



Chromaffin Cells in the Mammalian Adrenomedullary Tissue: Ultrastructural Aspects of Stimulus-Secretion Coupling – A Tribute to Odile Grynszpan-Winograd (1938–2023)

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1 ***Obituary***

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3 ***Chromaffin cells in the mammalian adrenomedullary tissue:***
4 ***ultrastructural aspects of stimulus-secretion coupling.***

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6 ***A tribute to Odile Grynszpan-Winograd (1938-2023)***

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Abstract

Odile Grynszpan-Winograd (1938-2023) was a French ultrastructural morphologist who worked in Paris in the second half of the twentieth century. She devoted her scientific life to the study of the adrenal gland anatomy. Through her countless electron microscopy images, in which she was undeniably an expert, Odile Grynszpan-Winograd pioneered the visualization of the liberation of chromaffin secretory granule content, providing an unequivocal anatomical demonstration of the exocytosis process. This seminal finding constituted a major advance in deciphering the stimulus-secretion coupling of the adrenal medullary tissue. By combining transmission electron microscopy with cryofracture, Odile Grynszpan-Winograd was also the first to illustrate gap junction plaques between chromaffin cells. However Odile Grynszpan-Winograd did not receive the entire benefit of her contributions, particularly with regard to the vesicular secretion of catecholamines, a subject that has been in debate for many years.



Fig. 1: Odile Grynszpan-Winograd, 1986

Odile Winograd was born in 1938 in Paris, France. She married in 1958 and obtained the same year her degree in Natural Sciences (Licence ès Sciences Naturelles). Henceforth Odile Diner, she was immediately appointed Lecturer and offered tenure as a Researcher at the Faculty of Sciences at the Sorbonne in Paris where she taught biology and cytology to both undergraduate and graduate students, until her retirement. At the same time, she joined the Laboratory of Cytology at Pierre and

Marie Curie University, headed by René Couteaux, a well-recognized 'ultrastructuralist'. As a doctoral fellow, Odile Diner became interested in the development of neural tissue, such as the sciatic nerve and the adrenal medulla. It was at the Electron Microscopy Centre of the Salpêtrière Hospital in Paris that Odile Diner obtained her first micrographs. Benefiting from René Couteaux's expertise in the field of nerve histocytology, and in particular of the neuromuscular junction (sharing many common features with the splanchnic nerve-chromaffin cell synapse), Odile Diner combined transmission electron microscopy (TEM) approaches and histological staining techniques to decipher the development of the adrenal medullary tissue, first in rats and then in hamsters, mice and cats.

Odile Diner's work pertaining to the innervation of chromaffin cells in the rat adrenomedullary tissue was first briefly described in 1961 in a review article by René Couteaux on the various types of synapses [1]. The meticulous observation of more than thousand micrographs led Odile Diner to publish several observations in the field of neuroendocrinology, providing new data on the differential innervation of adrenergic and noradrenergic chromaffin cells. Her work was regularly reported in the *Comptes-Rendus hebdomadaires des séances de l'Académie des Sciences* [2-5].

After a divorce in 1965, she remarried in 1969 (she met her husband at the Sorbonne during the turmoil of the 'academic and student revolution of May 1968'), later giving birth to three children, two girls and a boy. From then on, she authored her articles under the name Odile Grynszpan-Winograd. In 1972, under the supervision of René Couteaux, she defended a State Doctorate thesis in Natural Sciences¹, entitled 'Morphological study of the adrenal medulla of the hamster: innervation, vascularization, release of secretory granules'.

From the onset of her doctoral research, Odile Diner's work was spiced up by a major challenge: tackling the 'vesicular secretion' hypothesis and identifying the sites of hormone liberation. During that period, many TEM observations were published, on the ultrastructure of hormone-storing cells such as mast cells, adrenal cells, pancreatic cells, and many others. Although the concept of exocytosis had emerged from biochemical and cell biology experiments, morphologists were still looking for a direct proof of the secretory mechanism. This issue has been the subject of much debate, both within Couteaux's team and abroad. For several years, Odile Diner searched for the presence of exocytosis sites in rat adrenomedullary tissue, but failed. She then turned her attention to another mammal, the golden hamster, already studied in other laboratories, and she succeeded in visualizing omega-shaped sites of 'exocytosis', or rather 'reverse micropinocytosis'. Indeed, this was how Odile Diner referred to her observations, which she presented at the joint meeting of the French and Belgian Electron Microscopy Colloquium in May 1967, and in an abstract published the same year in the *Journal*

¹ The former French State Doctorate encompassed both the current PhD degree and the accreditation to supervise research.

