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Grimelius L. A silver nitrate stain for α_2 cells in human pancreatic islets. *Acta Soc. Med. Upsal.* **73**:243-70, 1968. [Department of Pathology. University of Uppsala, Sweden]

A simple silver-staining method was developed whereby most endocrine cells in the digestive tract, certain endocrine pancreatic cells, thyroid parafollicular cells, and so on, can be demonstrated. Only well-characterized and easily obtainable chemicals are required. The method is used mainly in routine histopathology for discriminating endocrine from nonendocrine tumors. [The SCI® indicates that this paper has been cited in over 475 publications since 1968.]

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"One of my first research projects as a young assistant at the Institute of Pathology in Uppsala was to investigate the endocrine pancreas in diabetic rats treated with anti-diabetic agents. Good staining methods were available for demonstrating insulin-producing cells and this held for D(A)₁ cells, which were later found to contain somatostatin.^{1,2} But how could the A(A₂) cells be visualized? I tested several silver techniques, but the results were not very satisfactory, except with one method developed by Bodian.³ Here a silver proteinate was used for impregnation. Unfortunately, the quality of this substance varied considerably both between different manufacturers and between different batches from one producer. Out of about ten silver proteinates that I tested, only one gave a satisfactory result, and then only after modification of the Bodian technique. When I tried to order more silver proteinate from the manufacturer, I was informed that this batch was sold out. I was also told that one seldom succeeded in preparing silver proteinate with

the same staining properties from one occasion to another even when the manufacturing process was the same.

"Instead of dropping the research project, I decided to develop a silver technique of my own with the same staining properties as the Bodian method but where only well-characterized and easily obtainable chemicals were required. The different factors in the staining steps should also be well controlled, so that the results would be reproducible. After four years' work and more than 2,000 staining experiments, I managed to arrive at a simple silver (argyrophil) technique which comprised only two stages — silver impregnation and the reduction process. The staining was also developed such that counterstaining was unnecessary. Subsequent analyses of the technique showed that the silver-positive cells in fact represented A cells. Electron microscopic studies⁴ revealed that the silver-positive reaction is due to precipitation of silver particles in the secretory granules (storage sites for hormones) of the endocrine cells. As with the Bodian technique, cells in the gastrointestinal mucosa were also stained. Later studies, by many other authors, showed that my technique stained almost all endocrine cell types in the digestive tract (cf. Grimelius and Wilander⁵). It was also found that the parafollicular cells in thyroid, and the endocrine cells in the respiratory tract, for example, could also be visualized. The demonstrable cells belonged to the APUD system.

"Originally, I developed the technique for the pancreatic A cells, but a few years later I modified it somewhat so that it would also give optimal staining of endocrine cells in the gastrointestinal tract.⁵

"The reasons this technique has become so widely established are probably that it is simple, that all chemicals are easily obtainable, and that the results are reproducible. The method has come into use in routine histopathologic diagnosis for discriminating endocrine tumors of APUD type from other endocrine and nonendocrine tumors.

"The research project with the diabetic rats was never completed!"

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