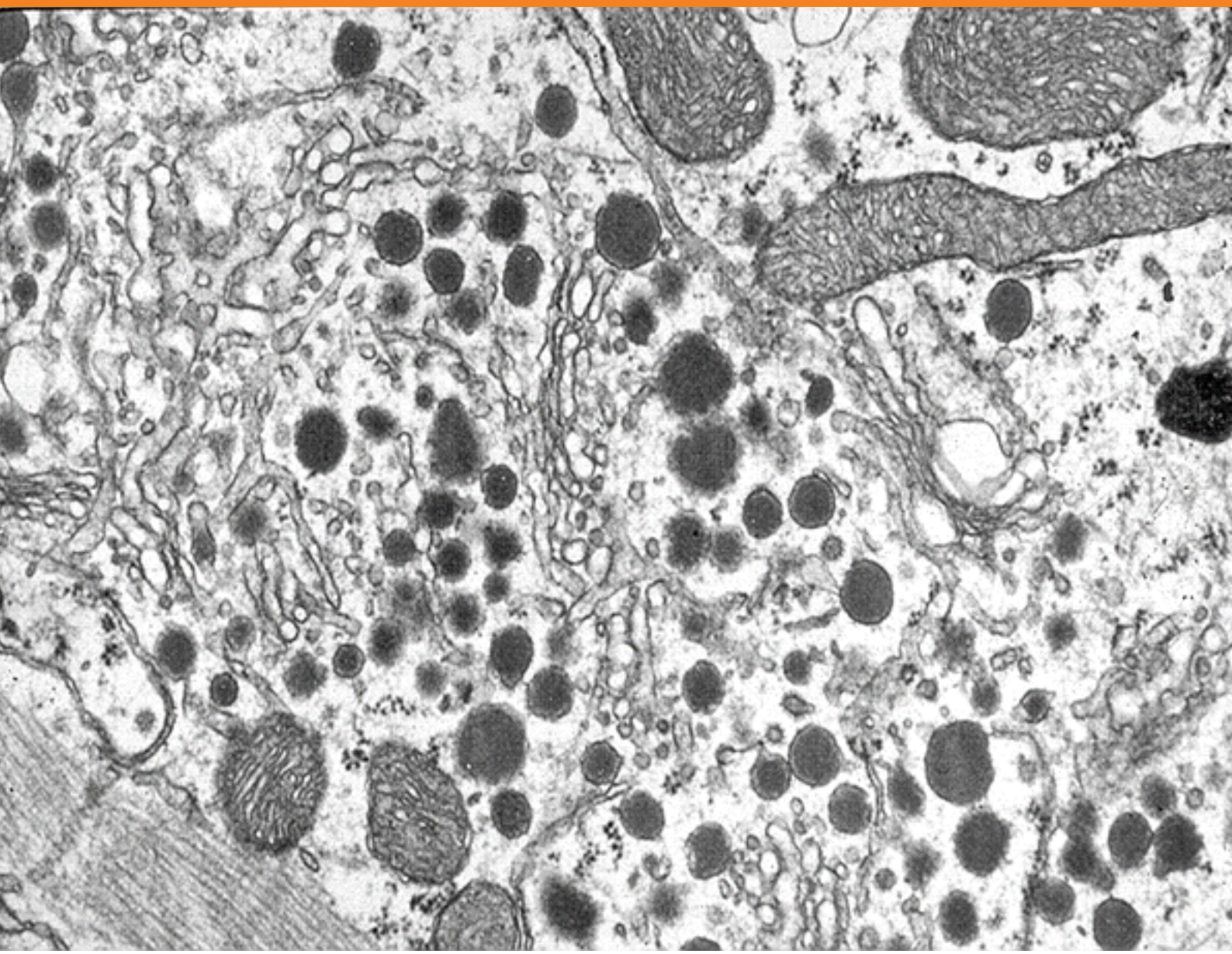
SPECIAL EDITION

TIMELINE OF PHYSIOLOGICAL DISCOVERIES

Milestones that paved the way to today's knowledge

Physiological Mini Reviews

1st NUMBER



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Physiological Mini Reviews

SAFIS
Sociedad Argentina de Fisiología

Dear Friends:

As already announced in our general Assembly and in the first issue of this volume, this year we are opening a new special session describing the way by which different researchers achieved outstanding discoveries in Physiology. Initially we thought to describe Latin American achievements. We realized now that this will artificially restrain our main intention which is to know the different journeys followed by scientists in the accomplishment of a discovery. We decided therefore to extend these descriptions to physiological milestones attained all over the world with the expectation of further growing and deepening our knowledge of physiological sciences and scientists.

The first issue of this special session is a delightful description of the discovery of the endocrine side of the heart made by its own discoverer, Dr. Adolfo De Bold.

We hope that this session contributes to inspire and bring enthusiasm to our scientific community, in particular to the new generation of physiologists.

