

Eighty years of research on islet amyloidosis in Uppsala

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Introduction

Science is said to be free in Sweden in the sense that scientists are allowed to do research on any subject, but there is one very serious limitation: the work has to be funded. The latter aspect has become increasingly evident during later years. In many ways, research was more free decades ago when more money was directly allocated to universities and their departments. In this way, islands of research could more easily develop around a successful scientist, and if he (it used to be men, at least in medical research) was lucky he might recruit a number of pupils who could take the research further. The most well-known example of this scenario in Uppsala of course is Carl von Linné (1707–1778) with his large group of famous disciples. On the other hand, it is a risk that a scientific line fades out rapidly when a leading and charismatic person dies or moves. There is a multitude of examples of this phenomenon.

The first demonstration of islet amyloid in Uppsala

During last decades amyloidosis has become a hot subject in medical research. Quite evidently, the number of scientists working on different aspects of amyloid has increased, also in Uppsala. But how was the situation earlier at Uppsala University? The first scientist interested in amyloid seems to have been a professor of pathology, Nils Gellerstedt (Figure 1). Gellerstedt was known as an excellent clinical pathologist and seems to have had quite wide interests in a number of different conditions. He and some of his pupils published several papers on age-related ('senile') amyloid forms. Most interesting is perhaps an article published in 1938 dealing with amyloid in the islets of Langerhans (1). That article by Gellerstedt was initiated by his observation of the presence of islet amyloid in two patients with diabetes mellitus. The scientific activity in Uppsala Medical Society (Uppsala Läkareförening) must have been very high at that time with many oral presentations each month. In the record of a meeting on 13 December 1935 it appears that Gellerstedt gave a talk entitled 'Elective amyloidosis of islet of Langerhans as a cause of diabetes' (Figure 2). Unfortunately there is no written report from that meeting, but Gellerstedt refers to these two cases as a background to his following, extended study. According to the title of his presentation in Uppsala Gellerstedt regarded the found islet amyloid as the cause of diabetes in these two patients. In his more elaborated investigation from 1938 he studied sections from the pancreatic tail in 181 consecutive autopsies among which 110 were from subjects aged 50 years or more. Gellerstedt most certainly was disappointed by the new findings of his larger study. As expected, he found islet amyloid in subjects with diabetes but perhaps more surprisingly he found the alteration to be quite common among non-diabetic individuals as well, some of them with quite pronounced depositions. Gellerstedt finally concluded that it was not possible to see a direct causal relationship between islet amyloid



Figure 1. Nils Gellerstedt. Professor of Pathology, Uppsala University 1948–1954.

and diabetes. This view has since then been the predominating one in the field of diabetes research until the last 10 years or so.

Remarks by Gellerstedt on the histological appearance of islet amyloid

For a very long time there was a discussion on whether hyaline in the islets of Langerhans was to be regarded as amyloid or not. Amyloid was at that time only defined by its appearance in the light microscope, i.e. a hyaline substance with some tinctorial properties, particularly metachromasy with certain aniline dyes and affinity for Congo red. The latter is still very much used in research and clinical practice although our present definition of amyloid is more sophisticated and based on protein molecular arrangements. Gellerstedt entered that discussion

