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The building up of a culture of standardization at the Institut Pasteur, 1885-1900

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The initial wave of conception and production of animal and human vaccines culminating with the human usage of vaccine against rabies (1885), supervised by Louis Pasteur (1822-1895), although considered a great achievement in France, had met with several theoretical, methodological and technical weaknesses that threatened the reproducibility of the results as well as part of the validity of the approach. The problems were pointed out mostly by German scientists, but by French critics of Pasteur's action as well. At first, problems were denied but answers to them were progressively brought in, years often after the first announcement of a success, according to a strategy that could be considered a kind of *a posteriori* re-construction of data that could have led to the initial positive conclusions. Such a progressive construction of acceptable practices, participate in a muddled attitude concerning the methodology used on medical issues during the period that Mirko Grmek has named the "heroic age" of the Institut Pasteur¹. An entirely different scientific attitude, now involving quality control, quantification at every step of a production process, closer contacts with medical structures and a more positive attitude towards German science, was by contrast obvious at the beginning of the 1890s. The development of serotherapy as a specific treatment of diphtheria, is the earliest example of that change: it involved the introduction of quantification and norms largely adapted from German procedures and which were approximate in earlier Pasteur's protocols. It ultimately led to the building up of an Institut Pasteur - associated bio-industry that could compete with the German one.

The present paper thus primarily deals with the manner a "culture of quantification and standardization" has been introduced at the Institut Pasteur or, more precisely, has been imposed by Emile Roux (1853-1933) and his co-workers, as part of Roux's general project on the approach to infectious diseases, based on the direct coupling of research in microbiology to the production of drugs of biological or chemical origin by the institute, and to medical care in Pasteur's hospital located on the campus itself.

The weaknesses of Pasteur's approach to vaccination were known of his fellow scientists.

The distinctive place occupied by Pasteur in the scientific landscape of the French Third Republic has largely been described elsewhere², as have been the conditions and uncertainties of Pasteur's approach to vaccination, following the unveiling of Pasteur's laboratory notes in 1971³. The present paper focuses on the evolution of laboratory practices at the Institut Pasteur during the 10 years that followed its creation in 1887, as it appears to reflect a deliberate strategy of French researchers to narrow the gap existing between Pasteur's statements and Koch's methods. The Koch-Pasteur dispute has been extensively discussed elsewhere⁴, and it can here be assumed that Koch's arraignment of Pasteur's approach to vaccination should primarily be considered as the enunciation of rules of good laboratory practices and methods in microbiology, thus a methodological discussion the benefit of which had been somehow obscured in France by the violence of the exchanges, by hagiography and nationalism. It has however been perceived as such by foreign scientists as well as by scientists around Pasteur⁵.

A short discussion of the anthrax and rabies cases can help defining the scientific problems as these have been expressed. The etiological agent of anthrax⁶ had been characterized by Robert Koch (1843-1910) in 1877, cause of a conflict with Pasteur on priority of discoveries⁷. Several “vaccines” against the disease had been tested in France before 1880, with no clear-cut evidence that effective protection could be achieved. The results of the vaccination of sheep against anthrax obtained by injecting animals with oxygen-attenuated anthrax bacilli were first communicated on February 28, 1881⁸. The experiment was publicly repeated on sheep in May 1881 at Pouilly-le-Fort and its results were highly publicized in France as a success-story. However experiments carried out in 1881-1882 in Germany met with no success and German scientists particularly Koch and Friedrich Loeffler (1852-1915) remained more than sceptical about the efficacy of Pasteur’s vaccine and its conception. Aside emotional and nationalistic comments in France and in Germany, several scientific criticisms were enunciated. It was pointed out that the observed protection might have merely been non-specific, thus addressing the question of specificity of the protection, if any. Other criticisms were elicited by the irreproducibility of the results of vaccination. The initial oxygen-based attenuation process did not reproducibly inactivate the bacilli. Moreover, the protocol for attenuation of the anthrax bacilli used in the Pouilly-le-Fort experiment was published as late as in 1883. A chemically induced attenuation procedure previously rejected by Pasteur but used by Chamberland and Roux had been used without saying; the conditions for the substitution of techniques were rendered public only 50 years later⁹. Finally, the statistical analysis of the results was merely based on the comparison of large numbers, letality in the absence of vaccination being only a rough estimate. Actually, several years¹⁰ of work using Chamberland’s attenuated vaccine enabled Institut Pasteur’s researchers¹¹ to collect a large enough number of cases to conclude at the International Congress of Hygiene in Vienna in 1887 that cattle was protected against anthrax by vaccination to a level of 80-90%¹². In summary, and irrespective of the fact that complete protection against anthrax is still nowadays more difficult to obtain than claimed in the 1880s, doubts concerning Pasteur’s vaccine were due to uncertainties about the quality and the protocol for preparation of the reagent, premature release of significantly “positive” data and insufficient statistical discussion of the results. Co-workers of Pasteur were at least aware of the first two of these problems.

Similar methodological problems emerge out of the rabies case¹³. A critical discussion of the conditions of preparation of the successive anti-rabies vaccines and the conditions of their first use on humans in 1885 has been extensively reported elsewhere¹⁴. Despite, the opposition of Roux to a premature extension to humans of the experiment, despite an immediate controversy in France on the effectiveness and danger of anti-rabies vaccination, the outcome of the first injections to two badly bitten children were understood in 1885 as the defeat of the disease, a strong emotional move that led to the creation of the Institut Pasteur in 1887 and later on, of numerous other Pasteur Institutes elsewhere¹⁵, all primarily devoted to vaccination against rabies. However, the treatment did not always prove efficient against the disease. The occasional absence of efficiency was attributed to the vaccine being injected too late after infection or to the localization of bites on the body. Since 1885 the techniques of preparation of the “virus-vaccine” were modified several times; the protocols used to inject the drug were subjected to major changes (“intensive” vs. “normal” treatment). Finally, no statistical analysis could be carried out in the obvious absence of negative controls and the lack of data concerning propagation of rabies in humans. The summing up of “cured cases” was considered as the proof of vaccine effectiveness, as shown by the monthly description of treated cases in the *Annales de l’Institut Pasteur*. The weakness of the demonstration was evident enough that it needed to be addressed to and was so by N. Gamaleya, vice-head of the Bacteriological Institute of Odessa, the frequency of rabies being much higher in Russia than

