The designing of anti-diphtheria serotherapy at the Institut Pasteur (1888-1900): the role of a supranational network of microbiologists

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SUMMARY: 1.—Introduction. 2.—Shuttling of research on diphtheria between France and Germany. 3.—The designing of French standards and norms in accordance with German ones. 4.—The rapid decline of strong anti-German feelings at the Institut Pasteur.

ABSTRACT: The development of anti-diphtheria serotherapy at the Institut Pasteur immediately follows the crisis known as the Pasteur-Koch debate. Research on diphtheria in Paris is indicative of the importance granted by Pasteurian scientists to Koch's school criticisms. After 1887, relations between French and German bacteriologists become more relaxed. A scientific and social network develops between them. It later extends to other fields of research at the Institut Pasteur, particularly therapeutic chemistry. The evolution of Franco-German relations at the Institut Pasteur is placed in the general framework of the way French universities considered German science.

PALABRAS CLAVE: Difteria, seroterapia, relaciones franco-alemanas, red científica, Roux, von Behring.

KEYWORDS: diphtheria, serotherapy, Franco-German relations, scientific network, Roux, von Behring.

1. Introduction

A violent debate between Louis Pasteur (1822-1895) and Robert Koch (1843-1910) broke out in 1881 on the issue of the vaccination against anthrax soon after the International Congress of Medicine in London held in August of that year. It became openly aggressive after the IV International Congress for Hygiene and Demography (Geneva, September 1882). Koch's and Loeffler's criticisms, not to say indictment, were actually directed against the entire

methodological approach of Pasteur to microbes, microbial diseases and induction of a refractory condition and *in fine* to the validity of the results. The Koch-Pasteur debate, the differences in procedures in Pasteur's and Koch's laboratories, have been studied extensively elsewhere, and will not be examined once more [1]. The main methodological questions raised by Koch in his 1882 paper [2] as summarized in 1883 by distant observers [3], were: claim of the priority of discovery; questioning of the purity of the bacteria used to prepare the vaccine; questioning of the existence of «general laws» of attenuation; abusive generalization of the results of some experiments; abusive publicity given to the anthrax vaccine; strong doubts concerning the efficacy and reproducibility of the vaccine and the secrecy kept on preparation protocols. Several non-scientific reasons are very likely to have boosted the bitterness of the dispute. They are certainly rooted in Koch's and Pasteur's strong respectively anti-French and anti-German feelings [4], and also in the economic interests involved in the success or failure of vaccination of cattle against anthrax [5].

The important point for us lies in the fact that the Pasteur-Koch debate was the first critical discussion of the definitions and methods to be used to identify microbes, prove their role in causing diseases and combating

1. SALOMON-BAYET, Claire. *Pasteur et la revolution pasteurienne,* Paris, Payot, 1998; CARTER, K. Codell. *The rise of causal concepts of disease: case histories*, Burlington, Ashgate, 2003; GEISON, Gerald. *The private science of Louis Pasteur*, Princeton, Princeton University Press, 1995; GRADMANN, Christoph. *Krankheit im Labor. Robert Koch und die medizinische Bakteriologie*, Göttingen, Wallstein Verlag, 2005; MOULIN, Anne-Marie. Le *dernier langage de la médecine*: *histoire de l'immunologie, de Pasteur au Sida*, Paris, PUF, 1991; BROCK, T. D. *Robert Koch: a life in medicine and bacteriology,* Washington, ASM Press, 1998; CARTER, K.C. The Koch-Pasteur dispute on establishing the cause of anthrax. *Bull. Hist. Med*., 1988, *62,* 42-57. A recent English version of the main papers by Koch and by Pasteur dealing with the dispute over anthrax can be downloaded from *www.foundersof science.net*
2. Translated in CARTER, K. C. *Essays of Robert Koch,* New York, Greenwood Press, 1987.
3. Dr Robert Koch latest estimate of Pasteur's methods and discoveries and the present position of the general inoculation problem. Editorial. *Boston Medical and Surgical Journal,* 1883, *108* (3), January 18. The answer of Pasteur to Koch is published in the same journal, (9, March 1).
4. For a discussion of the context by a close witness, on the Pasteur side, of the Koch-Pasteur dispute, see METCHNIKOFF, Elya. *Trois fondateurs de la médecine moderne Lister, Pasteur et Koch*, Paris, Felix Alcan, 1933.
5. Anthrax, or *charbon* in French, is an infectious disease caused by a sporulating bacterium, *Bacillus anthracis*. The disease exists in animals and humans. In the absence of vaccination, the death toll of the disease is about 10% among cattle. This explains in part why discussions about the efficacy of the vaccine were dominated by economics: to be useful, vaccination of cattle had to decrease animals' death toll to below 1-2%.

