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I welcome this opportunity to address the opening session of this Congress. It is a pleasure to greet you in the name of the Catholic University of Chile, and to wish you all that you may enjoy an interesting meeting and a pleasant stay in this country.

I am grateful and honored by the invitation to speak on Fernando De Castro on this year of the centennial of his birth. It is fitting that he should be now remembered. It was him who about seventy years ago, made a major advance in the knowledge of chemoreceptors by discovering that the glomus caroticum should be counted among them. I myself have a large debt of gratitude toward De Castro who initiated me in research work as a histologist, and I feel thankful to have the occasion of acknowledging this debt and paying tribute to his memory.

The story of De Castro's discovery has been told a number of times. A short time after becoming an assistant of Santiago Ramón y Cajal, he began studies on what was called at the time the intercarotideal gland, an organ which was commonly believed to be comparable to the adrenal medulla. De Castro had become at that time interested in the structure of the sympathetic nervous system and related structures. I suspect that behind this choice lay the desire to find some field of neurohistology which might have been left comparatively unexplored by Cajal. De Castro revered his master, but as will happen with creative disciples he wanted his own place in the sun. And this did not seem easy to achieve. Cajal's contribution had been incredibly extensive and varied,

and the weight of his authority was immense. On the other hand, his neuron theory was being seriously challenged in the autonomic nervous system. Complex networks of exceedingly fine fibers are found here, which were rendered visible mainly by silver impregnation techniques well-known to be fraught with artifacts. These fields of uncertain structure and difficult study were of course paradise for a legion of fanciful anatomists inclined to see things to the very limit of the visible, and then far beyond. In this case the distinction was often not made between preganglionic and postganglionic processes, and they were all interpreted as parts of a web of anastomosing cytoplasmic outgrowths. The SLIDE is taken from one of De Castro's early papers and shows the entanglement of dendrites of two sympathetic neurons with a very fine fiber correctly identified as preganglionic.

De Castro's work soon showed that the intercarotideal gland differed from the adrenal medulla. He found that the granules inside the cells did not stain in the same way as in the adrenal, and he discarded the idea that the glomus was a paraganglion. His histochemistry was quite primitive, and he was probably wrong when he dismissed any kinship between glomus caroticum and adrenal medulla, but in a sense this was a lucky mistake, because it raised the obvious question of what the main role of this small gland might be.

De Castro was struck by the unusual fact that this seemingly glandular tissue was very richly innervated, both in the parenchymal cells proper and in the blood vessels. The SLIDES taken from his work show abundant fine axons and nerve endings together with bundles of myelinated fibers. Serial sections by the thousands made on cranial structures of mice which had been block-stained by notoriously capricious silver impregnation techniques, resulted in images beautifully reproduced in the drawings made by De Castro himself. SLIDE. Here the intercarotideal nerve, a branch of the glossopharyngeal is shown to provide the major part of the fibers to the glomus. It is apparent that De Castro had availed himself of a trick introduced by Cajal. He chose very young small animals even foetuses which afforded a chance of getting a view of a large territory in a single adequately oriented section.

The myelinated bundles constituted a puzzle. De Castro appears to have thought at first that the glossopharyngeal innervation of the gland did not pass through an extracranial synaptic relay, and was consequently an exception in the organization of the autonomic nervous system. On second thoughts however he tested the hypothesis that these branching myelinated fibers might be sensory in nature, with the corresponding cell bodies located at the petrosal ganglion. In successive papers, De Castro showed that the nervous endings in the gland were not affected by extirpation of the sympathetic chain, that they were completely wiped out by extra cranial section of the glossopharyngeal, and that they did not suffer after intracranial section of the nerve. Thus the whole of the evidence pointed toward their being sensory fibers, and toward the glomus being a receptor, probably connected to reflex arcs involved in circulatory regulation. De Castro saw also that the endings were entirely different from the pressoreceptors in the arterial wall. Based on anatomical considerations he attributed to them a role in the detection of qualitative changes in the blood. On the whole, this was a brilliant achievement.

