Arch Virol 140 (1995)

VDN
Virology Division News

A century of veterinary vaccinology: the Mérieux initiative

Ph. Desmettre

A young chemist, disciple of Louis Pasteur ...

In 1894 Marcel Mérieux, a young chemist who had graduated from the Ecole de Chimie de Lyon (Lyon's School of Chemistry), entered the Institut Pasteur in Paris as an assistant to Doctor Emile Roux (Fig. 1). Emile Roux, who was himself assistant of Louis Pasteur, had just presented his work on the use of serum from horses immunized against diphtheria in the treatment of this disease in man in Budapest (in September 1894), following the initial observation made by Behring and Kitasato in 1890 on the diphtheria and tetanus toxins and antitoxins.

Fig. 1. Marcel Mérieux (*standing, third from left*) joined the Institut Pasteur in 1894 as assistant of Emile Roux (*sitting, third from left*) who was himself the assistant of Louis Pasteur (*sitting in the middle*)

The potential applications of this knowledge should have a strong influence on Marcel Mérieux' future life. Since he could not establish a diagnostic laboratory in Paris as Pasteur had advised him, he returned to Lyon, his birthplace, and founded the Institut Bactériologique de Lyon in collaboration with the veterinarian Henri Carré; his objective was to produce anti-streptococcal serum to protect against puerperal fever and tuberculin, which had been first prepared in 1890 by Robert Koch.

Marcel Mérieux and Henri Carré's partnership did not survive the commercial failure of these products. The two men separated and their institute disappeared.

While Henri Carré successfully pursued research in veterinary microbiology, Marcel Mérieux, an entrepreneurial man in the attics of Lyon's hospital, established a laboratory to which he gave his name. He also organized teaching in medical bacteriology. In 1907, Charles Mérieux was born, who would play a key role in the development of the Institut Biologique Mérieux which had been created ten years earlier. This institute, which then had only one employee, was involved in the diagnosis (mainly of diphtheria) and production of sera (antitetanus, antidiphtheria sera and desiccated serum for local application) and tuberculin. It was a sign of destiny and of the role that veterinary medicine would play in the Institut Biologique Mérieux's further expansion that just before World War I the institute had to leave its premises and to move to Bourgelat street, the place where Claude Bourgelat had installed the first veterinary school in the world in 1762. World War I put the Institut Biologique Mérieux in a difficult financial situation. However, this did not undermine the strong motivation of its founder who purchased 25 hectares of void land in Marcy l'Etoile (west of Lyon), where the industrial activities of the institute would develop later on.

Charles Mérieux, an heir of the Pasteur tradition

In 1926 Marcel Mérieux widened the range of products by adding Koch's new tuberculin and bovine anti-foot-and-mouth disease virus serum. In 1927 Charles Mérieux began his study of medicine at the University of Lyon and in the meanwhile helped his father to develop the institute, being more particularly involved in the veterinary range of products. In 1932 he attended the "Grand cours" of microbiology at the Institut Pasteur in Paris in order to learn the theoretical aspects of microbiology and add them to the practical aspects which he had already acquired. He then untertook several journeys that took him all over Northern and Eastern Europe. In addition to satisfying his inextinguishable curiosity – not only in the field of science – these trips showed him the importance of relationships which could be established within the international scientific world.

In 1934, Charles Mérieux met H. S. Frenkel who, while working on foot-and-mouth disease virus, had discovered a means of cultivating it *in vitro*. This encounter would be essential to the subsequent development of the animal health activities of the institute. In 1937, when his father died, Charles Mérieux assumed full leadership of the institute, the future of which was far from being secure. He would untiringly expand it, with passion and pugnacity, without respecting borders between human and veterinary medicine and following Pasteur's tradition he had inherited from his father. One year later, he defended a Doctor of Medicine thesis on tuberculin.

During World War II, in addition to the productions which were common at that time he prepared in the Lyon-Gerland slaughterhouses bovine serum intended for children who

suffered from malnutrition. At the same time, he secretly made human serum available to the "maquis" underground movement forces.

The dawn of industrial biology

In 1945, Charles Mérieux went on an official mission to the USA and discovered industrial biology and its potential for business development. In the starving Europe of 1946, farmers tried to restore their cattle herds, but foot-and-mouth disease caused such heavy losses that only a vaccine could stop.

Charles Mérieux then had the idea of creating a foot-and-mouth disease institute where the necessary doses of vaccine could be produced on an industrial scale. Due to the lack of financial resources, this idea took shape only in 1947 thanks to the support received from a competitor, Henri Vallée, who was also running an institute in the field of animal health (Institut de Sérothérapie de Toulouse).

