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EDITORIAL

In focus in HCB

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One of the great aspects of being a scientist is the ability to constantly "tweak" well-established protocols to meet contemporary standards of safety and environmental considerations. Just when we think that microscopy methods established more than 60 years ago are so entrenched in our collective minds that we would never think of altering them, current conditions and, in some cases local regulations require us to seek alternative, less hazardous reagents for our protocols. In this Editorial, we will highlight a Short Communication describing just such a modification: a safe, non-toxic and non-radioactive alternative to the use of uranyl acetate for sample contrast enhancement for transmission electron microscopy. Additionally, we provide a brief synopsis of two Original Papers describing the influence of fibroblast growth factor 9 (FGF9) on the width of long bones in the mouse, and the intracellular immunofluorescence localization of lysosomal-associated membrane protein1 (LAMP1) in many different mammalian cell lines. Enjoy!

A "contrasting" alternative to uranyl acetate

Traditional protocols for preparing biological samples and sections for viewing in the transmission electron microscope require contrast enhancement with heavy metal stains (Hayat 1989). This typically includes sample treatment with the very toxic osmium tetroxide prior to embedding in a plastic resin. The osmium will stain membranes and some lipids, rendering them very dark (electron dense) upon interaction with the electron beam in the microscope. Other reagents, including uranium salts and tannic acid, for instance, can also be used to render samples more electron dense during "en bloc" tissue staining. After thin sections have been prepared, they are typically further "contrasted" with the toxic heavy metal-containing uranyl salts and lead salts. Unfortunately, in addition to its toxicity, uranyl acetate is also radioactive, thus necessitating complex and costly waste disposal

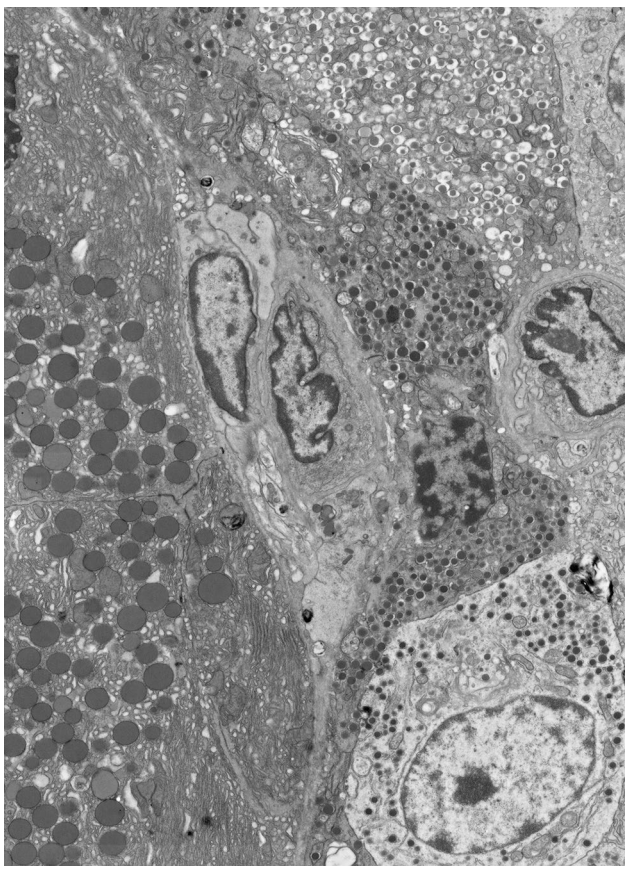

Fig. 1 Ultrathin section from resin-embedded rat pancreas contrasted with neodymium acetate and Reynolds lead citrate. The outermost cell layers of an islet of Langerhans with insulin B-cells and glucagon A-cells and part of exocrine pancreatic acinar cells are depicted. Neodymium results in an electron contrast of the various cellular organelles which appears to be equal to that obtained with uranyl acetate. Micrograph by courtesy of Kuipers and Giepmans, University of Groningen, The Netherlands

