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THE BEGINNINGS OF EXPERIMENTAL NEPHROLOGY IN SPAIN: THE CATALAN SOCIETY OF BIOLOGY, 1913-1934

LUIS RIERA CLIMENT
Universidad de Valladolid

RESUMEN

El presente trabajo estudia los comienzos de la nefrología experimental en España llevados a cabo por la Escuela Catalana de Biología, con amplias referencias a los estudios de Ramón Turró, Leandro Cervera y José María Bellido Golferichs y aportación fuentes y bibliografía.

ABSTRACT

This works presents the beginnings of experimental nephrology in Spain carried out by the Catalan School of Biology, with references to the studies of Ramón Turró, Leandro Cervera and José María Bellido Golferichs.

Key Words: Nephrology, Medicine, Spain, 20th Century.

The introduction of the experimental methods in Spanish medicine took place in the last third of the 19th century, from the diffusion of the work of Claude Bernard, who began to influence the peninsula in the ten-year period from 1870-1880. The first Institute of Physiology was created in Barcelona after 1907, thanks to the Barcelona Town Council. This centre housed the first School of Catalan Biology, with wide repercussions in the fields of Physiology and Experimental Bacteriology, with such prestigious figures as Jaime Ferrán y Clúa, August Pi y Suñer, Ramón Turró and Darder, among others.

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Throughout the journal «Papers of the Society of Biology» (1913-1934), over 500 papers on experimental investigation were published, some chapter of which correspond to renal physiology, hypertension and the adrenal glands, on which this paper is based. I would like to point out especially the work on renal physiology of Full Professor José M.ª Bellido, work in which among others, Augusto Pi-Suñer, B.A. Houssay, Leandro Cervera, W.B. Cannon, Rosendo Carrasco and members of this scientific institution collaborated. The atmosphere, which arose in 1934 in Spain for political reasons, caused the suspension of the journal, and the beginnings of the Civil War (1936-1939) led to the exile of the Catalan scientists, especially to South America. This is turn caused the disappearance of the most brilliant School of Biology in Spain in the first third of the 20th century.

Between 1801 and 1900 the Spanish bibliography on renal physiology is quite scant. J.L. Barona's review[1] of Spanish Physiology in the 19th century includes 109 publications on Nephrology, distributed into the following areas: urinary apparatus (1), diuresis (3), absorption (7), urinary bladder (14), ureter (1), kidney (13) and urine and diuresis (70).

Experimental Physiology in Spain was born with delay with respect to the rest of Europe. The beginnings of experimental positivism were due to the diffusion of the work of Claude Bernard after 1880. The first centres of physiological investigation in Spain date from the first years of the current century. Two schools stand out, the group of physiologists of Barcelona, whose head was Ramón Turró; and the School of Physiology of Madrid, whose founder was José Gómez Ocaña. Professor Gómez Ocaña began his work around 1904, and was followed by his brilliant disciple Juan Negrín, until the exile motivated by the Spanish Civil War of 1936. Gómez Ocaña was responsible for the projection of Spanish Physiology beyond national frontiers. He is one of the first physiologists who formed part of the Committee of the Congresses of Physiology of Heidelberg (1907), Vienna (1910), and Groinga (1913). He maintained a scientific relation with Ivan Petrovich Pavlov and Charles Richet, and collaborated in the *Dictionai re de Physiologie* (1895-1923) directed by Charles Richet. Gómez Ocaña was responsible for the treaty of *Theoretical and Experimental Human Physiology* (Madrid, 1915, 5th edition), in which the knowledge of renal physiology existing in Spain at the beginning of the 20th century is expressed with great clarity. He dedicated special attention to renal physiology, physiological mechanisms of excretion and secretion, described the urinary apparatus according to Ramón y Cajal, as well as vascularization and renal innervation.

The most important physiologist of the University of Madrid of the first third of this century was Juan Negrín (born in 1892). He stood out as a physiologist and politician; as a Full Professor of the University of Madrid, he held the office of President of the Spanish Republic in the Civil War. Negrín studied Medicine in Kiel and Leipzig, where he received his doctorate in 1912, after which he returned to Spain. He received a scholarship from the Board of Studies, returning again to Germany, where he studied Chemistry in Leipzig. The First World War obliged him to return to Spain in 1915. He also worked in the Rockefeller Institute for Medical Research under the tutelage of W Professor Meltzer, and in the Laboratory of W. Trendelenburg. Negrín's work had special resonance in relation with the investigation on the adrenal capsules. To him we owe several valuable studies on the «sting» of the fourth ventricle. In 1914, Negrín and Brücke proposed a method for determining the content of the chromatin substance of the adrenal capsules, which permitted testing the loss of this substance, after the ventricular sting.

