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Obituary

J.-M. Ghuysen

Bacteriology has recently lost one of its great contributors. Jean-Marie Ghuysen left us suddenly last August 31, 2004, as the result of septicaemia. He had devoted most of his research career to the (bio)chemistry of bacteria and to molecular microbiology.

Born in Blégny-Trembleur, a large village next to Liège, on January 26, 1925, Jean-Marie Ghuysen was raised in the local pharmacy owned by his father. As a boy, he went to the primary school in Blégny and then to the Saint-Hadelin secondary school in Visé, a small town north of Liège.

Before he was 18, the young Jean-Marie had virtually no contact with scientific matters except through his father's activities. The only influence he could remember was when his father, the mayor of the village, decided around 1933–34 to install a water supply in the village to replace the central well. Together they wandered around the countryside collecting samples from springs, and analysing them in a small laboratory next to the pharmacy. As a youth, Jean-Marie lived in a literary world (old Greek, Latin, philosophy, novels), but he also dreamt of becoming a sailor. His adventurous nature led him, when he left high school, to head for Antwerp with the hope of boarding a ship. This plan was foiled when he was arrested by the police (in war time) and brought back home.

He then had no other choice but to follow his father's steps and to become a pharmacist. He discovered the Sciences at the University of Liège and became fascinated by chemistry and physics. His passion for chemistry was so great that he decided to study both pharmacy and chemistry. He obtained his degree in pharmacy in 1947 and finished the chemistry curriculum in 1948, presenting a final term essay on the 'Isolation and purification of RNA'.

During the occupation, Jean-Marie was not regularly registered as a university student in order to avoid being sent to Germany as a 'voluntary worker'. Instead, he joined the resistance movement where he helped as a messenger and a bomb maker. His degrees were authenticated after the war was over.

He obtained a research fellowship, from 1948 to 1951, to pursue his work on RNA with Prof. V. Desreux in the department of chemistry. He published four articles that formed the core of a PhD thesis on 'The study of the heterogeneity of RNA' that he presented in October 1951 and for which he received the Stas-Spring award, the first of many prizes.

He was approached by the Labaz Laboratories, a company that was planning to create a biochemistry and microbiology facility. They offered him the post of director of the laboratory and while the laboratory was being completed, he decided to improve his microbiological knowledge. With the support of the IRSIA and the Labaz Laboratories, Jean-Marie worked with Prof. M. Welsch, director of the general and medical microbiology unit of the medical faculty of the University of Liège. A young chemical engineer was hired at the same time as his deputy. The team studied bacteriolytic enzymes that were known to be part of the *Actinomycetine* secreted by some Streptomycetes. Quite rapidly they identified and separated several proteolytic activities as well as different bac-

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teriolytic enzymes. Two enzymatic fractions FI and FII were subjected to detailed study and shown to have different peptidase activities on bacterial cell walls. Results collected over a 6-year period were published in 12 articles and in a thesis for the Agregation Diploma in Pharmaceutical Sciences, which was awarded in 1957. In the meantime, Jean-Marie had received, in 1955, the Louis Empain prize.

When the unit at the Labaz Laboratories was ready, Jean-Marie initiated research on brain glutamate decarboxylase. However, he resigned from the company after a few months and returned to university research in January 1958. In negotiations with the University, it was agreed that he would not be involved in practical courses for the students but be free to manage his research as he pleased. With the help of M. Welsch and the rector of the University, Prof. M. Dubuisson, he was rapidly promoted and became associate professor in April 1966.

From 1958 to 1969 his research focused on determination of the chemical structure of bacterial cell walls, using the various bacteriolytic enzymes he had purified and characterized previously. This was a period in which he collaborated extensively with a number of workers in related fields: his collaborations with Milton Salton (structure), Jack Strominger (biosynthesis) and Gerald Shockman (lytic enzymes) were particularly fruitful and established the main thrust of his research for the next two decades (Ghuysen and Strominger 1963; Tipper *et al*., 1964). The investigations he carried out were an essential contribution to the understanding of the chemical structure of the bacterial cell wall. In 1966, at a round-table organized during a Symposium of the American Chemical Society in Detroit, Jean-Marie proposed, with colleagues including G.D. Shockman, the word 'peptidoglycan' to define the macromolecular structure which forms the skeleton of the cell wall and is the target of autolysins and other lytic enzymes. These results were gathered in an important review entitled 'Use of Bacteriolytic Enzymes in Determination of Wall Structure and their Role in Cell Metabolism.', which was published at the end of 1968 in Bacteriological Reviews (Ghuysen, 1968). The work was developed by K. Schleifer and O. Kandler who, in 1972, used the different peptidoglycan types as taxonomic criteria for bacterial classification.