in France¹⁶. Thus the notion of success in humans was a kind of extrapolation of data obtained on genuinely vaccinated animals rather than a rigorous demonstration of the development of a protection in presumably infected humans¹⁷. As for anthrax, one could note a combination of uncertainty concerning the vaccine and its administration, the premature release as a success of a treatment on humans and the absence of a proper statistical analysis and comment of the treated cases, all problems that were tentatively answered by the empirical adjustment of procedures and reagents, without, however, bringing the definitive proof of the efficiency of the procedure in man. Of course, microbiology was under construction and the shift to immunology had not occurred yet¹⁸. The description of the host-microbes relationships and the proper methods of study, including the usage of animals, were far from the high rationality prevailing in physiology¹⁹. Statistical analysis of results was far from being widespread. The lack of precision described above could thus have merely been a feature of a discipline emerging from a kind of chaos of observations and results. Whatever it had been due to, the restrictive or protective attitude of Chamberland, Roux and Duclaux strongly suggests they were aware of the problems from the beginning, though they never publicly interfered with Pasteur's approach of vaccination and with his emotional ways of communicating with the public²⁰. Would any doubt be left about the awareness of scientists around Pasteur, the scientific policy prevailing immediately after the creation of the Institut Pasteur and the near retirement of Pasteur, is so sharply contrasting that it implies that the above-mentioned weaknesses had been identified as serious enough to threaten scientific, medical and economical developments, particularly faced to research carried out by Institut Pasteur's main competitor, Koch's Institute of Hygiene in Berlin.

A new environment for French microbiology

The building of the Institut Pasteur has been financed by a public subscription launched by the French Academy of Medicine in 1886 and aimed at creating a rabies research and care centre. The Institut Pasteur inaugurated in 1888 actually obeyed a different project and was much more of an institute of general microbiology, with at most one-sixth of its surface devoted to rabies consultation and vaccination. The rest of the buildings, excepting a kennel located in the animal facilities (*hôpital des animaux*), was attributed to research in general microbiology and to teaching. It was distributed into several well identified laboratories each headed by a senior scientists²¹. From the very beginning of Institut Pasteur's history, Roux has been in charge of defining plans with the architects and supervising the construction. Escaping the original devolution of the building to rabies studies, Roux designed the institute so that the different aspects of microbiology could be dealt with within the same building (the present *batiment historique*). Actually, Pasteur's declining health had forced him to gradually resign after 1887 from his official positions and pushed Roux, who was 35 years old in 1888, towards leading intellectual and administrative positions within the Institut Pasteur²². It is difficult to ascertain why Roux was distinguished among others, particularly since he had opposed Pasteur by considering premature the experimentation on humans of the rabies vaccine. Rabies was the only place of the institute where medicine was practiced, rabies consultation being set apart from the mainstream of research: Jacques-Joseph Grancher (1843-1907), *Chef de service* at the *Hôpital des Enfants-malades* (a Paris hospital located in the vicinity of Institut Pasteur) a highly praised physician specialized in infectious diseases particularly diphtheria and tuberculosis was in charge of rabies consultation and ensured a link with hospital medicine and hygiene.

The plans and the organization of the institute reflect the major change that had occurred in the manner research was conducted around Pasteur. Before the years 1887-1888, research was carried out in Pasteur's own laboratories and facilities at the *Ecole Normale Supérieure* under

his close personal supervision, or at the veterinary school of Maisons-Alfort where Nocard had established a kind of branch of Pasteur's laboratory. The hierarchical structure was rather rigid. In contrast, from 1888 onwards, laboratory space was nominally allocated to heads of laboratories, the publications of which indicate they have become scientifically independent. They were all rather young: Roux was 35, Chamberland 36, Metchnikoff 42, Grancher 44; Yersin, the youngest, was 24 and Duclaux, the oldest, was 47. Duclaux was elected Director of the institute in 1895, after Pasteur's death, but Roux and Metchnikoff clearly were the scientific leaders. The new team leaders were all civilians and have an open attitude towards European, particularly German, science. Roux, a former army physician who had left the army for insubordination, brought medical thinking into a research that he conducted with a rigorous approach to science, including technological developments (photography, temperature controlled incubators etc). Metchnikoff, a zoologist of Ukrainian origin, had long stayed in several European laboratories (1864-1866 in Germany: Heligoland with Kohn, Giessen with Leuckart; 1867 in Naples with Kovalevsky; then from 1869 to 1873 in several marine laboratories in France, Spain and Italy in addition to Russia, which he left in 1888). Metchnikoff brought in a wealth of knowledge on diverse biological systems, including the invertebrates defense system against microbes. Yersin had first studied medicine in Marburg and attended Koch's lessons in Berlin in 1889. Emile Duclaux (1840-1904) a chemist specialist of fermentations, taught biological chemistry at the Sorbonne and offered the link with the university. Chamberland, a physician and biologist close associate of Pasteur on the anthrax issue, was familiar enough with German science to be sent to Germany in 1887 to discuss results with Koch and Loeffler²³. A number of non-permanent members of the Institut Pasteur worked in the laboratories or attended the *cours de microbie technique* given by Roux since 1889, and comprised numerous foreigners including Germans. In a few years, the Institut Pasteur had become a kind of interdisciplinary and international research centre. In general, the years 1880 have in many fields seen a turn towards less tense relations between France and Germany. On the science side, missions to study the functioning of German universities, initiated by chemists before the Franco-Prussian war, had resumed since 1877 and their reports, for example on chemistry or embryology were published and widely discussed in the context of the reform of French universities²⁴. It was assumed that France had been defeated by German science and technology and as a matter of consequence, German universities were considered as models for the strengthening of French university research and teaching and ultimately for revenge. Reports were most often enthusiastic about the level of the teaching, the organization of faculties and the relations between professors and students. Concerning researchers in microbiology at the Institut Pasteur the attitude of Pasteur engaged in conservative political circles and strongly anti-German, contrasts with that of most other scientists working with him. Part of the change in the attitudes may have been associated with the political beliefs of scientific leaders. As seen from official statements and from personal letters, none of them showed any particular chauvinistic attitude. Roux and Behring were friends and Roux and Metchnikoff were the godfathers of a Behring's son. Letters exchanged between Roux and Metchnikoff and between Roux, Behring and Metchnikoff, indicate that Roux and Metchnikoff were highly confident in Koch's scientific knowledge and competence. Metchnikoff and Yersin even submitted to him data or preparations in order to obtain confirmation of some of their most important results, though the two of them were rather ironic concerning the absence of personal opinion of scientists gathered around Koch²⁵. Most of Institut Pasteur's researchers were politically close to socialists or radicals. Roux defended Dreyfus. Duclaux was more of a pacifist, very sensitive to human rights problems. He also defended Dreyfus as early as 1898 and contributed to the creation of "*La ligue des droits de l'homme*". Chamberland had been elected Deputy to the parliament in 1885 and belonged to the *républicain radical* group and was active in defending

laws on public hygiene at the parliament, as did Grancher who belong to the same political party. Metchnikoff was a convinced socialist and pacifist, later a theosophist and had experienced serious problems with the tsarist administration in Odessa. Thus the scientific staff of the Institut Pasteur dominantly belong to the republican meritocracy of the Third Republic impregnated with social-democrat thinking and more open to foreign influences.