The Institut Français de la Fievre Aphteuse (IFFA – French Institute for Foot-and-Mouth Disease) was thus set up on the site of Lyon-Gerland slaughterhouses, at the very place where Charles Mérieux had prepared bovine serum for underfed children during World War II. The approach used for manufacturing the vaccine was the Vallée-Schmidt-Waldman method (Fig. 2) which included:

1. infecting live cattle by inoculating their tongues with foot-and-mouth disease virus,
2. slaughtering them when the vesicles appeared,
3. collecting lingual epithelium which was homogenized and constituted the active ingredient of the vaccine after inactivation.

However, this method did not make it possible to produce the vaccine in sufficient quantities; 500 kg cattle would only provide 50 doses of 45 ml of a trivalent vaccine, at a high cost price, and which was difficult to use due to the volume to be injected.

The Frenkel method

Charles Mérieux remembered his encounter with H. S. Frenkel in 1934 and asked him to introduce the foot-and-mouth disease virus *in vitro* culture technique he had developed. It was based on the multiplication of the virus in lingual epithelium of cattle, which were collected immediately after slaughter and kept alive in suspension in a relatively simple medium (Fig. 3). Following the virus multiplication step, the epithelium and debris were eliminated by centrifugation, the suspension was treated with chloroform, inactivated with formaldehyde and then adsorbed on to aluminium hydroxide. The virus concentration technique led to a reduction of the dose volume first to 30 ml, a few years later to 15 ml and finally to 5 ml.

The multiplication of foot-and mouth disease virus in large-volume steel vessels was the first example of the industrial production of a viral vaccine worldwide. Thanks to the use of Frenkel's method, the Institut Français de la Fievre Aphteuse (IFFA) could help in controlling the terrible foot-and-mouth outbreak which had occurred in Europe (320,000 foci were officially reported in France) in 1952.

Fig. 2. Vallée-Schmidt-Waldman's method for foot-and-mouth disease vaccine production. Cattle are inoculated with the virus in the tongues and are subsequently slaughtered when the vesicles appear. Lingual epithelium is then collected and ground. After inactivation, the crude suspension constitutes the active ingredient of the vaccine

At that time, the quantities of vaccine produced also enabled Charles Mérieux to supply Columbia with the requested 400,000 doses of vaccine which he delivered himself to Bogota by airplane. He was confident in the quality of the vaccine produced at IFFA and wanted it to be recognized by an independent institution; he therefore contributed to the creation of an offical control laboratory by paying to the French Ministry of Agriculture a lump sum on sales of each vaccine dose.

Rescue from financial disaster

During the following two years, there were only sporadic foot-and-mouth disease outbreaks, and the farmers did not vaccinate their cattle any longer: the number of vaccinated cattle declined from 5.9 million in 1952 to 0.9 million in 1954. IFFA went through a financial crisis and had no alternative but to make most of its staff redundant. Mérieux therefore decided in 1953 to set up a subsidiary in Argentina in order to "save IFFA" as he said. The Institut Franco-Argentin de Production Biologique (IFA – French-Argentinian Institute for Biological Production) started in 1955 to produce vaccine according to Frenkel's method.

In 1956 a new outbreak of foot-and-mouth disease occurred in France in unvaccinated and therefore particularly receptive cattle. Up to 6.5 million doses of vaccine were required.