The success of this work rested upon a judicious use of the degeneration undergone by nerves after experimental sectioning. It is easy from our perspective to think that this was mainly an achievement of surgical skill. But one should remember that some very influential anatomical schools raised at the time serious objections against the neuron theory and consequently against the validity of wallerian degeneration as an experimental tool for exploring the finest nervous plexuses. De Castro's contribution showed that severing of the sympathetic chain, as well as of the glossopharyngeal at various levels enabled him to assign the proper labels to different components of the plexuses

For many years De Castro was part in a heated discussion on the organization of the peripheral territory of the vegetative system. Men no lesser than Max Bielschowsky, professor in Berlin, Germany, and Philip Stöhr jr. professor at Bonn, Germany, were extremely skeptical about the neuron theory as proposed by Cajal, and they tended to subscribe the idea that nerve processes built up a

network of anastomosing fibers. Some researchers even thought that there was cytoplasmic continuity with effectors, much along the same lines followed by the famous Dutch histologist Jan Boeke for the motor end plate. From this belief in a terminal reticulum of processes, they were dubbed the "reticularists", an expression which amounted almost to a four letter word in the Cajal Institute. These views were put forward even in the most authoritative histological treatise of the late twenties and early thirties, the mammoth *Handbuch der Mikroskopischen Anatomie* edited by von Möllendorff, and which came to number if I am not mistaken about twenty volumes. Their arguments rested on superb staining methods for neurons which were interwoven with general biological theoretical considerations. I show here a SLIDE taken from one of Stöhr's papers which shows an arrangement comparable to the one in the figure by De Castro which I presented earlier. You may notice that processes do not end anywhere, neither are any preganglionic fibers to be found. The image is similar to that of De Castro but it represents an entirely different interpretation.

The period of intense activity around 1930, which had ended by the proposal of the idea that the glomus was a chemoreceptor, was followed by a lull. This may be due to the fact that his contribution was widely ignored as coming from Spain. He opened the way to Heymans' discoveries but it did not receive due credit even at the moment when the latter was awarded the Nobel Prize. I never heard De Castro himself refer to that circumstance, but his disciples and friends often did, and were somewhat bitter about it. These were also the years of Cajal's death, and of organizational changes in his Institute.

Then in 1936, the black period for Spanish science set in. It was first the civil war. Life in Madrid was rendered very difficult by the advance of the armies of Franco that came to camp on the outskirts of the city within sight of the laboratory. There were also waves of terror by irregular forces of the defenders inside the capital. The Cajal Institute - the new building that Cajal had never really liked - had been placed on top of a small hill, the "cerro de Atocha" which commanded a grandiose view of the Castilian highlands, which at that time were not hidden by the tremendous outgrowth of the suburbs which set in in the course of later

years. On one occasion the defenders of the city believed that messages had been sent to the enemy by means of mirrors, and they came to search the Institute. De Castro's account of the incident was interesting in that it showed the immense respect for the name of Cajal - a sort of scientific saint - that was prevalent among the Spanish people at the time. Upon becoming aware that these men were the collaborators of that glory of Spanish science the aggressive mood of the visitors changed. What might easily have become a punitive raid, ended as a guided tour for the "milicianos", led by De Castro, which included a visit to the heart of the sanctuary, Cajal's museum itself. A sort of bond became established between that group of irregular forces and the occupants of the institute, which was of considerable help on the occasion of other emergencies.

The end of the civil war was followed by political persecution. The Cajal group was dispersed and its remains were denied the means for research work to the point that this brilliant center of neuroanatomy became almost extinct. When one recalls that De Castro's political views were extremely moderate, it becomes possible to take full measure of how stupid and blind men can be made to become in the exercise of power. The consequences for Spain were sad indeed. The civil war was followed by the isolation of the country during World War II and during the post war period, all of which caused cruel poverty and cultural stagnation. Only in the early fifties, almost fifteen years after the onset of the civil war, was De Castro made Professor of Histology in Madrid. But even though better years lay ahead, for him most of the damage had already been done.