The translation effort of foreign scientific publications was by no means new at the end of the years 1880, but the specific effort made by the Institut Pasteur in the follow up of foreign science is shown by the excellent coverage of foreign publications in the the *Annales de l'Institut Pasteur* launched in 1887 by Emile Duclaux (1840-1904)²⁶. Although the rationale underlying the creation of the journal had clearly to deal with rivalry with Koch's journal (*Zeitschrift für Hygiene und Infektionskrankheiten*), with problems of priority and with the self-promotion of results obtained at the Institut Pasteur and related institutions²⁷, the style of the papers was not polemical any longer and the *Annales* reported summaries of all works carried out elsewhere, particularly in Germany, that had looked important enough to the editors: microbiological information published in Germany was made readily available to French microbiologists²⁸. Indeed, in their papers concerning diphtheria, Roux and his colleagues positively discussed the successive and decisive contribution of their German colleagues. As an example, the 1894 best known paper written by Roux and Louis Martin (1864-1946) and dealing with the preparation and testing of anti-diphtheria sera, was preceded by an extensive bibliography of papers published abroad and analysis of the articles by Emil von Behring (1854-1917), Shibasaburo Kitasato (1852-1931) and co-workers on the production of sera able to neutralize bacterial toxins. The authors acknowledged German contributions by plainly writing: *Nous pouvons déclarer que nos résultats confirment, dans ce qu'ils ont d'essentiel, ceux de M. Behring et de ses collaborateurs*²⁹.

In other words, the years 1885-1890 have seen the coming of Pasteur's microbiologists out of the "heroic age". Members of the staff of the Institut Pasteur had gained scientific and practical autonomy in the conduct of their work. Moreover, the work they carried out and the deliberations of the Board of administrators show that they were now conscious of the economical context and competition in which medical or veterinarian usage of biological derivatives was actually embedded³⁰.

Competition over antitoxins and usage of German standardization procedures

Rabies has been a political more than a medical target for Pasteur³¹. In contrast with rabies, other human infectious diseases, tuberculosis, diphtheria, tetanus, syphilis etc, were genuine major public health problems in Western Europe and thereby attracted active research in France as in Germany. Despite early promising results, work on tetanus did not progress well and was rapidly slowed down for the benefit of diphtheria. The work carried out on diphtheria at the Institut Pasteur bears the hallmark of Roux's personal approach. Actually, most of the work of Roux on diphtheria should be considered in a double context. Firstly, the context of a concurrence, a kind of a race with German scientists as pointed out by P. Weindling³²: analysis of publications shows that very similar or complementary results were obtained and published by German and French scientists nearly at the same moment during the development of serotherapy. The work also developed in a way suggesting it was influenced by the will to avoid the criticisms raised against anti-anthrax and anti-rabies vaccines: the work and the results had to be impeccable particularly with respect to the German approach of the same problem(s). Secondly, Roux' scientific strategy was underlaid by a rationalized medical project, proposed in 1894 as the conclusion of the successful trial on diphtheria and which resulted in 1900 in the construction of a dedicated hospital, the *Hôpital de l'Institut Pasteur* located on the Paris campus, in the vicinity of research laboratories³³. To a certain

extent, the first twenty years of existence of the Institut Pasteur have been largely placed in the context of Roux's medical project, which later extended far beyond diphtheria and soon included tropical diseases such as yellow fever, malaria and sleeping sickness as well as the test of their treatment³⁴.

The causative agent of diphtheria is the Klebs-Loeffler bacillus (*Corynebacterium diphtheriae*), identified in 1883 in the throat lesions of patients. In the absence of treatment, diphtheria was in 1892-1894 responsible for an annual death toll of 6-7 per 10000 persons in Europe³⁵. The suffocating form of diphtheria in children (named the "croup") inspired much fear among parents. As for rabies, diphtheria was not only a deadly disease but also was a socially signified target for researchers³⁶. Two main scientific breakthroughs have been made between 1885 and 1890 concerning the mechanisms of action of bacteria and of vaccines, respectively³⁷. It has been shown that the microbial agents of diphtheria and tetanus acted through soluble toxins they release in the infected organisms; Behring and Kitasato have demonstrated that protective immunity could be induced against the toxins by injecting the latter into the proper recipient animals, a notion soon extended by Roux and co-workers to other soluble molecules. Behring and Erich Wernicke discovered that protection was associated with the serum of the immunized animals and transferable to other animals. Serotherapy is the development of that initial and decisive discovery. The first period of research on diphtheria at the Institut Pasteur (1888-1889) corresponded to the verification by Roux³⁸ that the Klebs-Loeffler bacillus was the genuine cause of diphtheria and the demonstration that the symptoms of the disease could be reproduced by injecting animals with the poison secreted by *in vitro* grown bacteria. A year later, Roux and Alexandre Yersin (1853-1943) described some properties of the toxin³⁹. As soon as Wernicke's paper describing the cure of three diphtheria cases in children by injection of the sera of dogs that have survived toxin and bacteria injections, was known and discussed at the institute⁴⁰, the preparation of hyper-immune sera in horses was initiated at the end of 1891 by Edmond Nocard (1850-1903) at the veterinary school of Maisons-Alfort⁴¹. From the fall of 1894, large scale serum production was organized mostly in the annexe of the Institut Pasteur in Marnes-la-Coquette near Paris⁴². The first 1894 paper written by Roux and Martin deals with the preparation and test of the horse sera. The quality and protective features of serum produced in various animals had already been tested by German scientists, the dog serum initially used by Wernicke being a mere by-product of earlier experimental infection experiments. The choice of horses as serum producers suggested by German scientists was promptly verified in Paris: the choice of horses was the result of a compromise between the needs of large-scale production, the lowest spontaneous toxicity of sera, and the highest protective efficacy. In contrast with earlier articles on anthrax or rabies vaccines which were rather imprecise about the technical procedures used, the protocols concerning the isolation and inactivation of diphtheria toxin were extensively described making them reproducible elsewhere. Accordingly, the methods used to control the sequential steps leading to the selection and tests of serum by using *in vivo* assays on guinea pigs and rabbits, were fully described. The protocols of bacterial growth were adapted to the induction of the secretion of the toxin in the supernatant of long-term cultures. The toxicity of diphtheria toxin was defined *in vivo* as the volume of supernatant which kills a 500 g guinea pig in 48 hours. The chemical inactivation of the toxin using iodine trichloride was used and described. It was largely inspired by the disinfectants used by German army physicians during the Franco-Prussian war and systematically studied afterwards. Residual toxicity of inactivated diphtheria toxin was tested on rabbits. The protocol of injection in to horses of increasing doses of the inactivated toxin, then of pure toxin in order to prepare protective immune sera, was described in great detail: the health and behaviour of each injected horse was monitored in laboratory files⁴³ and some