84 *de Microscopie* [6]. Her results on the expulsion of granules from the adrenal medulla were published
85 as a full paper in the *Comptes Rendus Hebdomadaires des séances de l'Académie des Sciences* in August
86 1967 [7]. Although written in French, this article had a resounding impact on the field². Actually, it had
87 barely come off the press when William W. Douglas, a British endocrinologist world-renowned for his
88 seminal work on the stimulus-secretion coupling of chromaffin cells at Yale University, in a plenary
89 lecture held at the University of Cambridge in September 1967 referred to the work as one of the
90 compelling evidence that catecholamine secretion from medullary chromaffin cells occurs by
91 exocytosis [8].

92 In 1969, Herman Blaschko, the father of catecholamine metabolism, invited Odile Grynszpan-
93 Winograd to present a seminar in his laboratory at the University of Oxford. He suggested that she
94 consider a sabbatical year in a laboratory specializing in the study of endocrine glands. At the same
95 time, other institutions in Europe and the United States approached Odile Grynszpan-Winograd to
96 offer her such an opportunity. She declined the offers, choosing to continue her research and teaching
97 in Paris and to take care of her family.

98 To understand the importance of this pioneering work, we need to go back to the state of
99 knowledge when Odile Diner was looking into the organization of the adrenal gland innervation and
100 secretion. Let's take a brief look at the history of exocytosis in adrenal chromaffin cells. Until the mid
101 60's, the release of packed granule content by the exocytosis process was still poorly understood and
102 even reluctantly accepted, particularly in chromaffin cells. The idea, published a few years earlier, that
103 the membrane of a chromaffin granule can fuse with the plasma membrane in response to cell
104 stimulation, had emerged from the pioneering anatomical work of De Robertis and Sabatini [9],
105 Coupland [10] and Douglas and Poisner [11], but still stood as a hypothesis. In the early fifties, many
106 laboratories in the neurochemistry field had focused their research on the elucidation of hormone and
107 neurohormone source and properties. It was in 1953 that these molecules, present in adrenal
108 chromaffin cells, were found to be exclusively localized in subcellular membrane-limited particles.
109 These molecules, namely adrenaline and noradrenaline, described as the stress hormones (*i.e.*
110 mediators of the so-called 'fight or flight' response), are released massively from the adrenal gland
111 upon stimulation via the splanchnic nerve and recovered into the blood stream to exert their activity
112 on multiple target organs, including lung, muscles, heart, etc... The name 'chromaffin granules' was
113 used for the first time in 1957 by the group headed by Hermann Blaschko in the Department of
114 Pharmacology at the University of Oxford. Later on in 1961, in the same laboratory, the use of
115 radioactive phosphorous allowed the localization of ATP together with adrenaline and noradrenaline

² At that period, the '*Comptes Rendus Hebdomadaires des séances de l'Académie des Sciences*' were indexed in the *Current Contents*, the reference database for searching bibliographic content.