The School of Barcelona and Experimental Nephrology

The first journal of Spanish experimental physiology was the *Papers of the Society of Biology* of Barcelona. Between 1913 and 1934 it was the organ of expression of the most important Spanish school of physiological experimentation. Nephrology and the organ of urine found an echo among the Catalan investigators. The physiologist who dedicated greatest interest to Nephrology was José M.ª Bellido (1880-1952), the true pioneer of this area in Spain. He was born in Barcelona the 22nd of November, 1880, and died the 19th of July, 1952, in Tolosa de Languedoc. He received his doctorate in 1904 with a paper on Hypophysis, and was Full Professor of Physiology from 1918. He also taught Pharmacology in Barcelona from 1923 to 1936. His republican ideas forced him into exile in 1939, going to Tolosa, where he continued investigating Physiology and Pharmacology with Professor Soulá. In addition to Bellido, among the Catalan physiologists who realized major contributions to the field of nephrology were Augusto Pi-Suñer, Santiago Pi-Suñer, José Álvarez-Puche, Rosendo Carrasco and Roig-Raventós, among others. Likewise, some Catalan physiologists increased their knowledge in foreign universities in their studies abroad. It should be added that Carrasco worked in Boston with W.B. Cannon and Pi-Suñer with B.A. Houssay. This collaboration gave fruit to specific papers on hypertension, as will be shown. The Catalan physiologists studied the following areas: renal histophyisiology, techniques and methods of investi-

gation, renal secretion, stimuli and renal innervation, and uremia in relation with renal function. Other work was dedicated to the study of renal secretory alternation, adrenal capsules and hypertension.

Histophysiology, Techniques and Methods

In 1915, M. Serés[2] studied renal irrigation through techniques of radiological contrast. He tested the independent vascularization of the renal lobules, as well as the calices and the pelvis. Other authors such as Alvarez and Bofill[3] contributed to histophysiology using the tinctions of Rio-Hortega and Achúcarro. Their experiments with partially nephrectomised rats demonstrated alteration in the rhythm of urine secretion. Histological specimens evidenced alterations, among others the disappearance of argentofile granulations of the nephrocytes.

The technical resources of the Catalan school, in addition to partial nephrectomy in rats and dogs, employed crossed circulation[4] in dogs. Likewise, they used vascular ligations, denervation of the reno-vesicle plexus, chemical, mechanical and faradic stimulation of the bladder and the methods of radiological contrast. The techniques of graphic inscription were usual in this organic physiology consecrated to the reno-vesicle organs. J.M.[a] Bellido tested perfusion of organs, a method based on crossed circulation, with success.

Among technical contributions, the study of the tuberculous antigen[5] in urine stands out. Bellido and Serés experimented with this methods, inaugurated by Robert Debré in France, in Barcelona. In over a hundred experiments, they demonstrated the fixation reaction of the complement in urine contaminated by tuberculoses germs. Tests with methylene blue[6] showed its passage through the placenta and feal kidneys to amniotic cavity. These experiments, concluded Roig-Raventós, manifest the renal origin of amniotic liquid. This method would serve to diagnose fetal death and for prophylaxis of amniotic liquid infection.

Renal Secretion

This was the area studied most among the Catalan physiologists. Bellido[7] dedicated special interest to the study of the functional coordination of the urinary apparatus, which constituted an aspect of the organic correlational

investigation in fashion in the early years of this century. They considered the urinary apparatus as a single unit, among whose organs existed reciprocal functional relations. The investigations were primarily oriented towards the search for mutual influences among kidney-bladder-ureter, not only in vascularization, but also in all the connections through the nerve plexus. The methods of denervation permitted the demonstration of alterations in functional rhythm. The visceral distension and pressure conditioned the appearance of polyuria phases, in others olyguria. However, once the kidneys and hilliary plexus were denervated, experiments in dogs confirmed the absence of secretory alteration. The histophysiological studies performed by Bellido confirmed the vesico-renal correlation as established through the hilliary nerve paths. Parallel studies with faradic excitation confirmed the anterior physiological hypothesis.