exemplary cases were reported in the paper, including immunization of a cow. More importantly, the anti-toxic properties of the sera of each individual horse were tested *in vivo* on a panel of guinea pigs challenged with a defined quantity of toxin, along with the appropriate negative and positive controls. This enabled the definition of protective units. The definition of the units used by Roux and Martin in 1894 (one ml of horse serum able to neutralize 20 ml of a toxin solution, 0.1ml of the latter killing a 500g guinea pig in 48 hours) was preceded by a one-page description of the three successive types of units used by Behring and Paul Ehrlich (1854-1915). The need for international units was emphasized: Roux speculated that standardization would be of more general use if comparative studies could be carried out using Behring's toxin and sera⁴⁴. Finally, the amount of serum to be administered to children was defined on a weight- (guinea pig) to-weight (child) basis. The entire production and test process thus obeyed a defined and published protocol. The units may have been different in France and Germany, the point is they were linked by a proportionality factor which made easy the comparison of their properties. Standardization of anti-diphtheria serum in France was definitively associated with the fact that German scientists had already worked out their own standards. Indeed, Roux wrote, *A vrai dire, nous n'attachons pas beaucoup d'importance à toutes ces définitions compliquées (...) Cependant il était nécessaire de parler de ces unités de mesure, puisqu'elles sont employées à chaque instant dans les travaux allemands.* Anyway, standardization of biological reagents became an integral part of Pasteurian culture as early as the beginning of 1894. The Direction of Hygiene of the Ministry of the Interior, in a note dated February 10, 1895, defined the conditions of delivery (20-ml vials) of the reagent by the Institut Pasteur and pharmacies, with no mention of other Institutions allowed to produce the serum. The 20 ml-dose was the dose injected by Martin into incoming patients prior to bacteriological diagnosis. It corresponded to the ability to neutralize 200ml of toxin supernatant in the *in vivo* guinea pig test⁴⁵. The anti-diphtheria serum became a drug with standardized usage in France as of April, 25, 1895⁴⁶.

A rational statistical analysis

The second important step in the usage of a therapeutic reagent is the proper statistical analysis of the results obtained on humans. We mentioned in an earlier section that statistical analysis was one of the weaknesses of initial Pasteur's tests of vaccines. Statistical analysis of results of therapeutic trials was not commonly used in medicine⁴⁷ but the progressive assessment of the efficacy of serotherapy by introducing properly defined cohorts and controls has led in 1898 in Denmark to what Ian Chalmers named the first random trial in human medicine⁴⁸. Concerning the definition of test cohorts, an important step took place in 1890 with Roux's decision to introduce systematic bacteriological diagnosis in patients suspected of having diphtheria: physicians were faced to difficulties in diagnosing true diphtheria because of the heterogeneity of the clinical signs shown by patients, thus leading to erroneous diagnoses. The introduction of bacteriological testing of diphtheria had two main consequences: one was clinical in nature, namely concerning prognosis and monitoring of the presence of the bacterium during the evolution of the disease, including during convalescence; the second was statistical in nature, allowing the sorting out of patients into better defined groups. The group of patients suffering from proven diphtheria was further subdivided into two groups, those with diphtheria bacilli alone and those with diphtheria bacilli associated with other pathogens, an association found to be of poor prognosis⁴⁹. In modern statistical terms, this means that cases could be organized in cohorts defined on a bacteriological basis. Bacteriological diagnosis was carried out in a laboratory implemented in the *Hôpital des Enfants-malades* by Louis Martin (1864-1946), a physician selected by Roux among

Grancher's students. The selection of groups among the large number of patients used to test serotherapy was based on bacteriological criteria⁵⁰.

The second 1894 article by Roux and Martin describes large-scale clinical trials of the previously defined anti-diphtheria sera⁵¹. There is no need to present once more the results communicated by Roux at the International Congress of Hygiene of Budapest in September 1894, except by noting that priority had been granted by a preliminary communication given in Lille in spring 1894. At the same Budapest meeting, Hans Aronson of Berlin confirmed that identical results had been obtained in Germany on diphtheria patients, a communication showing that the stage of development of serotherapy was primarily the same in France and in Germany. The discussion that followed oral presentations showed a general consensus on the efficacy of the therapeutic method and pointed to the highly significance of the numbers that have been reported, treated vs. untreated patients⁵².

It may be retrospectively surprising to note that no participant openly reacted to some ambiguities of the study. Roux and Martin compared two cohorts of patients identically defined in terms of bacterial infections: members of the treated group received serum in Grancher's own ward at the *Hôpital des Enfants-malades* in Paris, and the members of the second group, here used as a negative control, were hospitalised at the Hôpital Trousseau, East of Paris and did not receive any injection. However neither the patient population, nor the organisation of the hospital, the hygienic rules applied in the wards, the frequency of tracheotomy etc. were necessarily the same at Trousseau and at the *Enfants-malades*. Although intuitively suggestive of a several-fold decrease of the death toll in treated patients, the results could have been statistically less significant.