stored in these subcellular organelles, which could be isolated from the adrenal tissue by subcellular fractionation, a new technique at that time, implemented by high-speed centrifugation. The localization of adrenaline and noradrenaline in subcellular membrane-limited organelles questioned how, upon splanchnic nerve stimulation, they could be released into the bloodstream. In 1965, Peter Banks (University of Sheffield, UK) and Karen Helle (University of Bergen, Norway), working in Blaschko's laboratory, reported in *Biochemical Journal* that, upon stimulation of the adrenal gland, a granule protein is present together with catecholamines amongst the released material [12]. In parallel, Karen Helle developed a specific antibody against this chromaffin granule protein that was named chromogranin and, with the use of this antibody, she could demonstrate in 1966 [13] that the stimulation of chromaffin cells is followed by the release of chromogranin-immunoreactive protein, substantiating the original observation. To complete this crucial finding, on the following year, in a letter to *Nature*, the chromogranin protein was found to be secreted upon direct stimulation of the splanchnic nerve that innervates the adrenal glands [14]. The only suitable explanation for these observations was that the liberation of catecholamines requires the simultaneous opening of the granule membrane and the cell membrane towards the extracellular space. The interaction of the secretory granule with the cell membrane results in the fusion of the storage granule membrane with the cell membrane and the fusion produces the aperture of a pore through which the whole granule content, including catecholamines, proteins and smaller molecules such as ATP, ascorbic acid and others, is liberated into the extracellular space and then recovered in the blood stream. This process defines the exocytosis. The mention of the word 'exocytosis' in the title of a standing publication occurred for the first time in 1967 in an article published by Blaschko's group [15]. Interestingly, when they submitted their papers, late April 1967, neither Blaschko et al. [14] nor Schneider et al. [15] were aware that Odile Diner had already submitted an abstract a few months before her participation in the Brussels colloquium [6], mentioning the expulsion of isolated granules, and substantiating by direct morphological observations the forthcoming biochemical conclusion of the Oxford group.

In 1970, Herman Blaschko and Anthony David Smith invited several prominent senior scientists to participate in 'A Discussion on subcellular and macromolecular aspects of synaptic transmission'. Odile Grynszpan-Winograd, although still a young scientist, was also invited to this Symposium held in London, and contributed to the Proceedings published the following year in the *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* [16]. This article, which reports her key observations, became her most notable contribution. Indeed, it has been recognized among the 'adrenal medulla community' that Odile Grynszpan-Winograd (ex-Diner) provided 'the first convincing morphological evidence that exocytosis is responsible for secretion from chromaffin cells' [17], giving a substantial support to the exocytosis concept derived from biochemistry experiments.

She indeed reported a series of landmark TEM images showing secretory granules apposed to and fusing with the plasma membrane, in hamster adrenal chromaffin cells (shown in Fig. 2). A couple of years later in 1974, Hermann Blaschko and Anthony David Smith invited Odile Grynspan-Winograd to contribute to a chapter in the *Handbook of Physiology*, Endocrinology section [18].