In January 1950 I went to the Institute to begin my training as a histologist. My previous experience had been practically nihil, and I wished to convey an impression of commendable eagerness at the distinguished place where I was to start. So I ascended the modest Atocha hill at the south of Madrid, at what seemed a reasonable time of day for a first call. At about 10 AM I rang the bell, and had to do it for so long, so long that I was on the point of giving up when a sleepy employee clad in his pajamas opened the door and courteously observed that it was far too early and that if I wanted to talk to the professor in person, I

was to come back in the evening. Thus I discovered that De Castro was forced by necessity to pursue his research in the laboratory by working till late in the night, while his living came from surgical practiced which he performed by day. For many months to come I was to hear every evening the hurried strides of the tall athletic mountain climber, entering as a whirlwind, his loud voice rallying around him the small group of dedicated staff who made their utmost efforts to help him go along with his research.

De Castro's style of work was highly individualistic. You were expected to work hard, and to achieve something. In the measure in which you did, De Castro would care for you and for your results and provide with valuable suggestions. If you didn't, nobody would take notice of you. I came to know that I wasn't doing bad when he let me have a share of some mysterious fixatives the formulae of which were only known to him. It makes one smile to-day, but this was a common practice in many european histology laboratories of the time, which thus kept their small amounts of classified information which I suspect was not especially valuable. None of their great contributions may be rightly attributed to the power of the secrets they kept so well.

Some weeks after my arrival he suggested for me some work on the innervation of the gastric glands, and I became immersed in that cordial atmosphere of hard work which was pursued with very straightened means. The passion put by De Castro on his work made a lasting impression on me and was extremely useful for my beginnings. When I came back to Chile, I was given a painfully ill-equipped laboratory in a university that did not number more than five or six full-time staff members, and where according to a good friend who represented a great foundation in this country there was not the slightest chance that scientific research could ever become established. My memory of De Castro and of his style of work was an important inspiration, because I knew now that interesting research could be performed even if conditions might seem appalling. Moreover my contact with gastric glands came to be a very useful circumstance when some years later I became interested in the electron microscopy of the hydrochloric acid secreting cells.

A large room in the Madrid laboratory was occupied by cages with cats that had been subjected to some kind of cross anastomosis of cranial nerves. I think that with the dispersion of the Spanish physiologists De Castro was forced to find some means of exploring the functions of the structures he loved. And he hit upon a most ingenious procedure, which was to manufacture artificial reflex arcs (SLIDE), one of which is shown here, with the nodose ganglion of the vagus sending its peripheral branch through the sinus nerve, and its central branch to the superior cervical ganglion. When either blood pressure or CO₂ content of the blood were altered, the pupil in that cat's eye changed its diameter.

This is only an instance of the variety of these distorted nervous systems that De Castro had built. This particular experiment had considerable theoretical interest in that it showed that the response to a specific stimulus was due to the function of the glomic cells and could not be attributed to the type of the nerve fibers supplying it.

As for me these cross-anastomoses bring back pleasant associations. The contact with these techniques bore fruit in my own work when some years later, together with Vera and Luco we showed that adrenergic endings could be replaced by regenerating cholinergic fibers after suitable cross-anastomoses had been established.

Under the conditions prevailing in Spain and in the Cajal Institute, the gathering together of such a large number of skillfully operated animals amounted to a real treasure. At least that is how De Castro looked upon it. I will not readily forget the night in which one of the cats - an exceptionally prized one, a sort of Madonna of the cats - managed to slip away from the cage into darkness. De Castro was frantic, he literally bellowed, and under his leadership the surroundings of the institute became bristling with panicked collaborators, employees and students searching after the animal. The beast was finally discovered passably terrified and cornered near its own room. One of the employees engaged it in a fierce hand to claw battle putting all the time great care not to wound or damage the

cat, and after much biting and scratching he managed to get it back unhurt (the cat unhurt of course!) into its empty cage. I did not think that any one could have tackled a cornered cat with his bare hands, but the impassioned voice of De Castro forbade any attempt to retreat.