Anyway, since the fall 1894, anti-diphtheria serum was largely distributed in Europe by French and German institutes. An overall 2-5-fold decrease in lethality counts was observed through most countries in Europe during the following two years and was attributed to the use of antiserum⁵³, although the effects of new isolation rules in hospitals may have contributed to the decline of mortality. The adverse effects of serum-therapy (serum sickness) were known but barely questioned in France⁵⁴. Accidents however soon limited the usage of the antiserum, by casting some shadow on the benefit of using it. The genuine positive effects of the sera on the outcome of the diseases in diphtheria patients were only proven later, in 1898, by a Danish physician of the Blegdamskospitalet in Copenhagen, Johannes Fibinger (1867-1928). Fibiger wanted to determine if anti-diphtheria serum was worth using considering its serious side effects. He introduced a genuine random trial (same hospital, same diagnosis, one day incoming patients are treated, the other day incoming patients are not etc), used sera of Dane, German and French origin and then demonstrated the existence of a significant benefit for serum-treated patients thus confirming earlier conclusions⁵⁵.

Later on, proper statistical analysis of test results were of particular significance in the evaluation of the efficacy of several antisera and vaccines produced by the Institut Pasteur (against diphtheria, tetanus, typhoid, gangrene, typhus, staphylococci) and tested on the field during World War I by army physicians.

State-defined norms of quality, or Pasteur-control of quality on French producers of antisera ?

A fundamental question concerning the significance of the norms used in France stems from the condition of quasi-monopoly of production and trade of the antisera granted in France to the Institut Pasteur, a condition which contrasts with state-defined standardization procedures used in Germany. Put in other words, was the control of quality of the antiserum, the norms of which had been defined by Institut Pasteur before being confirmed by the Ministry of Interior, exerted by some independent supervisory office or institution? The serum committee of the

Academy of Medicine set up for that purpose in May 1895 by the health administration was largely controlled by influential members of the staff of the Institut Pasteur. It can be concluded that the Institut Pasteur was at the same time issuing norms and controlling the obedience to norms. It is beyond doubt that the Institut Pasteur occupied a privileged position in France concerning infectious diseases, and an original place in the French public health landscape⁵⁶. However, the situation that prevailed before World War I was less of an official monopoly granted to the Institut than a kind of institutionalised tutorship of the Institut Pasteur onto other private and public health institutions. Also, with the exception of the original agreement granted by government agencies to drugs and reagents of therapeutic use against infectious diseases, the production and trade of the latter were left to the initiative of private structures and local administrations, provided their activity was approved by the above mentioned committee, thus indirectly by the Institut Pasteur. In this respect, the Institut Pasteur had in general managed to place itself at critical places in the network of public health institutions organized as a response to the requests of different components of the French administration. As an example, the bacteriological diagnosis of diphtheria had to be carried out either at the Institut Pasteur or in trustworthy laboratories, such as that established in the *Hôpital des Enfants-malades*: this actually meant laboratories headed by people trained at the Institut Pasteur. This observation could in turn define the *Cours de microbiologie technique* created by Roux as early as 1888, not only as an original, high-level training course, but also as a powerful tool for the organization of a network of people and institutions associated in some way to the Institut Pasteur⁵⁷.

The production of anti-diphtheria serum was not co-ordinated at a national level or transferred to industry under the quality control of state agencies, even through delegation given to the Institut Pasteur. Production in Paris was made in response to local needs (Paris and surrounding areas). Dried serum could eventually be transported to more distant places in France and out of France, but local production of antiserum was preferred. However, that production remained under the supervision of the Institut Pasteur of Paris. In distant areas, the serum was produced under the supervision of people trained and approved by the Institut Pasteur on behalf of the committee. Several *instituts sérothérapeutiques* endowed with the production of serum, were created in France at the end of the 19th century (Marseille, Bordeaux, Rennes, Lyon etc.) and abroad, such as in Geneva⁵⁸. Some, because of their name, are not easily identified as such. As an example, *Institut Bouisson Bertrand* was created in Montpellier on February 6, 1897 due to a purely private initiative. The *Institut sérothérapeutique de Lille*, re-named *Institut Pasteur de Lille* in 1898, was created by Albert Calmette (1863-1933) to produce anti-diphtheria serum at the request of the municipality of Lille. Calmette was a well-known “Pasteurian” scientist who had created the Institut Pasteur of Saigon in 1890⁵⁹. In that particular case the use of the name “Pasteur” was granted to an institution placed under the administrative rule of the city of Lille and the Nord department. The interesting, well-documented case of Nancy, illustrates the complexity of the production and trade of anti-diphtheria serum in France⁶⁰. Nancy, a city to which the University of Strasbourg had settled after the Franco-Prussian war of 1870, had developed a strong tradition of hygiene and social care at the city level as well as teaching at the university. Following the 1894 meeting in Budapest, in a manner highly reminiscent of the national initiative of the newspaper *Le Figaro*, which had led to the creation of the annexe of the Institut Pasteur of in Marnes-la-Coquette, a regional subscription was launched by Pierre Parisot (1854-1938), a Professor of legal medicine at the University of Nancy, first to purchase serum from Paris, then to create a centre for its production and distribution. The serum obviously could have been purchased from nearby German factories. The initiative of creating a production centre in Nancy, Germany, may thus have been political as suggested by J. Simon⁶¹. It however appears to participate in a broader strategy which resulted in the creation of a number of

similar centres far from the German boundary and thus independent of nationalistic considerations. The *Institut de sérothérapie de l'Est* started producing anti-diphtheria serum in November 1894 by using toxin provided by Roux. Later, the toxin as well as the serum was produced locally and up to 100 horses could be immunized in 1900, providing serum to all departments of Eastern France. The new building of the *Institut de sérothérapie de l'Est, Fondation Osiris*, was located in Nancy's general hospital and opened in June 1896. It was placed under the supervision of Eugène Macé (1856-1938), a microbiologist trained in 1894 by Roux at the Institut Pasteur. The conditions of the opening and the administrative situation of the *Institut de sérothérapie de l'Est* were complex⁶². It appears to have been a semi-public institution, subsidized by the municipality of Nancy and the department, administrated by the board of directors of the "*Société privée de l'Institut sérothérapique de l'Est*," located in a general hospital and soon included in the *Institut d'Hygiène* of the University and later placed under the scientific and medical control of professors at the Faculty of medicine. It is not clear at this time whether the very same biological criteria as in Paris were used to define the protective units of the sera produced in Nancy. For example, vials containing smaller volumes of serum (10 ml, no titre indicated) were delivered to pharmacists. Nancy is exemplary of the manner procedures were taught person to person to other physicians in Institut Pasteur's laboratories and with the agreement of the local administration, rather than implemented under strict government rules or through industry and its controls. However effective may have been the initial control of the Institut Pasteur on the quality of the reagents and the training of people in charge of producing them, the multiplicity of the production and distribution centres is likely to have rendered difficult maintaining a strict standardization of the anti-toxic activity of the sera distributed throughout France and its colonies.

