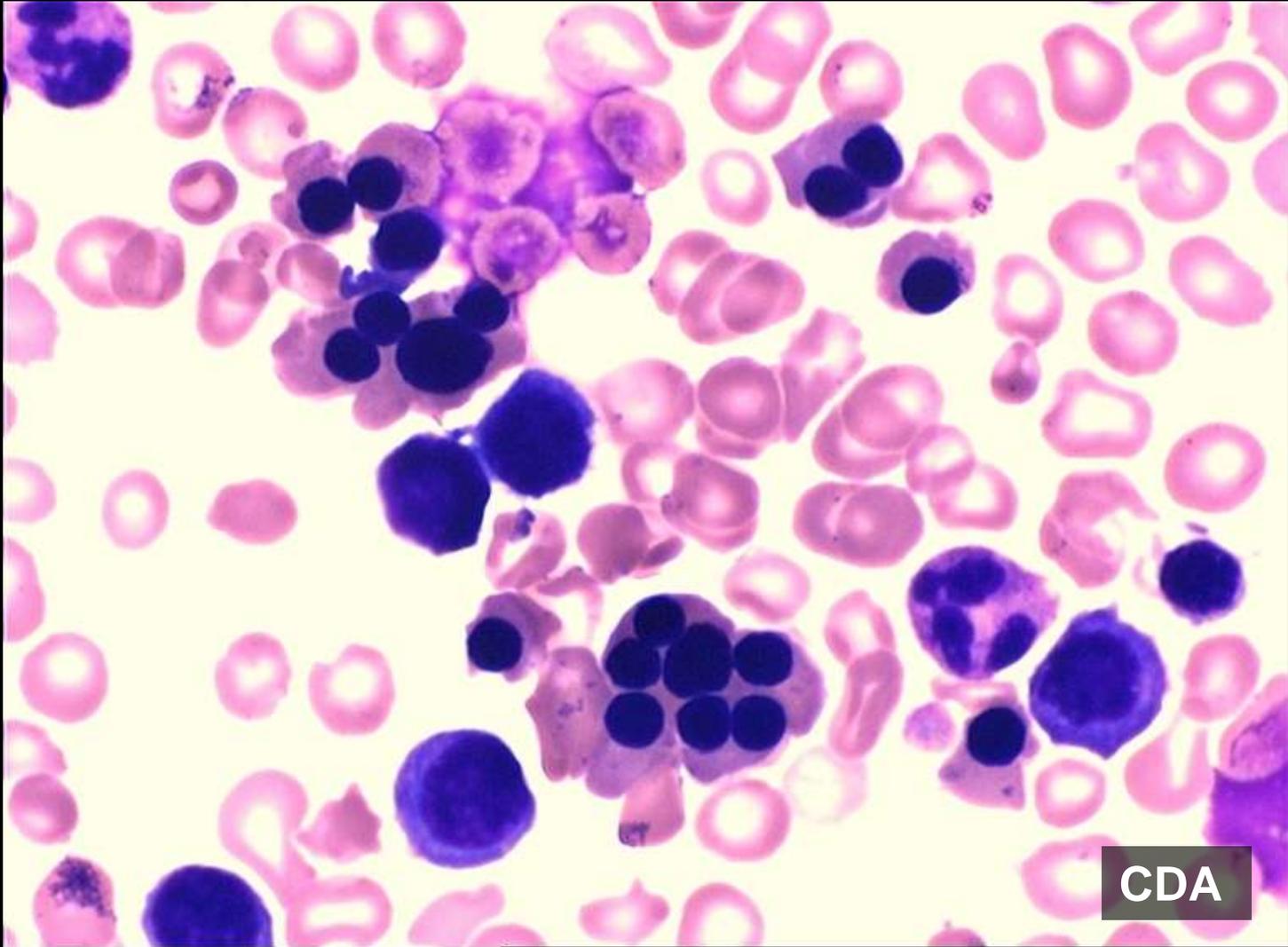


“The Microscope as a Tool for Disease Discovery”