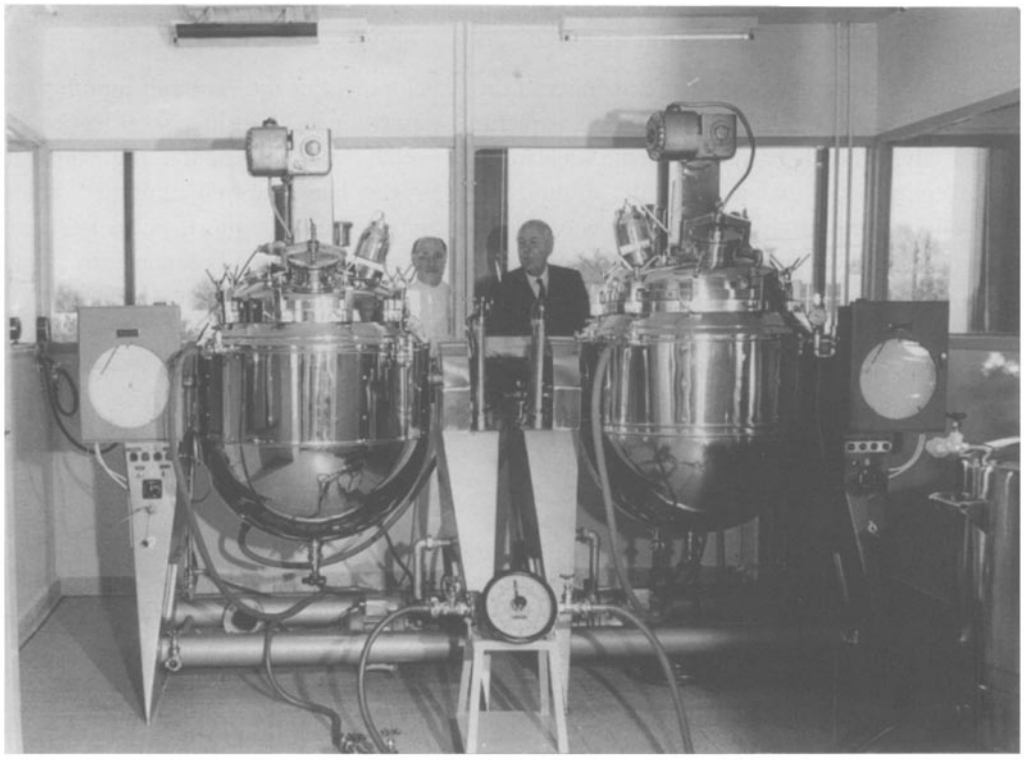

Fig. 3. Frenkel's method for the production of foot-and-mouth disease vaccine. Tongue epithelium of cattle collected immediately after slaughter is kept alive in suspension in stainless steel vessels and used for foot-and-mouth disease virus growth. This method gave rise to industrial virology. In the background Dr. Czelaw Mackowiak, DVM (*left*), former director of IFFA, and Dr. Charles Mérieux

To be in a position and meet such variable requirements while providing a vaccine of constant quality implied that highly-skilled technical staff had to be employed. Charles Mérieux then devised a 5-year vaccination contract between breeders, veterinarians and vaccine producers, under which the breeders committed themselves to vaccinate their cattle each year; the veterinarians and vaccine producers promised to provide vaccination at a lower price and to give the breeders priority for supply of vaccines in the case of an epidemic.

Foot-and-mouth disease vaccine improvements

In 1958, IFFA virologists achieved a major breakthrough by using saponin as an adjuvant, which enhanced the activity of the vaccine and made it possible to reduce the dose volume to 10 ml. Thanks to the vaccination contracts which in some regions resulted in a 80% vaccination rate of herds, foot-and-mouth disease was practically eradicated locally. The veterinary services could then make the slaughter of all susceptible animals compulsory in any new focus within 18 areas in the eastern part of France. For the first time, an area could this way be declared free of foot-and-mouth disease. In 1961, at the request of agricultural officials, vaccination of all cattle older than 6 months became compulsory in

the whole French territory; in any new focus, all infected or contaminated animals would be slaughtered.

Further technical achievements marked the development of the foot-and-mouth vaccine produced at IFFA, strengthening this institute's position as a world market leader. In 1965 a trivalent concentrated vaccine was launched which could be used at a single dose of 5 ml, regardless of the weight of the animal, and five years later, the first combined rabies and foot-and-mouth disease vaccine was approved by the French authorities. In 1971, an oil-adjuvanted vaccine for immunization of pigs was also approved. Much effort was simultaneously focused on foot-and-mouth disease virus inactivation and inactivating agents: after formaldehyde and glycydaldehyde, ethyleneimine was used. For safety reasons, inactivation controls were conducted both in cells (cytopathic effect test) and in the target species (intralingual inoculation and checking for absence of vesicles).

A milestone: virus growth in cell suspension

In 1975, a milestone was reached in the evolution of the production process of foot-and-mouth disease vaccines: the use of a cell line in suspension cultures for virus multiplication. The line IFFA 3 was derived from hamster embryo cells. The method was mostly used in case of epithelium supply problems. However, it did not replace the Frenkel technique which remained the most suitable method for rapid adaptation of field strains.

Vaccination became popular in France and throughout Europe, and the vaccine production process was further improved through the implementation of virus concentration by ultrafiltration and purification by chromatography. At the same time, the need for supply of the many countries infected with foot-and-mouth disease increased. Due to the impossibility to work in France with strains of foot-and-mouth disease virus regarded as exotic, vaccine production had to be decentralized.