Two conclusions can be brought out of the present survey of the introduction of norms at the Institut Pasteur as one of the consequences of the development of serotherapy. Firstly, less than five years had been required for all steps of production and clinical tests, to become standardized and included in a procedure endowed by the Institut Pasteur and transmitted to other French health institutions. A culture of standardization, a genuine breaking off earlier practices, was attained and persisted. The whole process had important consequences in the long term. Since the production of anti-diphtheria serum had been initiated in Marnes-la-Coquette in the fall 1894, a dual scientific culture has developed at the Institut Pasteur. Fundamental research in microbiology and related disciplines remained carried out primarily at the Institut Pasteur in Paris proper. Research aimed at producing standardized pharmaceutical reagents was primarily carried out in the Marnes-la-Coquette annexe which started as a mere horse-stable in 1894 and ended as an integrated production centre for a variety of sera and vaccines, largely before WWI. This so-called "applied" research, the associated production of a variety of reagents, although remaining under the supervision of scientists working in Paris *stricto sensu* particularly through the *service de sérothérapie* headed by Roux since 1894, was locally dominated by veterinarians. Among them, Alexis Prévot (? -1926) and Gaston Ramon (1886-1963) were the most prominent figures. Despite the genuine technological and scientific importance of the results obtained in Marnes (such as the discovery of anatoxins and the assay of specific antitoxin antibodies by flocculation tests, both discovered by Ramon soon after WWI), also despite the highly positive financial consequences of these activities for the Institut Pasteur, research carried out in Marnes was largely though unofficially, considered as requiring inferior scientific aptitudes and skills. These two clear-cut cultural identities co-existed until the disappearance of *Institut Pasteur Production* in the early 1970s. Secondly, not considering the administrative context which will be dealt with elsewhere, standardization of the whole process of production of anti-diphtheria serum needed the quantitative assessment of parameters and steps including the

characterization of the virulence of well-defined microbial strains, the toxicity (of toxins and of immunization reagents), controlled attenuation of virulence and evaluation of protective effects of the sera. In this respect, the answers given to the various objections raised against the first wave of Pasteur's vaccines have contributed to the construction of a step-wise, ordered approach to the production of therapeutic reagents. It has often been argued that the French merely had copied the Germans. Analysis of the laboratory practices rather shows a reciprocal usage of the data obtained on both sides, most probably made easier by a change in the attitude of scientists of the Institut Pasteur after 1887. The opposition between French and German scientists, aggressive during the "heroic age" of the Institut Pasteur, had indeed lost its acuteness after 1890 and in some instances, such as Roux, Metchnikoff, Behring and Pfeiffer the relations have reached a rather friendly stage. Although hostility may have remained rooted in some of the persons involved in the competition, the harsh discussions between German and French scientists significantly and positively contributed to improving the emerging concepts and practices of vaccination against infectious agents. The different steps in the production of efficient biological reagents were clearly individualized, and standards and controls were introduced at the proper levels more or less simultaneously in the two countries before merging through the use of Frankfort's standards. The preparation and use of anti-diphtheria serum at the Institut Pasteur thus largely benefited from that "epistemology in progress" which finally resulted in the emergence at the Institut Pasteur, and particularly in the *Service de sérothérapie*, of a kind of culture of quantitative evaluation of therapeutic reagents and clinical trials, which must be opposed to the attitudes prevailing before. Although such a conclusion may appear provocative, although the way of conducting research particularly in the context of nascent medical microbiology was different in Koch-Behring and in Roux-Duclaux laboratories, we suggest that the development, test and rapid diffusion of serum therapy against diphtheria in Europe are the consequences of reciprocal transfers of knowledge from a local context to another and vice-versa. The development of serum-therapy in France was not the mere mimicking or transplant of techniques and protocols set up in Germany, but rather a succession of adaptive moves. A very similar reciprocal transfer of knowledge between German and French laboratories has been studied on several other cases, immunology⁶³ and therapeutic chemistry⁶⁴ at the Institut Pasteur and experimental psychology at the Sorbonne⁶⁵. The history of serum-therapy is thus to be replaced in the general context of the ambiguous and finally positive relations between French and German researchers and universities which developed soon after the 1870 war until WWI⁶⁶.

Bibliography and notes

¹ Grmek, M. D. "L'âge héroïque : les vaccins de Pasteur" in *L'aventure de la vaccination*, dir. A.-M. Moulin, Fayard, Paris, 1996, p143-159

² Salomon-Bayet, C. *Pasteur et la révolution pasteurienne*. Payot: Paris 1998; Debré P., *Louis Pasteur*, Flammarion, Paris, 1994 ; Dubos R. , *Louis Pasteur, franc-tireur de la science*. La Découverte, Paris, 1995

³ Geison, G. *The private science of Louis Pasteur*, Princeton 1995

⁴ Brock, T. D. *Robert Koch: a life in medicine and bacteriology*. ASM press, Washington 1998; Carter, K.C. The Koch-Pasteur dispute on establishing the cause of anthrax, *Bull Hist Med.* 1988 62:42-57; Gradmann, C. *Krankheit im Labor. Robert Koch und die medizinische Bakteriologie*, Göttingen, 2005

⁵ Anonymous. Dr Robert Koch latest estimate of Pasteur's methods and discoveries and the present position of the general inoculation problem. Editorial of the *Boston Medical and Surgical Journal*, 1883 *Vol. CVIII*, N°3, *January 18*. The answer of Pasteur to Koch was published in the same journal, 1883 *Vol. CVIII*, N°9, *March 1*.

⁶ Anthrax, or *charbon* in French, is an infectious disease caused by the sporulating bacterium, *Bacillus anthracis*. The disease exists in animals and humans. In the absence of vaccination, the death toll is about 10% among cattle bred on infected areas. Spores persist for several years in the soil and are responsible for sudden outbreaks of anthrax. This explains in part why discussions about the efficacy of the vaccine were dominated by economics: to be of any use, vaccination of the cattle had to decrease death toll of cows and sheep to below 1-2%, values reached in 1886-1887.