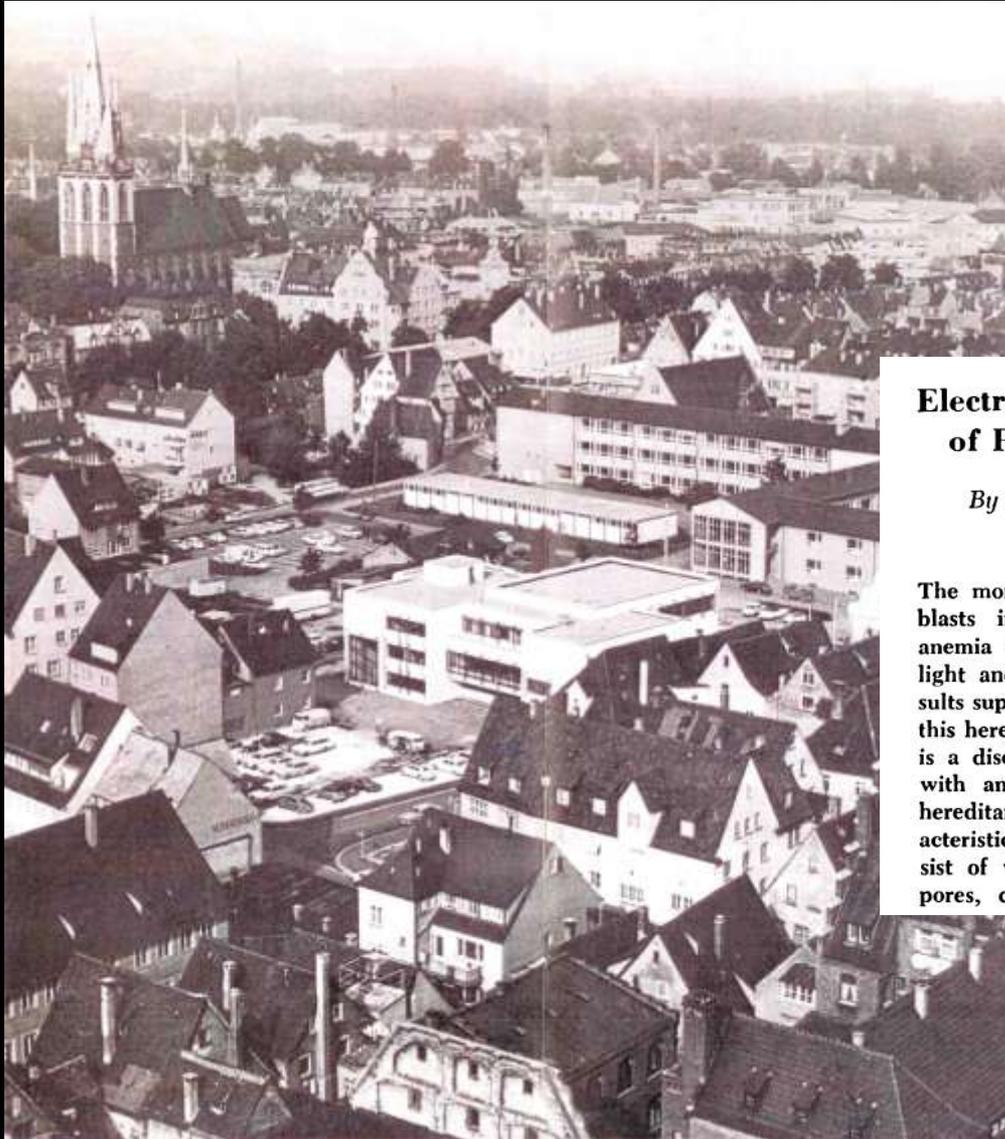
Elaine Jaffe

(Universidad de Barcelona, 2008)
Honoris Causa speech





CDA



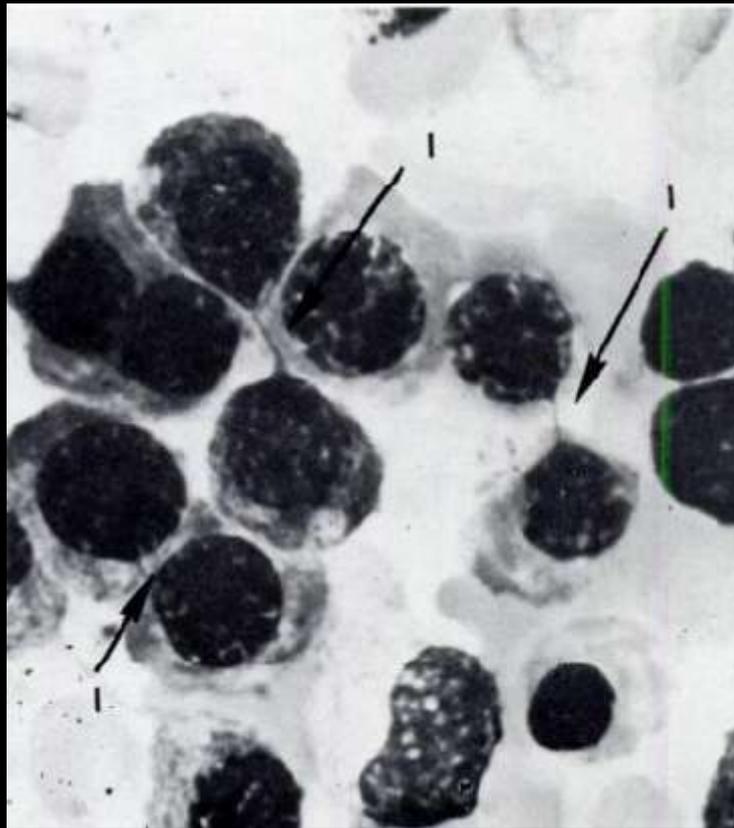
Electron and Light Microscopic Study of the Erythroblasts of Patients With Congenital Dyserythropoietic Anemia