the latter. Actually, and whatever the response of Pasteur himself to Koch could have been [6], criticisms of Pasteur's methods were not ignored by French microbiologists, although their work, including vaccination against rabies in 1885, somehow continued along their former methodological scheme. In that respect, the development of research on diphtheria in France after 1888 illustrates a movement away from the criticized Pasteur's methods. The work on diphtheria at the Institut Pasteur indeed appears to have developed in a manner indicating an underlying will to avoid the kind of criticisms raised against the anti-anthrax and anti-rabies vaccines. Actually, it also served the introduction of a culture of the norm and of quantification at the Institut Pasteur, in a scientific context which very rapidly lost most of its initial hostile feeling and instead has to be placed in the context of a kind of race between French and German scientists [7]. The aim of the present paper is thus to examine, by studying the diphtheria case, the influence German microbiology had on scientific procedures and attitudes at the Institut Pasteur.

2. Shuttling of research on diphtheria between France and Germany

Diphtheria is an illness affecting the upper respiratory tract, the nosography of which is described and the disease named (*diphtérite*) in 1826 by the French physician P. Bretonneau [8]. Diphtheria is characterized by sore throat, fever, and the progressive development, on the tonsils and the pharynx, of membranes adherent to mucosa, which in the most severe cases, leads to the obstruction of airways and suffocation. Diphtheria is a highly contagious disease spread by infected individuals, and is associated with epidemic outbreaks. During the 1888-1894 period, diphtheria was

6. PASTEUR, Louis. La vaccination contre le charbon. *La Revue Scientifique*, 20 janvier 1883.
7. WEINDLING, Paul. From medical research to clinical practice: serum therapy for diphtheria in the 1890s. *In:* PICKSTONE John V. (Ed.), *Medical innovations in historical perspective,* New York, St Martin's Press, 1992, pp. 72-83; and WEINDLING, P. Scientific elites and laboratory organisation in *fin de siècle* Paris and Berlin. The Pasteur Institute and Robert Koch Institute for Infectious Diseases compared. *In:* A. Cunningham and P. Williams (Eds.), *The laboratory revolution in medicine*, Cambridge, Cambridge University Press, 1992, pp. 170-188.
8. BRETONNEAU, Pierre-Fidèle. *Des inflammations spéciales du tissu muqueux, et en particulier de la diphtérite, ou inflammation pelliculaire, connue sous le nom de croup, d'angine maligne, d'angine gangréneuse, etc,* Paris, Crevot , 1826 (available on line at the Bibliothèque Nationale de France).

responsible for an average annual death toll of 6-7 per 10,000 persons in Europe [9]. Mortality could reach 80 % in children under 10. Consequently, the disease and particularly the suffocating form of diphtheria often observed in children (named the «croup») inspired much fear among parents. As for rabies, diphtheria was not only a deadly disease; it was also a highly significant social target for researchers [10].

Before the introduction of specific antidiphtheria serotherapy in 1894, treatment of diphtheria chiefly consisted of the disinfection of the throat in attempts to kill bacteria and dislodge membranes. One of the first effective treatments, introduced in the 1880s was the insertion of tubes into the throat through the membranes, to prevent victims from suffocating. In the most severe cases, tracheotomy was performed [11]. That therapeutic strategy, associated with hygienic procedures and disinfection, was in use in 1890 [12]. The same procedures later remain in use as adjuvant to serotherapy [13].

The causative agent of diphtheria is *Corynebacterium diphtheriae* (Klebs-Loeffler bacillus), a bacillus first identified in 1883 by E. Klebs (1834-1912) in throat lesions of patients and grown *in vitro* by F. Loeffler (1852-1915) in 1884, two German bacteriologists. The first period of active research on diphtheria at the Institut Pasteur (1888-1889) corresponds to the demonstration by E. Roux (1853-1933) that the Klebs-Loeffler bacillus is the genuine and only cause of diphtheria [14] (thus meeting the requirement of «one bacterial species-one disease» and that of purity of bacterial strains emphasized by Koch). Major scientific breakthroughs are made in Germany at the same moment: the bacteria responsible for two major human infectious diseases, diphtheria and tetanus, are proved to act through the soluble toxins they release in infected organisms. E.von Behring (1854-