⁷ Koch R. *Ueber die Milzbranddimfung*, 1882. Translation by Carter K. C. in *Essays on Robert Koch*, Greenwood Press, New York, 1987

⁸ Chamberland, Ch., Roux, E. et Pasteur, L., "le vaccin du charbon" *Comptes rendus de l'Académie des sciences* 1881 *CVII*: 666

⁹ The attenuation protocol used in Pouilly le Fort public experiment was not the one used in the earlier publication. The more efficient attenuation protocol, designed by Chamberland and Roux and substituted for that of Pasteur, was only published two years later. Ch. Chamberland and E. Roux, "Sur l'atténuation de la virulence de la bactériidie charbonneuse sous l'influence de substances antiseptiques" *Comptes rendus de l'Académie des Sciences*, 1883, *96*, 1088-1091 and 1401-1402. The existence of a difference in the procedures used in February and in June 1881 was divulged in 1938 (Adrien Loir *A l'ombre de Pasteur* Paris 1938). The usage of chemicals to attenuate bacteria, instead of using exposure to oxygen as speculated by Pasteur, is among the first evidence of the influence of German studies on generalized usage of antiseptic substances.

¹⁰ The list of publications of Pasteur, Chamberland and Roux concerning the vaccination against anthrax can be found in the *Annales de l'Institut Pasteur*, 1908, *22* : 377-380.

¹¹ Chamberland M. "Résultats pratiques de la vaccination charbonneuse" *Annales de l'Institut Pasteur*, 1887, *1*: 301. The content of the paper basically is that of the lecture delivered at the International Congress of Hygiene, Vienna 1887.

¹² A summary of the violent debates opposing Chamberland and others to Loeffler during the International congress of hygiene, Vienna 25 September to 2nd October 1887, can be found in the *Revue d'hygiène et de police sanitaire* 1887 *9* : 910 .

¹³ At the time of these studies, it was already known that the unseen agent of rabies was present in the brain and spinal chord of the affected animals and that brain extracts could transfer the disease to unaffected animals. Human rabies was rather rare in France.

¹⁴ Geison, G. L. "Pasteur, Roux and Rabies: scientific versus clinical mentalities" *History of Medicine and Allied Sciences* 1990 *XLV*: 341-365.

¹⁵ Although many used the name "Institut Pasteur", most were financially and administratively independent of the Institut Pasteur in Paris which had no control on them.

¹⁶ Gamaleya N. "Sur les prétendues statistiques de la rage" *Annales de l'Institut Pasteur*, 1887 *1*: 289

¹⁷ *Revue d'hygiène et de police sanitaire* 1887 *9*: 902-909.

¹⁸ Silverstein A. M. *A history of immunology* Academic Press: San Diego Ca, 1989

¹⁹ Gradman C. « Maladies expérimentales. Les expériences sur l'animal aux débuts de la bactériologie médicale » in Gachelin G. ed. *Les organismes modèles dans la recherche médicale*, Presses Universitaires de France, Paris 2006, p 75-94

²⁰ Neither Duclaux nor Roux ever expressed any restriction about Pasteur's works and hypotheses. The reason for keeping so long laboratory secrets can certainly be deduced by the general policy developed by Duclaux and Roux which gradually made the Institut Pasteur the dominant, not to say the unique, interlocutor of the French government concerning all aspects of microbiology, from hygiene to tropical medicine passing through the production and the control

of therapeutic material of biological origin. The two had experienced the emotional power of Pasteurian discoveries on health and it can be assumed that the Institut Pasteur could certainly not publicly recognize the uncertainties and risks associated to initial cures particularly faced to the growing challenge by medical universities.

²¹ Anonymous. L'Institut Pasteur. *Annales de l'Institut Pasteur*, 1889, 3, 1-17

²² In 1889, Roux, in addition to being the acting director of the Institute, was in charge of the "Cours de microbie technique", of his own laboratory ("laboratoire de microbie technique") which included a group of photomicrography, and of the animal colony. The three other heads of laboratories were Metchnikoff, Chamberland and Duclaux. DEA of Sandra Legout *La famille pasteurienne : le personnel scientifique permanent de l'Institut Pasteur de Paris entre 1889 et 1914*. Paris : Ehes, sept. 1999.

²³ Chamberland, Ch. "Résultats pratiques de la vaccination charbonneuse" *Annales de l'Institut Pasteur*, 1887, 1: 301

²⁴ Gachelin G. "The designing of anti-diphtheria serotherapy at the Institut Pasteur (1888-1900) : the role of a supranational network of microbiologists" *Dynamis*, in press

²⁵ Archives de l'Institut Pasteur (AIP), Fund MTC2 MTC2 correspondence and AIP, Yersin's letters to his mother.

²⁶ The editors were : Roux, Chamberland , Duclaux, Grancher, Nocard, Straus.

²⁷ The first article of the first issue of the *Annales* is a letter by Pasteur developing the success of the anti-rabies vaccination. "Lettre de M. Pasteur sur la rage" *Annales de l'Institut Pasteur* 1887 1: 1-18

²⁸ The *Bulletin de l'Institut Pasteur*, launched in 1903, was exclusively aimed at the discussion of foreign publications.

²⁹ Roux E. et Martin L. "Contribution à l'étude de la diphtérie (serum-thérapie)" *Annales de l'Institut Pasteur* 1894 8: 609

³⁰ As shown for example by the creation by the Institut Pasteur of a commercial society for the production and distribution of the anthrax vaccine.

³¹ Letter of Pasteur to Grancher, 4 septembre 1888.

³² Weindling P. "from medical research to clinical practice: serum therapy for diphtheria in the 1890s" in J. Pickstone ed. *Medical innovations in Historical perspective* St Martin's press : New York, 1992, pp 72-83

³³ Opinel A., "The anti-diphtheria apparatus at the Institut Pasteur (1890-1914)" *Dynamis*. In the press.

³⁴ Opinel, A. "The emergence of French medical entomology: the respective influence of Universities, the Institut Pasteur and Army physicians (1890 ca-1938)" *Medical History*, in the press

³⁵ Weissenfeld J. « die Veränderungen der Sterblichkeit an Diphterie und Schralach », *Centralblatt für allgemeine Gesundheitspege*, 1900, 318, cité dans la revue des journaux par F. H. Renaud , in *la Revue d'hygiène et de police sanitaire*, 1900, 22, 955-956.