The Editorial Committee

Dr. Adolfo J. de Bold

Adolfo J. de Bold, OC, FRSC (born 14 February 1942) is an Argentinian–Canadian cardiovascular researcher, best known for his discovery of atrial natriuretic peptide (ANP), a polypeptide hormone secreted by heart muscle cells. He was born in Paraná, Argentina, and obtained a BSc in clinical biochemistry at the National University of Córdoba. His MSc (1972) and PhD (1973) are from the Department of Pathology at Queen's University in Kingston. De Bold has received many awards for his work on ANP, including the Gairdner Foundation International Award (1986), Manning Innovation Awards Principal Award (1986), Royal Society of Canada McLaughlin Medal in Medical Research (1988), International Society for Hypertension Research Award (1990), CIBA Award of the American Heart Association (1994) and the American Society for Hypertension Research Award. He is a fellow of the Royal Society of Canada and of the American Association for the Advancement of Science. In 1992, he was appointed an Officer of the Order of Canada. In 2014, he was inducted into the Canadian Medical Hall of Fame. He received the Gran Prix Scientifique Lefoulon-Delalande, Institute de France, 2014.

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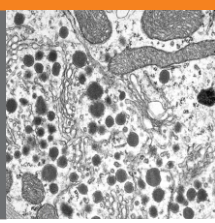

THE DISCOVERY OF THE ENDOCRINE HEART

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THE DISCOVERY OF THE ENDOCRINE HEART

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To travel down the memory lane with reference to the discovery of the atrial natriuretic factor (ANF) and hence of the endocrine function of the heart is a slippery slope. It is so because it takes me down to my very young years during which, somehow, I had decided to be a research scientist. And now I realize that this had nothing to do with my education, family influences or role models. It was just that: one wants to be a scientist since very early in life and nobody, including you, knows exactly why. Call it a vocation.

With that emotional package in hand I parted after completing my secondary education from my natal city of Parana, Entre Rios to Rosario, Santa Fe to study to become a clinical biochemist. Why biochemistry and not medicine? Because I somehow knew that biochemistry would provide a biomedical scientist with more basic tools that medicine would. How did I know that? I don't know. And here comes the part where the Providence really starts to show its hand.

A pathologist by the name of Oscar Sudilovsky (later of Case Western Reserve University at Cleveland, Ohio) invited me (don't know why) to his laboratory during the preparatory course that we had to take before entrance to the clinical biochemistry career itself. It was in that laboratory that I learned techniques to prepare tissues for microscopy; the same techniques that I would apply many years later in my studies on the heart. A student revolt in Rosario led to a strike that lasted enough to make us loose an academic year. That was the reason why I transferred to the Faculty (then Institute) of Chemical Sciences of the National University of Córdoba, where I caught up with my studies. The academic syllabus of the career of clinical biochemistry at the University of Córdoba was truly impressive. It included several chemistry courses, mathematics, physics, physical chemistries and others. In fact, the curriculum was more appropriate to train scientists than it was to train clinical biochemists. During the last two years of the career I became involved with another pathology department. The Department of Pathology at the Hospital Clínicas in Córdoba was headed by José Mosquera; a forward-looking pathologist that, among his innovations for the Department of Pathology, brought in electron microscopy and teaching assistants such as myself who contributed the biochemical expertise that the department was lacking. It was there that I developed my own little research projects and got notions of other techniques, including electron microscopy. There were two important outcomes arising from these activities. The first one was the rather sad conclusion that there were no significant prospects of learning how to publish at the international level in that environment and, secondly, I met who was going to be (and still is) my wife: Mercedes Kuroski. With her, we have spent almost 55 years of work sharing research laboratories. Nevertheless, these self-taught activities whereby I learned histological and histochemical technique in Córdoba would later be fundamental in my work leading to the discovery the endocrine function of the heart. In addition, I met Dr. Juan Lechago - a resident at the time - who emigrated to Cánada and would serve as nexus between myself and the Department of Pathology at Queen's University in Kingston, Ontario. In Cánada both my wife and I got into graduate programs in Experimental Pathology and eventually obtained our respective MSc and PhD degrees.

My theme for graduate studies was supposed to be on functional aspects of the pancreatic beta cell but I was attracted to the morphology of the atrial muscle cells of mammals that displayed a phenotype that combined the expected features of a cardiac muscle cell with that of a secretory cell. The latter included abundant storage granules known as specific atrial granules (SAG), a highly developed Golgi complex and abundant profiles of rough endoplasmic reticulum. The predominant hypothesis about the role of these granules - sustained by some published data – was that they were a storage site for catecholamines. This seemed to be the ideal theme to investigate and realize my dream of combing biochemistry with morphology. At this point in my “laboratory life” all that I had learned in Rosario and Córdoba either by myself or through courses, came very handy. I set out to develop a technique to