9. WEISSENFELD, J. Die Veränderungen der Sterblichkeit an Diphterie und Schralach, *Centralblatt für allgemeine Gesundheitspflege*, 1900, p. 318, cité dans la revue des journaux par RENAUD, F. H. *La Revue d'hygiène et de police sanitaire*, 1900, *22*, 955-956.
10. The success of the anti-diphtheria serum was largely divulged in France through engravings printed on the front page of newspapers in September 1894, just as for rabies.
11. BOURGES, Henri. *La diphtérie*, Paris, Bibliothèque médicale Charcot-Debove, 1892.
12. OPINEL, Annick. The Pasteur hospital as an element of Emile Roux's anti-diphtheria apparatus (1890-1914). *Dynamis*, present issue.
13. SEVESTRE, L.; MARTIN, Louis. *Diphtérie*. *In*: *Traité des maladies de l'enfance*, Paris, Masson et fils, 1896. vol. 1, pp. 616-623 (local treatment) and 655-705 (surgical treatment).
14. ROUX Emile; MARTIN Louis. Contribution à l'étude de la diphtérie. *Annales de l'Institut Pasteur,* 1888, *2* (12), 629-661.

1917) and S. Kitasato (1852-1931) demonstrate that protection against the toxin can be induced by injecting the latter into the proper recipient animals. Protection is associated with the serum of immunized animals and, more importantly, is transferable to other animals. This is followed by showing that the symptoms of the disease can be induced by injecting animals with the toxin alone. A year later, Roux and Yersin detail the properties of the toxin, show it has the features of a *diastase*, and confirm that immunity against the toxin can be induced [15], as it can against other soluble molecules. A therapeutic (still confused with prevention) target is thus identified, different from the bacterium proper: neutralization of the toxin by a serum, instead of attempts at killing bacteria, is now searched for in both countries.

Another important move takes place around 1890 with Roux's decision to carry out systematic bacteriological diagnosis in patients suspected of suffering from diphtheria. Indeed, physicians were facing difficulties in diagnosing «genuine diphtheria» because of the heterogeneity of the clinical signs displayed by patients, thus leading to erratic diagnoses. Also the presence of other bacteria, such as staphylococci and streptococci, could influence the outcome of the illness. 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; the second was statistical, enabling the sorting of patients into better defined, more homogeneous, groups. Only patients suffering from proven diphtheria were retained for subsequent clinical trials. That group was further subdivided into two sub-groups, those with diphtheria bacilli alone and those with diphtheria bacilli associated with other pathogens, an association found to result in a poor prognosis [16]. In modern statistical terms, this means that diphtheria cases could now be organized into defined cohorts, enabling a more reasonable statistical approach. Bacteriological diagnosis was carried out in a laboratory implemented in the Hôpital des Enfants-malades by Louis

15. ROUX, Emile; YERSIN, Alexandre. Contribution à l'étude de la diphtérie. 2e mémoire. *Annales de l'Institut Pasteur,* 1889, *3* (6), 273-288.
16. ROUX, Emile; YERSIN, Alexandre. Contribution à l'étude de la diphtérie. 3e mémoire. *Annales de l'Institut Pasteur*, 1890, *4* (7), 384-426.

Martin (1864-1946), a physician selected by Roux among Grancher's [17] students.

The ping-pong process between France and Germany persists. Between 1890 and 1892, Wernicke and Behring, following studies of serum transfer into infected animals, describe the cure of three diphtheria cases in children by injection of the serum of dogs that have survived experimental diphtheria. The strategy German scientists use is known and discussed in France before their final publication in 1893 [18]. Thus the serum of immunized animals can neutralize the toxin and contribute to curing the patients. The above-mentioned results initiate competition for both the most efficient production of anti-diphtheria serum usable in humans and for protocols of testing. In contrast with earlier experiments, the procedures, the data and the discussion are now given in great detail in the 1894 paper which accompanies the first French large-scale test on children. Before being injected into the serum-producing animals, the toxin has to be produced and inactivated. The Pasteur team is expert in defining the conditions of bacterial growth and toxin secretion. Concerning toxin inactivation, the French team abandons Pasteur's dogma of lowering the virulence by exposure to oxygen or oxidants, and instead uses chemical attenuation of the toxin with iodine trichloride, a procedure inspired by Ehrlich's work on iodine derivatives (who is quoted). The protocol of injection of increasing doses of the inactivated toxin into horses, then of pure toxin in order to prepare protective immune sera, is described in great detail. The health and behaviour of each injected horse is monitored in laboratory files [19], and several exemplary cases are reported in the paper. Roux acknowledges that preparation in horses of hyper-immune sera has been made in concordance with the work carried out in Germany: the quality of the production of serum by various animals had already been tested by German scientists, and the choice of horses as serum producers has been suggested by von Behring as a good compromise between the needs for large-scale produc-