He was appointed full Professor in Liège in 1969 and formed a nucleus of local research workers that included Melina Leyh-Bouille, Jacques Coyette, Martine Distèche, Jean Dusart and Jean-Marie Frère. The stimulation provided by Jean-Marie Ghuysen and his laboratory attracted a plethora of international scientists (microbiologists, biochemists, chemists). It is not surprising that such an invigorating atmosphere increased the enthusiasm of all participants and paved the way for future successes. At the same time, the general orientation of the research also shifted from the elucidation of the structure of the bacterial peptidoglycan (a problem Jean-Marie considered as solved in 1971) to that of the mode of action of penicillin, which was known to interfere with peptidoglycan biosynthesis. The enzymes produced by various strains of actinomycetes and that Jean-Marie had used as tools for the study of peptidoglycan structure became model proteins for the analysis of penicillin–target interactions (Leyh-Bouille *et al*., 1971). Model substrates and assay systems were developed to enable the complex peptidoglycan transpeptidation reaction to be studied using purified enzymes so that the kinetics of the interactions could be investigated. The studies were later extended to Penicillin-Binding Proteins (PBPs) from *Escherichia coli* and enterococci. In 1976, the team contributed a major advance in the field by demonstrating that penicillin acylated a serine residue in one of the *Streptomyces* penicillin-sensitive D-alanyl-D-alanine carboxy-transpeptidases (Frère *et al*., 1976), a reaction that was later found to account for the inactivation of all PBPs by β-lactam antibiotics. Surprisingly, this seminal paper is seldom cited, it seems that the result is now considered as self-evident! Not surprisingly, the 'sensitivity' of a PBP to a particular penicillin was found to be dependent mainly on the rate of this acylation reaction.

Further progress required the utilisation of new methods, and collaborations were initiated with crystallographers in Connecticut (Jim Knox and Judith Kelly) and Liège (Otto Dideberg). The first complete structure of a D-alanyl-D-alanine carboxypeptidase was solved in Liège in 1978 (Dideberg *et al*., 1980). It was a Zn^{++} metallo enzyme that, as a consequence, was not sensitive to penicillin. It was the first protein 3D structure to be solved in Belgium. Later, the collaboration with the Connecticut group resulted in the demonstration that penicillin-destroying β-lactamases and penicillin-sensitive DD-peptidases shared many important structural features (Kelly *et al*., 1986) At this time, Jean-Marie also felt the need for theoretical approaches and Georges Dive and Josette Lamotte joined the team to start a group specialising in molecular modelling and quantum chemistry.

The group had now become multidisciplinary, covering such diverse areas as microbiology, enzymology, protein crystallography and theoretical chemistry. New staff members, namely Paulette Charlier, Bernard Joris and Colette Duez obtained permanent positions. The need for large quantities of proteins became an important factor and, since the focus was still mainly on *Streptomyces*, Jean Dusart visited David Hopwood's laboratory in Norwich to acquire expertise in cloning streptomycete genes and in expression systems for the production of large amounts of streptomycete proteins. Several genes encoding D-alanyl-D-alanylpeptidases and β-lactamases were cloned to satisfy the requirements of the protein chemists and crys-

tallographers (Dehottay *et al*., 1986). The structural and functional characteristics of several enzymes were thus determined and attempts were made to establish correlations between both types of properties, an old dream of Jean-Marie. In 1990, when Jean-Marie reached the age of mandatory retirement, the Centre for Protein Engineering was created to avoid dispersion of the various experts that formed the team and of the equipment. The Rector of the University organized the appointment of Jean-Marie as the first director of the Centre until, at 70, he had to retire, though he remained active as a scientific advisor. Meanwhile, new collaborations had been initiated, mainly with Josef Van Beeumen, a protein chemist at the University of Ghent and with Léon Ghosez, an organic chemist at the University of Louvain.

Jean-Marie had an encyclopaedic knowledge of microbiology and biochemistry. He was generous with his time and keen to discuss their data or more general scientific problems with his younger collaborators: this often led to the birth of new ideas, many of which turned out to be seminal. In his most recent publications (out of a total of more than 350), he discussed the molecular basis of the lack of efficiency of penicillins against mycobacteria (the 'Mycobacterial Paradox') and the evolutionary relationship between penicillin-binding enzymes (Ghuysen and Goffin, 1999; Terrak *et al*., 1999; Goffin and Ghuysen, 2002).

Over the years, Jean-Marie was the recipient of a large number of prestigious awards: the Prix Joseph Maisin of the National Research Foundation of Belgium (FNRS), the Prix de l'Innovation Technologique of the Walloon Region (shared with four of his coworkers), the Gairdner Foundation International Award in Medical Science, the UNESCO Carlos J. Finlay Award in Microbiology, the Albert Einstein World Award of Science and the Bristol-Myers-Squibb Award in Microbiology. He was chairman of numerous symposia at international conferences and a much sought after lecturer at several Belgian and foreign universities and at a large number of meetings. He received 'Honoris Causa' doctorates from the Universities of Nancy, Debrecen and Montreal.

The last years were saddened by his wife's poor health. To the end, in August 2003, he devoted a lot of time, attention and love to her.

Jean-Marie leaves behind him a thriving Centre, composed of more than 70 scientists and technicians now headed by Jean-Marie Frère (not unexpectedly called 'Jean-Marie the Second'). He has made a lasting mark on Belgian and international science and his numerous contributions (total of more than 350) will long be remembered not only by scientists all over the world, but also by his three children and eight grandchildren, one of whom is . . . a pharmacist!

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