He loved Madrid, he was "madrleño" to the bones. He could not really envisage living at any other place. He loved mountain climbing. He had made a short trip to Chile and had remained with an overpowering feeling of the immensity and the abruptness of the Andean mountain range. The passionate strain in his personality kept him active, hard-working and aggressive even when for years the isolation in which he worked made him embark upon some dubious hypotheses. As he tried hard to improve the knowledge about the structure of nervous endings in the glomus he came to believe that the nerve fiber entered the glomic cell, even though remaining in full trophic dependence from the neuron [SLIDE]. The electron microscope has shown (as De Castro himself lived to study), that this was a mistaken view, possibly due to superposition of planes in the microscopical image. Unfortunately the error fitted well the idea of a bipolar glomic cell, and De Castro fell a victim to the temptation of seeing things which he believed should be there, a temptation alas to which many, many a distinguished anatomist has succumbed.

His new idea induced him to think that in many, if not in all synapses there was cytoplasm of glial cells interposed. He spent much time and effort in the pursuit of a demonstration for this hypothesis which did not survive the first electron microscopic images of synaptic boutons. This phase of his research required extremely refined silver impregnation techniques and in the early fifties his laboratory afforded an ideal place to become proficient in these. I remember it well because the technical ability acquired in the Cajal Institute was a good presentation card when I came to other research laboratories to continue with my training. For many years I remained interested in nervous tissue, wallerian degeneration and relationship of axon and glia in peripeheral nerves. I mention this and other work in which I became involved only to show how a

comparatively short period with a man like De Castro could go a long way toward shaping your research interests for years to come.

An account of life in the Cajal Institute in the forties and early fifties would be incomplete if nothing was said of the shadow of Santiago Ramón y Cajal which loomed upon the place. Cajal had not only been a formidable genius in nervous system research. He had served as the voice of conscience calling his country and his countrymen to the austere delights of rigorous science. A man of retiring habits, confined to his small laboratory, shunning honours and bored with fame, he had exerted a tremendous influence upon the whole of the cultural life in Spain. His influence on his collaborators had been of course enormous. De Castro's first contact with him had been as an undergraduate student in Histology. He used to tell a story about his exam, when asked a difficult question he developed an elaborate and - he thought- brilliant answer. Cajal looked at him from beneath his thick eyebrows and said: "Very interesting, very interesting...if only there was some support for it". When one reads the papers written by De Castro in the twenties, one finds the spirit of Ramon y Cajal: sturdy common sense, summary dismissal of nebulous theories, fine, precise technical achievements and a passion for discovery, all of this together with an almost unlimited capacity for work.

Some time after Cajal's death, De Castro was approached by a man, who had risen to wealth coming from the poverty of the Spanish lower middle class and who said he wanted to make a substantial contribution to the Institute. He had no knowledge of scientific matters, and was certainly not of an intellectual turn of mind. His explanation of his feelings for the group of Cajal was simply "Creo en la virtud del trabajo". "I believe in the virtue of work".

In this De Castro also believed and this faith helped him to shape a life full of zest and creative contributions in very difficult times.

I would like to finish by a small anecdote. De Castro was superb at drawing from the microscope. He claimed to be very good at microphotography. But I think he

was a rather poor portraitist. Once when asked for a photograph of himself, and being as he was, extremely careful about his personal appearance he engaged in a session of experiment with a big bellows camera, and requested his collaborators to sit for trial exposures. Thus it came to pass that the present speaker had a photograph of himself taken sitting at the hallowed place of the professor's microscope [SLIDES], just before De Castro obtained a plate which is not perhaps very good as photographs go, but which is for me a reminder of the time I spent with a great scientist and a great man.