Following the initial creation of the Institut Franco-Argentin de Production Biologique (IFA) in 1953, the Interifa-Uruguay subsidiary was set up. In 1963 vaccine production was organized at the Razi Institute in Iran, in order to protect Europe against a SAT 1 virus epizootic. In 1972, following an agreement signed with the Soviet government, a production laboratory was opened near Moscow.

In 1978, Botswana, a South African country whose agricultural resources came mainly from cattle breeding, was faced with a SAT 2 virus epizootic. At the request of the Botswanian government, a laboratory unit was sent by airplane together with a team of technicians who succeeded in producing a vaccine within a few months.

A collaborative agreement with the Republic of Iraq led to the construction of a state-owned laboratory in 1982 which, due to difficulties in epithelium supply, used the IFFA 3 cells for multiplication of the A, O and ASIA 1 types of foot-and-mouth disease virus. Finally, in 1990, following an agreement signed with the Thai government, a vaccine production laboratory was built and technicians were trained as part of a technology transfer programme.

In March 1991, when foot-and-mouth disease vaccination was banned in Europe, production was stopped in France and the laboratories were restructured. The acquisition of a production laboratory at Pirbright in England made it possible to continue foot-and-mouth disease vaccine production according to IFFA methods.

Institut Mérieux and IFFA: eliminating borders between human and veterinary medicine

Charles Mérieux' dedication to worldwide preventive medicine, without borders between human and veterinary medicine, led to the parallel development at Marcy l'Etoile of bacterial and viral vaccines intended for both man and animals, as well as of fractionating techniques used in the preparation of blood-derived products. Some of the products for human use (tetanus serum, tuberculin) had been first developed for veterinary medicine – an unparalleled phenomenon. Later on veterinary vaccines were developed, amongst others against blackleg, anthrax, contagious ecthyma, fowlpox, Newcastle disease, erysipelas, brucellosis. Its expertise in industrial virology led IFFA to develop and produce viral vaccines for both human (poliomyelitis, influenza, rabies) and animal use (hog cholera, canine distemper, myxomatosis). This is how one of the widest ranges of viral, bacterial and even parasitic veterinary vaccines arose, covering companion animals (dogs, cats, horses), farm animals (cattle, sheep, goats, pigs, rabbits), poultry (broilers, laying hens, turkeys, ducks) and fish.

Among the products which made a significant contribution to veterinary vaccinology, special mention should be made of the first rabies preparation produced in cell culture (1967). Another breakthrough was the combined rabies and foot-and-mouth vaccine, and a combined vaccine for dogs providing protection against rabies, canine distemper, hepatitis, and *L. canicola* and *L. icterohaemorrhagiae* leptospiroses.

Innovation relies on tradition...

In Charles Mérieux' opinion, tradition is not the enemy but the ally of innovation. After Frenkel's method, which laid the foundations of industrial virology, various techniques, such as *in ovo* culture, Roux bottle culture (Fig. 4) and more recently roller bottle culture, have been extensively used for the propagation of primary cells and lines. More potent techniques for cell cultivation on an industrial scale, e.g. suspension or microcarriers, followed when electronically regulated biogenerators or biofermenters had been introduced.

In the production of inactivated vaccines, concentration by ultrafiltration and purification by gel filtration or affinity chromatography played a key role in the development of methods for extraction and purification of antigenic fractions, which resulted in a unique range of subunit vaccines particularly used in the prevention of animal herpesvirus infections. Also, know-how accumulated in the field of immunoadjuvants (particularly oil adjuvants), in which the company was a pioneer, helped to increase the efficacy of "killed" preparations.

The advent of new biotechnologies

The company has traditionally been interested and involved in new technologies, in diagnostics based on monoclonal antibodies, antigens produced by recombinant DNA technology or peptide synthesis, als well as in recombinant DNA-based subunit or live vector vaccines. It thus has pioneered the development of a technology using poxviruses as expression vectors. As a result, a live vaccine was produced for oral vaccination of wild carnivores against rabies which used vaccinia virus as a vector. This preparation is currently