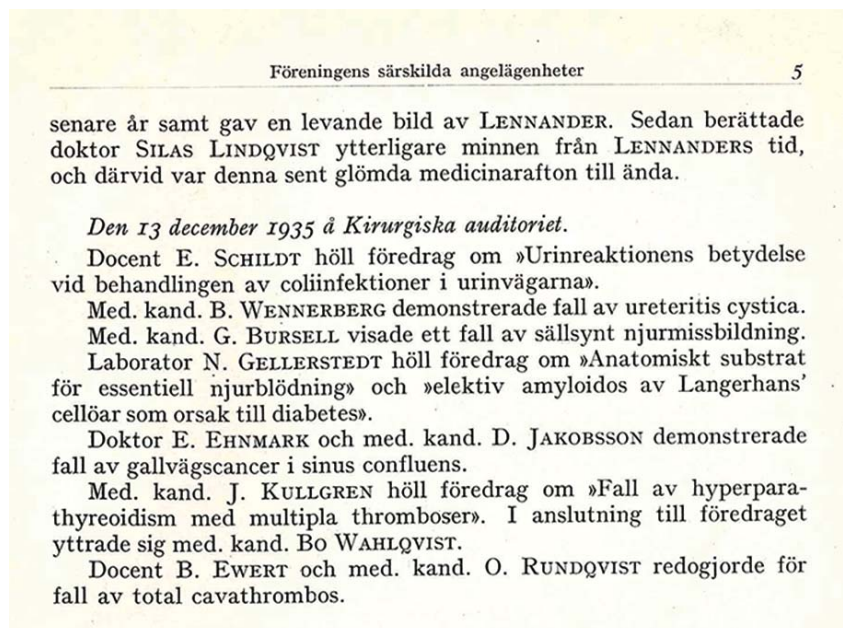


Figure 2. Extract from *Upsala Läkareförenings Förhandlingar*, 1935.

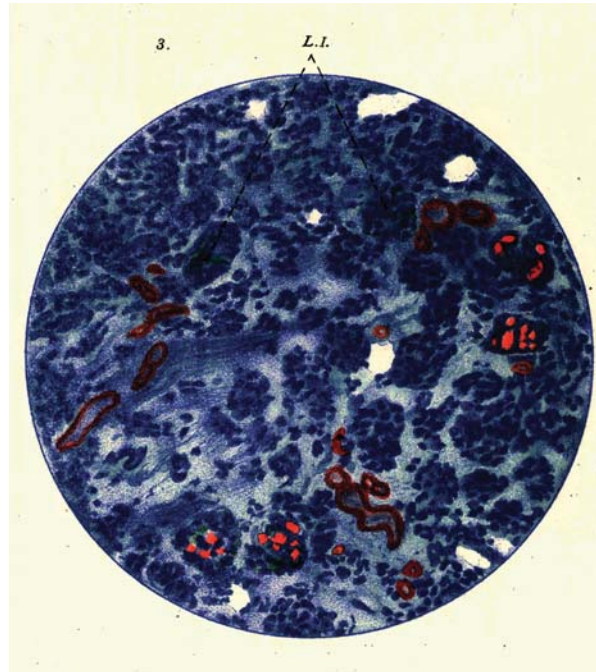


Figure 3. Figure from Gellerstedt's paper from 1938 showing islet amyloid in a patient with systemic amyloidosis. Although we do not know what type of systemic amyloidosis it is, AA amyloidosis was by far the most common form in Sweden at that time. Note the colour differences between the amyloids. The depicted section was stained with methyl violet.

and proposed that islet hyaline actually should be regarded as amyloid as judged from his experience with staining with methyl violet and Congo red, beautifully illustrated by hand-coloured pictures in his article. However, he must have felt some uncertainty, since he put in a 'para' in the title and gave the hyaline deposits the designation (para)amyloid. The amyloid nature discussion was not finished with this paper but principally went on (2,3) for decades until amyloid in general was defined by its cross- β -sheet fibrillar structure (4-6), shown to be valid also for the islet form.

The study by Gellerstedt contains several other interesting points. He noted that the islet amyloid shows 'classical colour reactions' and that the metachromasy with methyl violet is very clear but the colour slightly different from general amyloid ('allgemeine Amyloid', most probably AA amyloidosis), also shown in his illustration (Figure 3). This observation shows that with an open mind it is possible to find interesting facts without very sophisticated methods. Interestingly, the same colour difference can be seen with Congo red as we use this stain today (Figure 4). It is now understood that amyloid is not a single substance but a conformational