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methods. In a fitting tribute to last year being recognized as "International Year of the Periodic Table of the Chemical Elements", due to the sesquicentennial of the creation of the periodic table by Mendeleev (Mendelejeff 1869), Kuipers and Giepmans (2020) sought a safer, non-radioactive alternative to uranium for contrast enhancement by looking to the element neodymium (Nd; atomic number 60) sitting just above it in the table. They tested various concentrations, settling on a 4% NdAc solution for post-staining of ultrathin sections (Fig. 1), en bloc staining of tissue pieces, and negative staining of amyloid-β fibers. Their results and analyses demonstrated convincingly that neodymium acetate can be substituted as a non-toxic, non-radioactive alternative for uranyl acetate for all three of the electron microscopy staining techniques mentioned above. From a safety and environmental standpoint, it is to be hoped that this contrast reagent alternative to uranium will become the new "universal standard" in electron microscopy labs.

The "bona fide" details concerning bone formation

Bone formation through endochondral ossification during embryonic development is regulated by a number of growth factors and signaling pathways (Amizuka and Kitazawa 2018; see Special Issue of *Histochemistry and Cell Biology* on "Hard Tissue Biology", volume 149, number 4 April 2018). Members of the fibroblast growth factor (FGF) family, especially FGF9 are of particular importance during the process of mesenchymal condensation through the formation of a cartilage template, to final bone deposition. Harada and Akita (2020) have now investigated the regulation of the width of long bones during embryonic development using mice, with a specific FGF9-mutation (Elbow knee endochondral (Eks)) known to affect bone formation (Murakami et al. 2002). Entire skeletons and sections from humeri long bones from $Fgf9^{Eks/Eks}$ mice were evaluated using a wide variety of histochemical staining techniques, including Alizarin/Alcian blue staining of whole pups or embryos, conventional immunohistochemistry, RNA in situ hybridization, fluorescence cell proliferation with Click-iT EdU Alexa fluor, and multiple image analyses. Their comprehensive results showed that compared to control $Fgf9^{+/+}$ littermates, $Fgf9^{Eks/Eks}$ mice, (1) displayed wider long bones at birth; (2) the widths of chondroprogenitor expression domains, as determined by immunohistochemical staining for chondrocyte markers, were expanded; (3) the perichondrium of the humerus, determined by immunohistochemical staining was also widened; (4) an increase in the density of humeral chondrocytes; and (5) increased and expanded FGF signaling pathways during chondrogenic condensation, as assessed by immunohistochemical and in situ hybridization analyses of specific signaling pathway molecules. These impressive results taken together suggest that FGF9 is intimately involved in the regulation of the width of long bones, such as the humerus during embryonic development, via control of the overall width of early chondrogenic condensation.