Jorro Azcue[8], who demonstrated in histological sections the morphological changes of renal epithelia during urinary secretion, completed the work of these histophysiological studies. The modifications were distinguished on the border of the fibres and in the mitochondria of the bordered segments. Azcue thought that the first segment bordered was the principal in substance excretion, although in intervened secondarily in water excretion. Also, the second and third segments presented histophysiological alterations in the phases of secretory action.

Many other projects, with similar techniques, confirmed these experiments. Among these we should cite the effects of urethral distension[9] or vesical distension[10], as well as vesical excitation and its influence in renal secretion. To these experiments one can add those designed to demonstrate the effect of pelvic compression[11] on secretory function. More complex were the investigations on the action of homologous blood on renal function[12], or the renal role in ammonia formation[13], even in the presence of methyl-glioxal in diabetic urine[14]. Even more valuable were the studies on the role of the kidney in the regulation of acid-base balance[15].

Bellido's contributions stand out as the most direct student of experimental nephrology in Spain at the beginnings of the 20th century, as they said. In his early work he showed that vesical distension, by compression of the abdominal viscera, generated an impulse which, linked by the vegetative nervous system, acted on urine production. These stimuli were provoked by the faradic stimulation of the bladder. Likewise, the functional suppression of a kidney, produced by distension of the pelvis and ureter, gave place to a reflex,

which generated polyuria in the other kidney. All these reflexes are transmitted through the vegetative nervous system.

The experiments on renal secretion led Bellido and Muniesa to test endogenous injections of homologous blood, whose transfusion slightly increased diuresis.

Augusto Pi-Suñer demonstrated ammonia production in the kidney, a function tested in dogs, whose production must have renal innervation intact. The ammonia is produced, according to Pi-Suñer, to avoid the excessive acidity of urine, being a regulating mechanism of the acid-base balance. He studied experimentally acidosis and alkalosis in dogs, and faced with the changes in pH, Pi-Suñer described the renal response in the form of waves, as if it were a functional test. Bicarbonated intravenous solution, in experimental animals, decreased urinary acidity in waveform. These experiments were published in *The Doctrine of Internal Sections* (Barcelona, 1917), which Bellido, Pi-Suñer and Nubiola presented at the «Second Congress of Catalan-language Doctors». In this work the existence of a physiological correlation between the hypophysis, pancreatic hormone and renal function was pointed out.

Innervation and Renal Uremia[16]

As a technique, it was normal for the Catalan physiologists to use denervation in order to discover secretory system alterations. Functional alterations were studied with the method of Golgi tincture to verify the existence of structural changes. Renal denervation in dogs demonstrated the long-term appearance of lesions in renal epithelium, which would lead to excretory insufficiency, intoxication and death of the animals. Bellido demonstrated that the kidney functions of a chemical character are coordinated thanks to innervation. In the kidney, innervation fulfils atrophic function on the parenchyma, so that denervation leads to irreversible lesions in the epithelia. These cells degenerated, lacking nervous trophism, in time leading to a compromise of urinary secretion. There is a double renal innervation, according to Bellido: one with a vasomotor role and another trophic. The denervated kidneys reacted slowly to variations in the period of 12 days, and the urine did not reach the degree of acidity of normal kidneys. The anatomical study of dogs permitted Serés to show that this vesico-renal path is established through an odd ganglion located in the

ed in the medial line; the superior branches innervate the kidney, while the inferior branches head toward the bladder.

The studies of Pi-Suñer and Bellidol[17] graphically demonstrated the effects of uremic blood on renal function. With the method of crossed circulation, the injection of blood from a uremic dog to another normal dog influenced the urinary secretion of the second dog. Small quantities of uremic blood, or large quantities of slightly uremic blood, stimulated renal secretion. This effect was produced a minute following the transfusion, being maintained in variable form. However, the injection of large quantities of very uremic blood decreased renal secretion, and even provoked complete anuria. Toxicity of uremic blood was determined by the refraction index, a simpler method than ionic alterations of nitrogen (N_2) or potassium (K^+).

Álvarez-Puche performed some work on experimental uremia, attributing the symptoms provoked by uremia (vomiting, diarrhea, anorexia, bradichardia and death) not to the toxicity of the urea, but to renal failure and lack of its antitoxin purification function. In his opinion, the altered renal function in uremia cannot eliminate the catabolytes, which provoke cellular problems and death.