³⁶ The success of the anti-diphtheria serum was largely divulged in France through engraving printed at the front page of journals in September 1894, just as for rabies.

³⁷ Silverstein, A. M. op.cit.

³⁸ Roux E. et Martin, L. « Contribution à l'étude de la diphtérie ». *Annales de l'Institut Pasteur*, 1888, 2^e année n°12, 629-661

³⁹ Roux E. et Yersin A. « Contribution à l'étude de la diphtérie. 2^e mémoire » *Annales de l'Institut Pasteur*, 1889, 3^e année n°6, 273-288

⁴⁰ note de lecture des *Annales de l'Institut Pasteur*, 1893, 7, 833-837, signalant l'article de Erich Wernicke « contribution à la connaissance du bacille diphtérique de Loeffler et à la sérothérapie » first published in *Archiv für Hygiene*, 1893, t. XVIII.

⁴¹ Archives of the Ecole vétérinaire d'Alfort, kept at the Archives départementales du Val de Marne, Créteil, France. Some injections and bleeding may have been made in 1893-1894 on the

Institut Pasteur campus (J. Simon oral communication) and very likely at the municipal stables of Grenelle (Paris). Gaston Calmette (Le Figaro, “les étrennes du Figaro” 1er janvier 1895, ascertains the existence of 40 horses at Grenelle in the context of the production of free of charge serum to Paris hospitals. He also mentions that the first immunization was made by Nocard on December 1st, 1891 at Maisons-Alfort.

⁴² The initial name of the Annexe is *domaine de Villeneuve l'Etang*. A detailed study of the architecture and plans of the Marnes-la-Coquette production centre and of its link with the medical project of Roux and Granger is under preparation (Bottineau, Opinel, Rivoirard, Leniaud and Gachelin)

⁴³ Some of these records have been retained at the Archives de l'Institut Pasteur in Paris and at the *Musée des applications de la recherche* in Marnes la Coquette (France).

⁴⁴ In a first step, Ehrlich and his followers including Roux, used a standardization of the sera based on the toxin itself (which amount of serum is needed to neutralize 100 lethal doses of toxin, the lethal dose being the amount of toxin which kills in 4 days a 250h guinea pig). Because of the variability of the toxicity of the toxin supernatants, Ehrlich, and immediately after him the Institut Pasteur, turned to the use of serum standards, more stable than toxin standards. The procedure is described in detail in the course on diphtheria given by Roux and Martin at the Institut Pasteur (Archives de l'Institut Pasteur, fonds Ramon, box RAM-42)

⁴⁵ Roux E., Martin L. et Chaillou A « Trois cents cas de diphtérie traités par le sérum anti-diphtérique » *Annales de l'Institut Pasteur*, 1894 8: 640-661, 1894.

⁴⁶ Anti-diphtheria serum is registered in the *Codex medicamentarius gallicus* 1908, there was no Codex edition between 1884 and 1908. A law was issued on April 25 1895 defines the preparation and use of therapeutic sera (Ministère de l'Intérieur : *Sérums thérapeutique, et autres produits analogues. Législation et réglementation 1895*). A committee is created by decree of the Ministry of interior on May 15, 1895 under the auspices of the Academy of Medicine for controlling the authorizations to be granted to producers.

⁴⁷ Jorland, G., Opinel A. and Weisz G. ed. *Body counts. Medical quantification in a historical and sociological perspective*, McGill-Queens University press, Montreal, 2005

⁴⁸ Chalmers I. “Comparing like with like: some historical milestones in the evolution of methods to create unbiased comparison groups in therapeutic experiments” *British Medical Journal*, 1998 317: 1167

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⁵⁰ Chaillou A. et L. Martin « Etude clinique et bactériologique sur la diphtérie. Travail du laboratoire du Dr. Roux ». *Annales de l'Institut Pasteur*, 1894, 8, 449-478.

⁵¹ Roux, Chaillou et Martin op. cit.

⁵² *Revue d'hygiène et de police sanitaire*, 1894, 16, 784-798.

⁵³ J. Weissenfeld « die Veränderungen der Sterblichkeit an Diphterie und Schralach », *Centralblatt für allgemeine Gesundheitspege*, 1900, 318, cité dans la revue des journaux, F. H. Renaud, *la Revue d'hygiène et de police sanitaire*, 1900, 22, 955-956..

⁵⁴ Landouzy L., *Les sérothérapies* Masson, Paris 1898

⁵⁵ Fibiger J. « Om serumbehandling af difteria ». *Hospitalstidende* 1898, 6, 309-325 et 337-350. English translation of the lecture delivered by Fibiger at the Congress of Medicine at Moscow 30/11/1897 has been published in Bmjournals.com/content/317/issue/7167. Fibiger 's paper is discussed by Hrobjartsson A., Gotzsche C. and Glud C. « The controlled clinical trial turns 100 years : Fibiger's trial of serum treatment of diphtheria » *BMJ*, 1998, 317, 1243.

⁵⁶ The *Ministère d'hygiène, de l'assistance et de la prévoyance sociales*, was created by the decree of January 27, 1920. In earlier times, a “*Direction de l'hygiène publique*” was in charge of public health at the Ministry of Interior since 1889.

⁵⁷ The “*Association des anciens élèves de l’Institut Pasteur*” reflects well the emotional and scientific link which persists between Institut Pasteur and former students of the Institut Pasteur.

⁵⁸ Kaba M. “La diphtérie à Genève à la fin du XIXe siècle: l’entrée en scène de la bactériologie et l’emploi de la sérothérapie”. *Gesnerus* 2004 61: 37-56

⁵⁹ Guénel A. “The creation of the first overseas Pasteur Institute, or the beginning of Albert Calmette’s Pastorian Career” *Medical History* 1999 43:1-25

⁶⁰ Parisot P., *Revue médicale de l’Est* 1894 26:641-642; id 962-963

⁶¹ J. Simon, oral communication

⁶² The annexe of Marnes la Coquette had an administration (Service de sérothérapie), partly independent of the Institut Pasteur, which it however belonged to.

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⁶⁴ Debue Barazer C. «Quand l’admiration d’une culture conduit à l’innovation thérapeutique. Ernest Fourneau et les Allemands » *Gesnerus* 2007 in the press

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