Not all LAMPs are the same–at least by location

The *trans*-Golgi network (Rambourg and Clermont 1997) as a ubiquitous subcompartment of the Golgi apparatus is involved in a variety of cellular functions, including the sorting of lysosomal enzymes into the lysosomal pathway. Lysosomal-associated membrane proteins 1 and 2 and CD63 are major components of the lysosomal membrane and are typically localized to late endosomes and lysosomes (Luzio et al. 2007; Saftig and Klumperman 2009) as revealed by immunolabeling in cultured cells. Baba and colleagues (2020) have analyzed the subcellular distribution of different lysosomal-associated membrane proteins (LAMP) in kidney-derived rat NRK, golden hamster BHK-21 and human 293 cells, Chinese hamster ovary-derived CHO-K1 cells, mouse muscle-derived C2C12 cells, mouse lymphoma-derived YAC-1 cells, and human lung-derived A549 cells. With the exception of BHK-21 and CHO-K1 cells, EGFP-tagged LAMP1, LAMP2 and CD63, as well as endogenous LAMPs were observed as small spots distributed throughout the cytoplasm and codistributed with LysoTracker Red. Unexpectedly, in BHK-21 and CHO-K1 cells, the three LAMPs accumulated in the same particular cytoplasmic area near the nucleus and displayed only minimal codistribution with LysoTracker Red. This was not related to species differences of the *LAMP1* gene since transient expression with expression vectors encoding golden hamster, rat, Chinese hamster and human LAMP1-EGFP resulted in the same "aberrant" distribution pattern. It was also excluded that C-terminal tagging with EGFP or DsRed-monomer potentially interfering with LAMP glycosylation influenced the proper localization of LAMP1 in BHK-21 cells and CHO-K1 cells. Through a codistribution analysis with established Golgi subcompartment markers, it could be shown that the perinuclearly accumulated LAMP1-DsRed in BHK-21 cells matched up with the distribution of TGOLN2-EGFP, a *trans*-Golgi network marker. The authors hypothesize that the analyzed LAMPs may be transported back from endosomes to the *trans*-Golgi network due to unusual recognition by tethering factors and conclude that BHK-21 cells and CHO-K1 cells appear to be unique tools for investigating the intracellular trafficking of LAMP family proteins from the *trans*-Golgi network.

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News from the Society for Histochemistry

Dear Colleagues,

On behalf of the board of the Society for Histochemistry, it is our great pleasure to invite you to the world 16th International Congress of Histochemistry and Cytochemistry (ICHC), to be held on August 30 – September 2, 2020, in Prague, Czech Republic.

The ICHC is held every four years under the auspices of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), which continually strives to provide grounds for communication and cooperation among scientists all over the world in the areas of cyto- and histochemistry, cell and tissue biology, microscopy, pathology and other relevant fields.

The city of Prague, also known as the heart of Europe, provides easy access for scientists from all over the world. The congress venue, Cubex Centre Prague which offers technologically and visually unique space, promises to leave everyone with an unforgettable experience. Of course, Prague prides itself with its beautiful historical architecture, technical monuments, celebrated cafés, great food, and beer. This will be underlined by the ICHC gala dinner in the famous Art Nouveau Municipal House, and a free beer party organized in the premises of the Staropramen brewery.

We hope that you will join us in Prague to discuss together your latest achievements and that the venue will provide great opportunities for specialists at all levels of their career, bringing lots of opportunities for strengthening international collaborations. Special attention will be therefore given to the presentations of students. We also expect a rich commercial exhibition where new and emerging technologies will be presented.

Looking forward to meeting you in Prague,

Klara Weipoltshammer, President of the SfH
Pavel Hozak, Chair of the Local Organizing Committee, Secretary of the SfH

Contacts
Home: www.ichc2020.com
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If you have any questions about abstracts, please contact: abstracts@ichc2020.com
Other inquiries and comments about the conference, please contact: info@ichc2020.com

ANNOUNCEMENT

The Society for Histochemistry

Invites scientists to apply for the 2021 Robert Feulgen Prize. The prize is awarded for an outstanding achievement in the field of histochemistry.

The contributions may be either towards the development of new histochemical and cytochemical techniques or in the application of existing technology towards solving important problems in biology and/or medicine. Addressed are scientists working in microscopical sciences (in the widest sense) as well as in biochemistry, cell biology, endocrinology, in situ molecular techniques, and neurosciences. Scientists in their mid-career (assistant or associate professor, priv. doz.) are encouraged to apply. The prize is not intended for lifetime contributions.

The Prize consists of a monetary prize of €2,000

All applications should be submitted before January 31, 2021 via the electronic submission system at: https://www.greception.com/form-login-window/191a281d/

The application should contain a short curriculum vitae, a 1,000 word summary of the contributions of the applicant and PDF reprints of the pertinent publications. Full description of conditions is available on the Society website: http://histochemistry.eu/description_of_conditions_.html