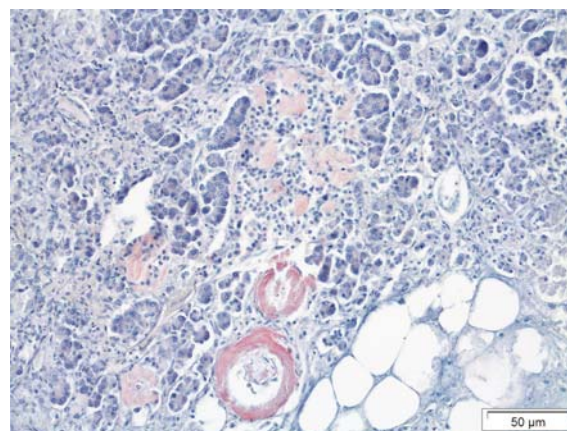


Figure 4. A modern pancreatic section from a patient with AA amyloidosis due to rheumatoid arthritis and with islet amyloidosis of IAPP nature. A similar colour difference as in Figure 3 is seen in the two amyloid forms. Congo red.

Das Problem der „senilen Amyloidose“ schließt manche interessanten Fragen in sich, deren Studium vielleicht neue Wege der Forschung eröffnen kann. Das Hauptgewicht liegt hier wiederum auf dem Gebiete der Serologie. Es müßten viel eingehender als es bisher geschah, die serologischen Veränderungen des Seniums untersucht, die immunbiologischen und allergischen Reaktionen des alternden, gesunden oder somatisch erkrankten Organismus auch im Tierexperiment erforscht werden. Die Untersuchung müßte ferner auch auf psychiatrischem Gebiet ausgebaut werden. Hier verbergen sich interessante Probleme über die Möglichkeit der Entstehung der senilen und präsenilen Psychose auf Grund von Antigen-Antikörperreaktionen usw. — Aber der pathologisch-anatomische Forschungsweg ist auch nicht zu vernachlässigen. Verf. ist geneigt zu glauben, daß eine systematische und genaue Untersuchung der verschiedenen Körpergewebe auf das Vorkommen einer atypischen Amyloidablagerung bei alten Menschen, vielleicht auch Tieren¹⁾, weitere, nicht seltene Befunde ähnlicher Natur entdecken ließe.

Figure 5. Gellerstedt's prophetic statements on p. 11 of his paper (1). In translation: 'The problem "senile amyloidosis" contains many interesting questions, studies of which perhaps may open new ways in research. The most important is again serology. It is important to put more efforts than earlier into studies of the serological changes in the senium as well as immunobiological and allergic reactions in the healthy as well as sick organism, also in animal experiments. The studies must also widen into the psychiatric field; there are interesting problems regarding the development of senile and presenile psychoses due to antigen-antibody reactions and so on. — However, the pathological-anatomical research is not to be disregarded. The author tends to believe that a systematic and careful study of the different body tissues for presence of atypical amyloid deposits in old humans, perhaps also in animals, could discover additional, non-rare findings of similar nature'.

state that at least 30 human proteins can obtain *in vivo*, resulting in deposition of protein fibrils (7). Although these fibrils look similar by means of electron microscopy and have the same general protein conformation and some properties in common, it is clear that there are differences (8). However, Gellerstedt did not speculate much about the nature of the amyloid material although he mentioned the possibility of an antigen-antibody reaction. This was a common theory concerning the nature of amyloid in general at that time. The article ends with the prophetic statement that 'The problem "senile amyloidosis" contains many interesting questions, studies of which may open new ways in research' (Figure 5). This was certainly true as we will see in the following.

Reappearance of islet amyloid research in Uppsala

Gellerstedt published some additional articles on senile amyloidosis but nothing more about islet amyloid. After his decease in 1954 Gellerstedt's knowledge capital on amyloid seems to have faded, and when one of us (P.W.) started his career in pathology in the late 1960s no one at the department knew anything about amyloid or even knew that someone had been interested in that subject. It was instead quite by accident that studies on islet amyloid started from a project on mast cells in diabetes (9). In an early quantitative study published in *Uppsala Journal of Medical Sciences* it was shown that islet amyloid is initiated in a few islets but as the degree of amyloid infiltration augments the number of affected islets increases as well (10), as confirmed in a later study (11). Another important finding, obtained by serial sectioning, was that deposits usually are spread throughout islets, particularly along capillaries, even in cases with comparably little amyloid (10). These novel and quite intriguing findings fit very well with the present conception of formation of amyloid as a nucleation-dependent process (12) and that amyloid actually can spread between islets in this way (13).