By HERMANN HEIMPEL, JERONIMO FORTEZA-VILA, WOLFGANG QUEISSER
AND EGON SPIERTZ

The morphological changes of erythroblasts in congenital dyserythropoietic anemia type I have been analyzed by light and electron microscopy. The results support our earlier assumption, that this hereditary disorder of erythropoiesis is a disease entity and is not identical with any of the previously described hereditary hematological diseases. Characteristic morphological aberrations consist of widening of nuclear membrane pores, condensation, vacuolization and

disintegration of the nuclear chromatin, structural changes of the nucleolus, appearance of myelin figures, and final autolysis of the cells. All changes are restricted to erythroblastic cells beyond the stage of the proerythroblast. The biochemical basis of this abnormality is not known. It leads to severe ineffective erythropoiesis with anemia, increased total hemoglobin turnover, and secondary hemochromatosis.

BLOOD, VOL. 37, NO. 3 (MARCH), 1971



Bone marrow smear, Pappenheim Stain

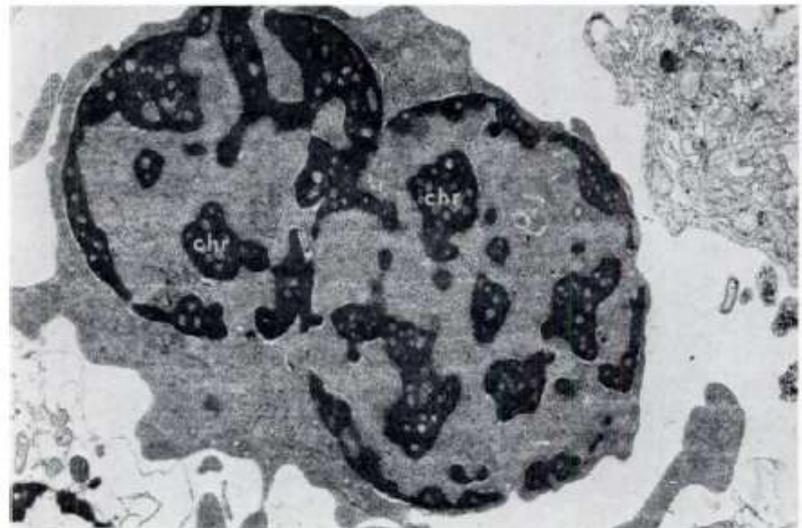