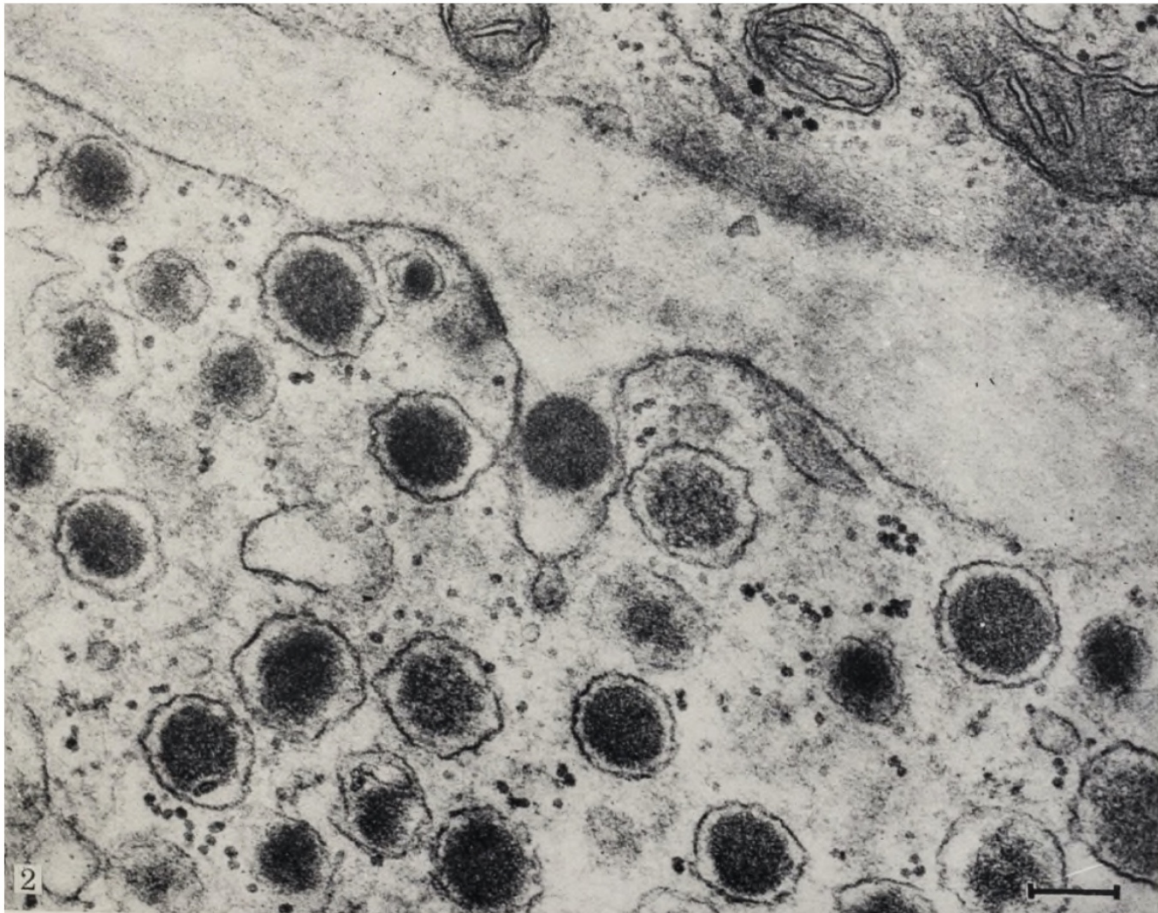


Fig. 2: Representative exocytosis figure in a chromaffin cell. Example of a granule-containing membrane invagination in an adrenaline-secreting cell (from [16], with permission).

The ultrastructural observations of Odile Grynspan-Winograd paved then the way to many other works using later more sophisticated ultrastructural techniques, *i.e.* freeze-fracture cytology [19], immunogold staining [20], or confocal microscopy, all of them confirming the liberation by exocytosis of catecholamines but also other hormones and neurotransmitters from various tissues. We now know that exocytosis is the mechanism by which hormones, neurohormones and neurotransmitters are released upon stimulation of endocrine/neuroendocrine cells, nerve endings and neurons. It is a concept well established and fully recognized although the intimate mechanism of

fusion between the two membranes, granule membrane and cell membrane, waits to be resolved.

However, in her professional environment, Odile Grynszpan-Winograd had to face some skepticism, owing to a group of neurochemists who did not accept the 'vesicular secretion'. These scientists believed in a non-vesicular release and put all their efforts in attempting to demonstrate that there was a controlled diffusion of neurochemical molecules through specific pores, simultaneously occurring on both the granule (or vesicle) membrane and the cell membrane. A specific protein element was then believed to play such a role and was named the 'mediatophore' or 'vesigate' [21]. This hypothesis however has been definitively rejected at the beginning of the 90s when the mediatophore was found to be related to a transporter protein, close to the H⁺-ATPase involved in the translocation of catecholamines from the cell cytoplasm towards the inside of chromaffin granule [22]. Nonetheless, this skepticism has poisoned the neurochemistry community, and particularly the French one. In this conflictual atmosphere, we think that Odile Grynszpan-Winograd did not receive the entire benefit from her observations that she would have deserved. Nonetheless, her papers from the 1970s are still cited today to illustrate the secretion of endocrine glands. Her article published in the *Philosophical Transactions of the Royal Society of London, Series B, Biological Sciences* [16] has been cited around a hundred times. Her contribution to the exocytosis concept was really a brick into the wall.

In the 1980s, Odile Grynszpan-Winograd also experimented with cryofracture (a microscopy technique which had become 'fashionable'), but she quickly realized that it was only useful if observations were performed in parallel with conventional TEM. The combination of these two microscopy techniques led to another major contribution by Odile Grynszpan-Winograd to the understanding of stimulus-secretion coupling in the adrenal medulla, through her work describing the intercellular junctions between chromaffin cells (focal tight junctions and gap junctions), in several animal species [23]. At the time of publication in 1980, this work received less attention than that describing exocytosis sites, but it was nonetheless the first to illustrate gap junction plaques between chromaffin cells, using the freeze-fracture technique (shown in Fig. 3). Today, this pioneer observation remains the morphological substrate for more recent work demonstrating the physiological role played by gap junctions in adrenal catecholamine secretion, *ex vivo* in rat adrenal slices [24], and *in vivo* in anaesthetized rodents [25].