Functional kidney alternation[18]

Renal alternation was another area addressed in the Catalan School of Physiology by J.M.ª Bellido. Using chloralose as an anesthesia in dogs, he demonstrated that during the first two hours of anesthesia no variations in quantity of urine segregated by each of the kidneys was observed. However, later experiments led him to conclude that the true alternations were produced in certain cases, such as in renal faradisation when the kidney has to purify crystalloid substances, and when certain reflexes act on the kidney. Through his experiments Bellido concluded that circumstances of chemical and nerve order can modify renal function. Abnormal excitation or osmotic overcharges fatigue the epithelia and the renal nervous system, making rest periods follow those of excretion. Bellido considered that three groups of intermittence of alternations exist in renal secretion and excretion. The first group is easily registered, but the last are more difficult to perceive. From his experiments, it can be deduced that nervous and chemical causes exist which alter the rhythm of renal function.

Hypertension and physiological experiments[19]

The Catalan School of Physiology addressed the experimental study of hypertension. Among other authors we should cite Leandro Cervera and B.A. Houssay, who collaborated during his stay in Barcelona, as did W.B. Cannon and Rosendo Carrasco. Likewise, the names of J.M.ª Bellido and J. Álvarez, among others, must be added. Cervera and Houssay studied the hypertensive effects of adrenaline discharges provoked experimentally. In contrast to the opinions of Gley, who denied the vasoactive action of adrenaline, Houssay and Cervera managed to demonstrate experimentally the vascular effects of adrenaline. Splanchnic stimulation provoked an increase in arterial pressure, but this increase was greater if the irrigation of the adrenal capsule was conserved. In contrast, the effect was less if adrenal vascularization was ligated. They demonstrated that splacnic excitation produced direct vasoconstriction and adrenal discharges of adrenaline in addition, whose effects was added to vasoconstrictive hypertension. Carrasco-Formiguera also demonstrated experimentally in animals, above all dogs, Claude Bernard's model of «sting» to the fourth ventricle. Such a sting produces an increase in cardiac frequency in a denervated heart. However, the tachycardia is not produced if an adrenal gland is extirpated and the vessels of the other are ligated. Complete ligature of the two adrenal glands also impedes tachycardia, but this is produced if the adrenal blood flow is permeabilised. Carrasco concludes, in his interesting paper, that a sting to the fourth ventricle produces a dischange of adrenaline or of an identical substance. W.B. Cannon, working with Carrasco-Formiguera, likewise studied the secretion of reflex and asphyxiant adrenaline. They showed that following section of hepatic nerves, tachycardia is due to adrenaline secretion. In this sense the vascular blockage of the adrenal glands prevents tachycardia and reestablishment of blood flow makes the effect reappear. The asphyxiated animal increases its cardiac frequency at 45 seconds, due to the adrenaline liberation. Dr. J. Álvarez-Puche also worked in this line of investigation, studying the effects of ephedrine in dogs, previously anaesthetized with chloralose and vagotomised. However, at high dosages of ephedrine, 3-3.5 gr/kg of weight, the antagonistic effect of arterial hypotension is produced. This, in Álvarez-Puche's judgment, is the difference that separates the effects between adrenaline and ephedrine. One of the most interesting areas in which contributions were made by the Catalan physiologists, first Pi-Suñer and later Álvarez-Puche, was the nervous reflexes, which act on arterial pressure. They described three regulating mechanisms: one which they considered a non-spe-

cific sensitive receptor and cardiovascular effector; the second a specific receptor as the carotid sinus; and thirdly, the mechanisms of anticipation when we prepare to make an effort.

The first attempts in Spain to initiate the experimental study of hypotensor drugs are due to Bellido. Among his experiments his work with amyl nitrite and acetylcoline stand out, whose immediate, early effect had no long-term repercussions. In addition, he performed experiments with odorless potassic as a hypotensor.

The experiments continued in Barcelona until the Civil War of 1936-39. The obligatory exile led the professors of the Institute of Physiology of Barcelona to disperse, seeking refuge in universities of Latin America. However, their work was interrupted as a group. The examples, which attract our attention most strongly, were J.M.ª Bellido, exiled in Tolosa, or Jaime Pi-Suñer in Venezuela. Fascist military personnel, such as Rosenco Carrasco Formiguera, executed some of these outstanding doctors. Spanish physiology and nephrology from 1939 on is an area which would require another communication.

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