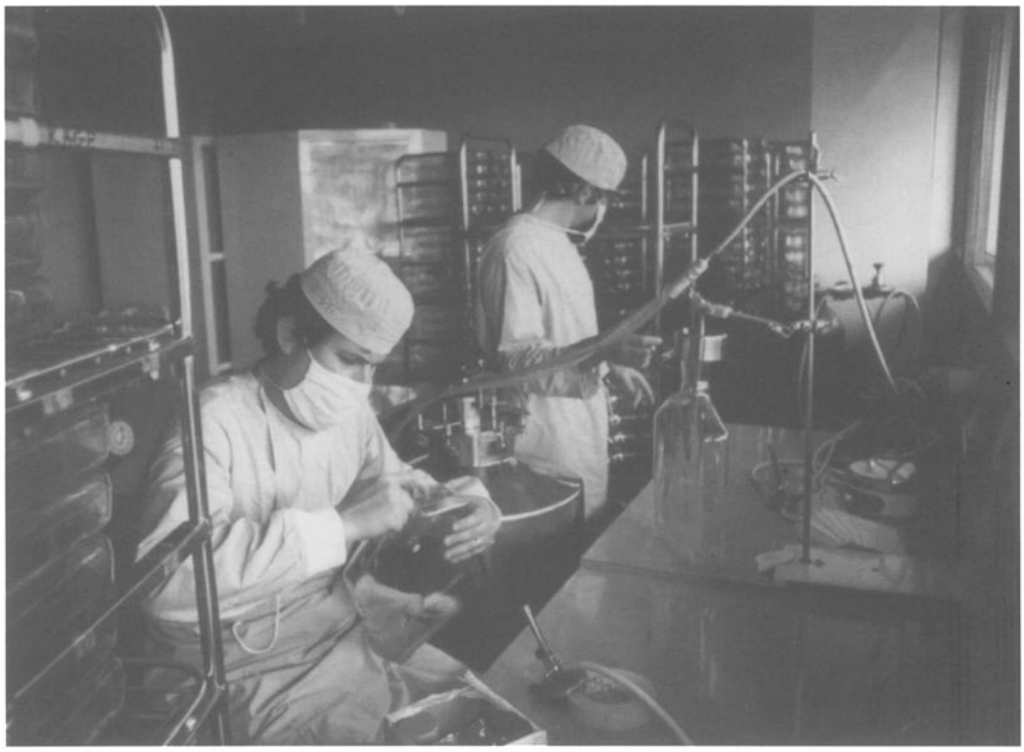

Fig. 4. Roux bottle cell culture. This technique has been replaced by roller bottles and more recently by culture on microcarriers

marketed in Europe and the U.S.A. As the first example of a registered live viral vectored vaccine, the rabies vaccine will be followed by others which are either under registration or in an advanced phase of development. As the company is anxious to strengthen its technological advance, it is also involved in the development of synthetic vaccines and polynucleotide vaccines.

From Institut Mérieux and IFFA to Rhône Mérieux

While the sales of foot-and-mouth disease vaccine boosted the expanding activities of Institut Mérieux in both human and veterinary medicine, through what Charles Mérieux used to call "aphto-financing", the sales of vaccines against rabies and brucellosis, and of combined vaccines for dogs and poultry (in particular Marek's disease vaccine) were key factors in the successful development of Institut Mérieux' veterinary division.

In 1968, the takeover of the Institut de Sérothérapie de Toulouse, its partner at IFFA's creation, has enhanced the potential for the development and production of bacterial, in particular anaerobic vaccines. IFFA joined the Institut Mérieux and the animal health department was called IFFA-Mérieux. In the same year, Charles Mérieux sold equities of the company to the French chemical group Rhône-Poulenc which became and still is the majority shareholder. In 1983, Rhône Mérieux was created from the merger of the veterinary operations of Institut Mérieux and Institut de Sérothérapie de Toulouse, its subsidiary, with Roger Bellon and Specia, all belonging to Rhône-Poulenc. Other acquisitions include

the US-based Select Laboratories in 1988, the Italian IVAZ and Bioteke companies and the Japanese Phylaxin laboratory in 1992. Finally, in 1995, the acquisition of Sanofi Animal Health's activities in America and Asia was in line with the company's growth objectives to be achieved by the end of the century.

Verleger: Springer-Verlag KG, Sachsenplatz 4–6, A-1201 Wien. – Herausgeber: Dr. F. A. Murphy, School of Veterinary Medicine, University of California Davis, Davis, CA 95616, U.S.A. – Redaktion: Sachsenplatz 4–6, A-1201 Wien. – Satz und Umbruch: Thomson Press (India) Ltd., New Delhi. – Offsetdruck: Adolf Holzhausens Nachfolger, Kandlgasse 19–21, A-1070 Wien. – Verlagsort: Wien. – Herstellungsort: Wien. – Printed in Austria.