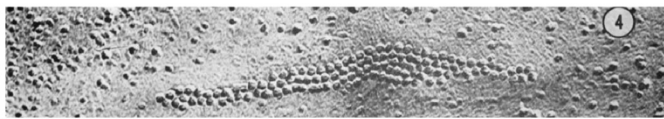
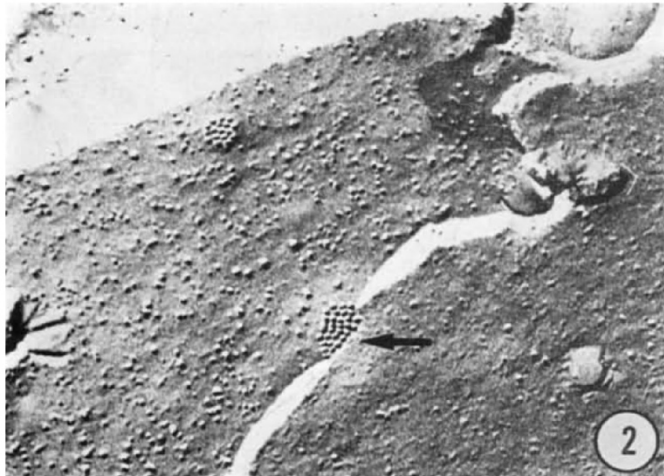
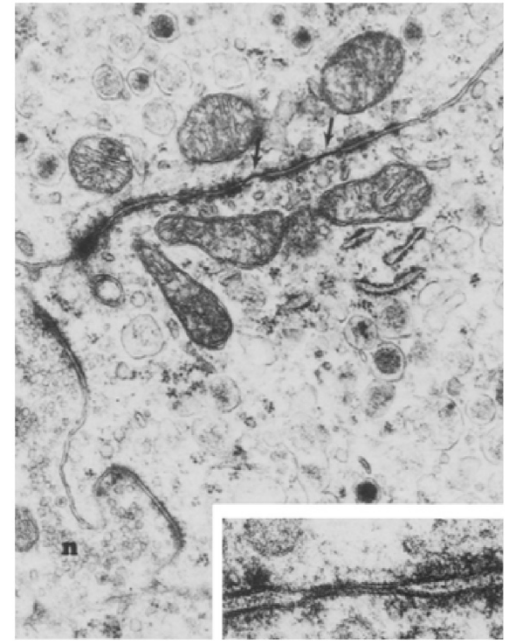
A**B**

Fig. 3: Electron microscopy pictures illustrating gap junction plaques between chromaffin cells. A. Freeze-fractured specimens illustrating gap junction plaques in hamster chromaffin cells (from [23], with permission). Most gap junctions are organized in small clusters (upper panel). Large gap junction clusters (lower panel) are less frequently observed. **B.** Electron microscopy picture of a junctional complex between two adjacent adrenaline-containing chromaffin cells in the mouse (from [26], with permission).

Although electron microscopy, the cutting-edge technique commonly used in biology in the 1950s-1980s, has gradually lost ground to other histological approaches, the experimental data generated by this technique are still the benchmark in various fields of biology today. The spatial resolution achieved by this cell/tissue microscopy remains a major advantage. In the case of adrenal chromaffin cells, Odile Grynszpan-Winograd's micrographs will continue to set the standard in the field.



Fig. 4: Odile Grynspan-Winograd at a banquet in honor of René Couteaux. From left to right: C. Bouchaud, R. Couteaux, J. Koenig, S. Tsuji, M. Israel, J. Gautron, J. Auber, ?, A. Barets, Odile Grynspan-Winograd, J. Taxi.

We are pleased to offer this tribute to Odile Grynspan-Winograd's achievements in the field of the histology/morphology of the adrenomedullary tissue. Her observations added a major brick in the wall of knowledge.

Dr. Odile Grynspan-Winograd passed away on September 7th, 2023, in Paris.

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