IAPP and the precipitation of islet amyloid

Gellerstedt, like most other researchers at that time, thought that amyloid hardly could be a cause of diabetes since it occurs in non-diabetic subjects as well and there are always a number of unaffected or less affected islets left in diabetic patients (1). At that time the nature of amyloid was only a matter of speculation, and its origin from blood vessels or fibroblasts was much discussed. Later electron microscopic studies showed a close relationship between islet amyloid and β -cells (14). This finding was important in two respects. First, it indicated that islet amyloid is a

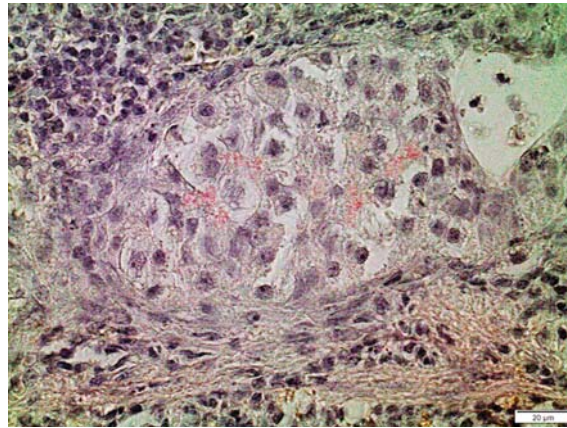


Figure 6. Human islet from a non-diabetic subject transplanted into the spleen of a nude mouse. Pronounced amyloid deposits had developed within the islet after 1 month. Congo red.

product of β -cells. Second, it showed that even if deposits were slight, they were widely spread within the islet and resulting in virtual holes in β -cell membranes, something that should result in functional loss. However, not much happened on islet amyloid until its composition of a previously unknown regulatory peptide was untangled (15). This peptide, named islet amyloid polypeptide (IAPP, or amylin) was shown to be a 37-amino-acid residue peptide, most closely related to calcitonin-gene related peptide (CGRP). It was demonstrated to be expressed by β -cells where it is stored and released together with insulin (reviewed in (16)). Initially, IAPP was believed to cause insulin resistance in type 2 diabetes, but when it turned out that a superphysiological plasma concentration is needed to initiate such an effect the interest in function of IAPP declined somewhat.

However, the interest in IAPP has increased considerably again, but now as an amyloid-forming peptide rather than for its effects via receptors. Deposition of IAPP amyloid is associated with a decline in the number of β -cells in human beings (17,18), even in the absence of diabetes (17). Presence of islet amyloid is associated with β -cell apoptosis, and no apoptotic cells are detected in non-amyloid containing islets (11). Why islet amyloid cytotoxicity is limited to β -cells and α -cells are spared (19) may suggest differences in cell surface-associated molecules. The mechanism is unclear, however. Like the situation with Alzheimer's disease and the $A\beta$ peptide, the interest has moved from the mature amyloid fibrils to pre-fibrillar aggregation states, often named oligomers or protofibrils. Such aggregates of misfolded peptides, including $A\beta$ and IAPP, have been shown to exert toxic effects on cells in many systems *in vitro*. Therefore, there is strong evidence that a major cause of the reduction of and impaired function of β -cells in type 2 diabetes is aggregation of IAPP in the islets of Langerhans.

Amyloidosis in transplanted islets

Studies based on transplantation of pancreatic endocrine tissue into nude mice—diabetic or non-diabetic—were performed in one of our laboratories in the 1970s. Thus, for instance isolated human islets were injected intraperitoneally (20), and porcine islet-like cell clusters (21) or human fetal pancreas (22) were grafted under the renal capsule. When isolated human islets became more regularly available, thanks to the collaboration with the laboratory in Brussels, we carried out extensive studies on the long-term *in vivo* effects of high glucose concentrations (23) and alloxan (24) on human islet tissue. It was then logical to look for the possible development of amyloid deposits in the same type of grafted tissue. To some surprise such deposits were demonstrated even in islet grafts from young, non-diabetic donors (25). These first studies were carried out on renal, subcapsular islet grafts, but extended studies revealed that islet amyloid developed in intrasplenic (Figure 6) and liver grafts as well (26). We were also able to demonstrate the deposition of amyloid in transplanted islets prepared from transgenic mice expressing human IAPP (27).