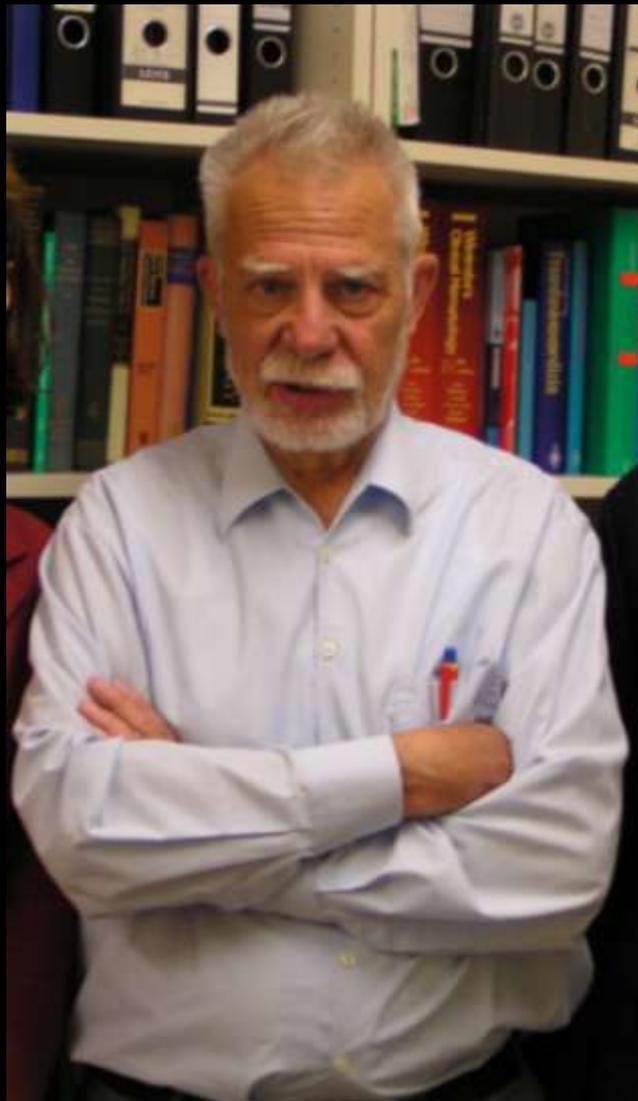
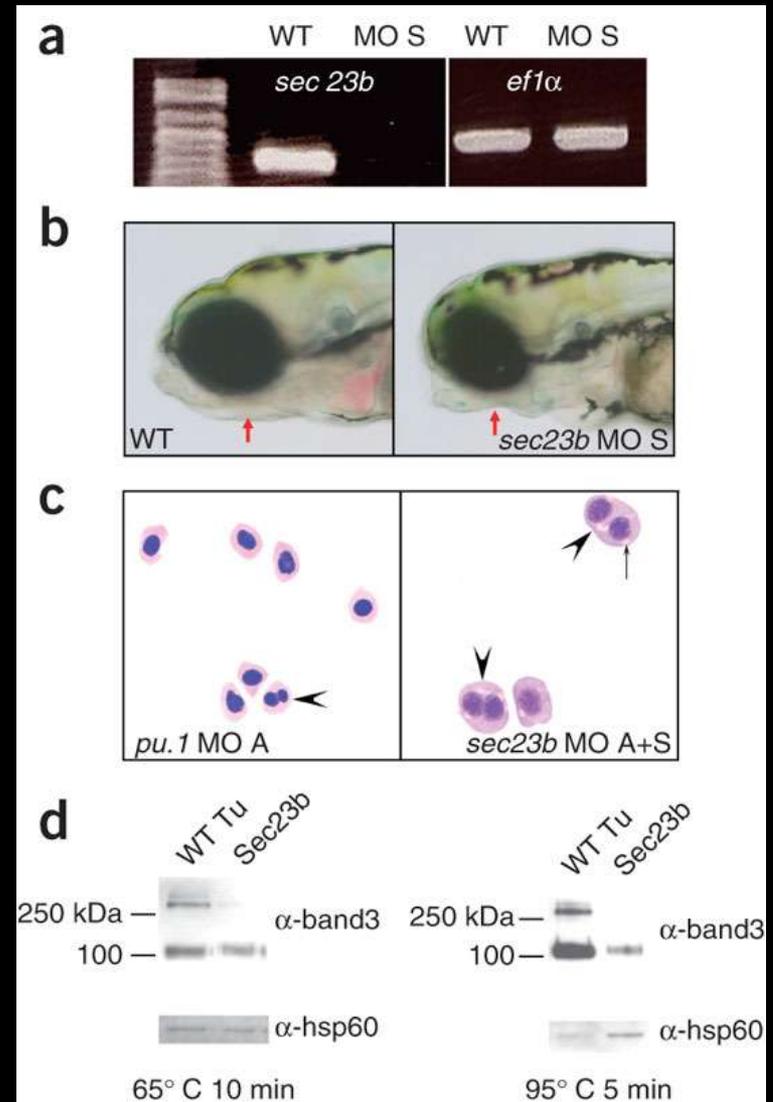


Fig. 8 (Upper).—Bridge of dense chromatin sheathed by cytoplasm (→). $\times 50,000$.
Fig. 9 (Lower).—"Normoblast." Cell with abnormal chromatin (chr) shows incomplete division of nuclei. $\times 12,000$.



Prof. H. Heimpel (Ulm, Alemania)

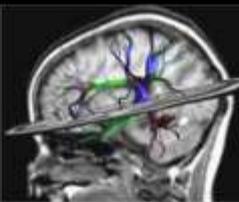
German Registry on Congenital
Dyserythropoietic Anemias (CDAs)



Mutations affecting the secretory COPII coat component SEC23B cause congenital dyserythropoietic Anemia type

One picture as worth ten thousand words

Frederic Barnhard (1927)



Magritte 1936 "La Clairvoyance"



A scenic landscape featuring a calm lake in the foreground. The left bank is lined with trees in vibrant yellow autumn foliage. The background shows a dense forest of green trees on a hillside. The sky is not visible, and the overall lighting is soft, suggesting a late afternoon or early morning setting.

CONCLUSIÓN:

La microscopía electrónica es necesaria para la comprensión de la enfermedad.

¡MUCHAS GRACIAS POR SU ATENCIÓN!