isolate the SAG in a preparative scale using differential and density gradient centrifugations. The idea was to measure catecholamines in the isolated granules and thus confirming or denying the catecholamine storage function hypothesis for these organelles. There were two logistic barriers in this endeavour. One was that, since there was no knowledge as to the content for the SAG that could be used as a marker of the behaviour of the granules during isolation, the only way to follow the behaviour of the granules during the several trials of isolation (each consuming the atria of 60 rats sacrificed by decapitation), one could only use electron microscopy to follow them. The easier way would have been to use light microscopy to identify the SAG in the different fractions and sub-fractions, but no one had ever developed a light microscopy staining technique for the granules. The procedure was tedious, slow and expensive. I developed a quick method to process these fractions that took about 45 min as opposed to the 24-48 h that was necessary under conventional processing for electron microscopy. Finally, an ultracentrifugation technique was arrived at using a discontinuous sucrose gradient of a post mitochondrial fraction that yielded a purified SAG fraction. Analysis of the purified SAG fraction so obtained revealed that it did not contain significant amounts of either epinephrine or norepinephrine [1]. The good news then was that we had succeeded in isolating and purifying SAG but now we had no hypothesis to work on these granules. Very little work had been done on the SAG using histochemical techniques and thus very little information was available regarding their chemical make up. Whatever was known was derived from observations at the electron microscopic level, which suffers from a paucity of cytochemical techniques. The histochemical techniques learned in part in Argentina came very handy to investigate this aspect of the atrial granules. Several histochemical techniques, this time at the light microscopic level were applied and from what we gathered we learned that the granules contained a basic polypeptide with a random coil conformation and with a significant a mount of sulphur-containing amino acids and tryptophan [2-4]. Autoradiographic studies suggested that the SAG had a high turnover as would be expected for most endocrine granules [3]. Suggested by my wife, we also found a stain for the granules that allowed for the first specific demonstration of the granules at the light microscopic level [5, 6]. Such demonstration was used to develop the first unbiased morphometric method to determine the degree of granulation in atrial cells and the effect of different experimental manoeuvres in the rat to see if such procedures affected the number of granules [6]. From that work it became obvious that some experimental procedures that affected water and electrolyte balance in rats affected the degree of granulation of the atrial cells [7]. Hence the hypothesis that the SAG contained un polypeptide involved in water and electrolyte balance. To test this hypothesis, we prepared atrial tissue homogenates in our laboratory and shipped them to Dr. Harald Sonnenberg laboratory in Toronto who had a rat preparation to test for substances that modified renal function. The injection of the atrial extracts into such preparation was of such potent natriuretic/diuretic response that at first, we disbelieved the results. Altogether, the work showed that the atrial extract contained a factor (Atrial Natriuretic Factor, ANF) that can induce a powerful natriuresis and hence diuresis, did no affect renal potassium excretion and lowered blood pressure [8].

Because of the properties of ANF were of obvious importance for hypertension and chronic congestive heart failure research, publication of these results in 1981 unleashed a huge amount of work in dozens of laboratories around the world. Our laboratory was comparatively small, but Providence once again intervened. I found myself in charge of an HPLC that was purchased at that time to make blood dosages of theophylline. This machine I coopted at nights for peptide isolation, allowing our laboratory to be the first to isolate to chemical homogeneity a peptide with the properties of ANF. Providence intervened once more when the government of Ontario instituted a program to encourage research and discovery. Through this program we purchased the first gas-phase sequencer ever to come to Cánada and with this machine we were the first to publish the peptide sequence of ANF (now referred to as ANP) in the year 1983, ahead of everybody including the fiercely competitive Japanese laboratories who published sequence data a few months later in 1984 [1, 9-12].

Chemical synthesis of ANP allowed for the development of radioimmunoassays and the factors that affected its secretion from the atria as well as very many investigations on the role of this hormone in homeostasis thus expanding the role of ANP to aspects beyond the maintenance of water and electrolyte balance. The discovery of the natriuretic peptide brain natriuretic peptide or BNP [13] led to the mistaken view that the source of this hormone in mammals was the cardiac ventricles. BNP in fact, is produced in the atria together with ANP. The measurement of BNP and ANP in different pathologies

that affect water and electrolyte balance as well as blood volume, open the door to the utilization of ANP and BNP blood levels as biomarkers, particularly useful to diagnose heart failure. It is now a required measurement in the work up of such diagnosis in many countries.
Studies on the metabolic fate of circulating ANP and BNP led to pharmaceutical searches to find a pharmacological approach that would decrease their catabolism and thus to an increase in the circulating levels of these peptides and so take advantage of their beneficial effects. Novartis developed a new medication that is a mixture of valsartan, an angiotensin receptor blocker, and sacubitril which is a neprilysin inhibitor that inhibits catabolic clearance of ANP and BNP.
The cardiac natriuretic peptide discovery is as good a history as any showing the value of basic research leading to new concepts in physiology [14] and to applications in medicine [15].

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Electronic microscopy of a rat atrial cardiomyocyte. Atrial specific granules can be observed as high density spheroids. These organelles contain ANP and, in a lesser extent, BNP. They are surrounded by numerous elements from the Golgi apparatus and mitochondria. At the lower left corner of the picture, a sarcomere portion denotes the muscular nature of the cell.

Courtesy of Dr. De Bold