Against this strong evidence of an exaggerated development of amyloid in transplanted human islets it was with tense expectations that we undertook the first investigations of liver sections from a deceased diabetic patient grafted intraportally with isolated islets about 5 years prior to his death (28). Quite a few islets were identified, and interestingly enough many of them (40%) contained IAPP amyloid deposits as assessed by means of Congo red

staining and immunolabelling with specific antibodies. In a follow-up study there was further evidence for amyloid deposition in clinical pancreatic islet grafts in that one patient from Edmonton and one patient from Milan also had such deposits in their islet grafts (29). Since there is a decline in function in such islets (30) it is tempting to believe that islet amyloid formation is an important cause (16,29,31).

Our findings of amyloid in human islets, both grafted and cultured specimens, have been repeatedly reproduced. Perhaps the most informative one of these reports is that of Potter et al. (32) demonstrating that while human islet grafts suffer from the deposition of amyloid after transplantation into nude mice, porcine islets do not. The explanation for this is that porcine IAPP lacks the ability to form amyloid due to its amino-acid sequence. Therefore porcine islets might be useful for clinical islet transplantation.

Islet amyloidosis and Alzheimer's disease—is there a link?

In this context it may be relevant with a reflection. Alzheimer's disease is an age-related condition which is associated with deposition of amyloid as plaques in the cerebral cortex and in cerebral blood vessels. These pathological traits have been known for a long time, but the importance of the alterations has not been well understood. Alzheimer research was for a long time very much focused on disturbances in cerebral transmitters, and not much happened in the field. Thus, when Glenner (33,34) and Beyreuther and Masters (35) discovered the A β peptide it meant a sudden, unexpected, and completely new direction in Alzheimer research. This field completely exploded and almost everyone moved to A β research. Why did the same not happen with islet amyloid and IAPP? One may suspect that researchers in the diabetes field have had difficulties to liberate themselves from old ideas and to accept new ones, and this is probably true generally in science. Another likely possibility is that for a long time type 2 diabetes was regarded as a disease dependent on peripheral insulin insensitivity with no direct β -cell lesion other than that these cells 'burnt out' due to constant stress exerted by high plasma glucose concentrations. In a way this may not be completely wrong since hyperglycemia is believed to drive IAPP overexpression, impaired processing of proIAPP, and dissociation from parallel expression with insulin (reviewed in (16)). However, slowly, IAPP amyloid or pre-amyloid assemblies are increasingly accepted as important players in the pathogenesis of type 2 diabetes.

Interestingly, several epidemiological studies support a link between type 2 diabetes and Alzheimer's disease, two conditions that have local amyloid formation in common as a component in their respective pathogenesis. Comparison of A β and IAPP amino-acid sequences point to a 50% identity which is slightly higher in regions identified as important for amyloid formation (36). In *in vitro* studies on amyloid seeding, IAPP and A β can cross-seed (36) and injections of preformed A β -fibrils to human IAPP transgenic mice trigger islet amyloid formation (13). Further studies on brain tissue from patients with Alzheimer's disease with the antibody-based proximity ligation assay revealed co-localization of IAPP and A β in both diffuse and dense plaques and in amyloid deposits present in cerebral vessel walls. Therefore, it is possible that cross-seeding could be a mechanism that links the two diseases.

Our increasing understanding of the importance of IAPP aggregation in the pathogenesis of type 2 diabetes as well as the development in the Alzheimer field underlines that Gellerstedt was right when he in 1938 wrote 'The problem "senile amyloidosis" contains many interesting questions, studies of which perhaps may open new ways in research' (1).

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