17. Jacques Joseph Grancher (1843-1907) a physician responsible for the department of infectious diseases at the Hôpital des enfants malades in Paris, was from the beginning a follower of Pasteur. A succinct biography can be found at: http://www.pasteur.fr/infosci/archives/f-bio.html
18. Note de lecture des *Annales de l'Institut Pasteur,* 1893, *7*, 833-837, signalant l'article de WERNICKE, Erich. Contribution à la connaissance du bacille diphtérique de Loeffler et à la sérothérapie. (Translation of the paper published in *Archiv fur Hygiene*, 1893, vol. 18).
19. 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).

tion, the lowest spontaneous toxicity of sera, and the highest protective efficacy. Vaccination of horses was initiated in 1891 by Edmond Nocard (1850-1903) at the veterinary school of Maisons-Alfort [20]. Starting from the fall of 1894, serum production is organized in the annexe of the Institut Pasteur in Marnes-la-Coquette, near Paris [21]. It is worth noting that the first 1894 paper written by Roux and Martin dealing with the preparation and testing of protective horse sera acknowledges not less than 23 major contributions by Ehrlich, Behring, Wernicke and Aronson on the topics. This obviously indicates an excellent knowledge of the German literature as expected from the general circulation of scientific information, but should also be taken primarily as the overt recognition of German contributions to the work carried out at the Institut Pasteur.

The second 1894 article by Roux and Martin describes the results of a large-scale clinical trial using previously produced and characterized immune horse sera [22]. The trial was carried out in Grancher's ward at the Hôpital des Enfants-malades in Paris. Grancher had already been closely associated with Pasteur's and Pasteurian work. His ward was devoted to the treatment of children with diphtheria and was organized accordingly [23]. The selection of the test groups out of a large number of patients, as described in the paper presenting results of large-scale serotherapy (see below), was based on bacteriological criteria [24]. There is no need to discuss once more the results communicated by Roux, first in Lille in the spring of 1894 and then at the International Congress of Hygiene in Budapest in September 1894. It is worth noting that at the same meeting, Hans Aronson of Berlin reported identical results concerning the treatment of diphtheria patients. The stage of development reached was primarily the same at the Institut

20. Archives of the Ecole vétérinaire d'Alfort, kept at the Archives départementales du Val de Marne, Créteil. Some injections and bleeding may have been made on the Institut Pasteur campus (J. Simon personal communication).
21. 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 center and of their link with the medical project of Roux and Grancher is under preparation (Bottineau, Opinel, Rivoirard, Leniaud and Gachelin).
22. ROUX, Emile; CHAILLOU, Auguste; MARTIN, Louis. Trois cents cas de diphtérie traités par le sérum anti-diphtérique. *Annales de l'Institut Pasteur*, 1894, *8*, 640-661.
23. OPINEL, note 12.
24. CHAILLOU, Auguste; MARTIN, Louis. Étude clinique et bactériologique sur la diphtérie. Travail du laboratoire du Dr. Roux. *Annales de l'Institut Pasteur*, 1894, *8*, 449-478.

Pasteur and in the Hoechst factory in Germany [25]. The two studies and the discussion that followed show a general consensus on the therapeutic method, as well as on the meaning of the numbers reported [26]. The proceedings did not mention any critical discussion between German and French contributors concerning procedures and uses of serum against diphtheria, but rather noted a consensus. A consequence of that consensus is the extremely fast, world-wide diffusion of serotherapy and the development of the corresponding bio-industry.

Insufficient statistical analysis is one of the weaknesses of Pasteur's initial trials. An important step in the evaluation of a reagent for therapeutic use is the designing of adequate tests cohorts and proper statistical analysis of the results. Roux and Martin compared two cohorts of patients identically defined in terms of symptoms and nature of bacterial infections; subjective and social biases are, however, not excluded. In any case, that type of analysis fitted a use of statistics based on the direct comparison of numbers, which was rather common at the moment and was accepted by all participants of the meeting, including the Germans. The adverse effects of serum-therapy (serum sickness) were known [27] but neglected as considered minor. The genuinely positive effects of immune sera on diphtheria patients are finally proven in 1898, by a Danish physician of the Blegsdamhospitalet in Copenhagen, Johannes Fibinger (1867-1928) [28], in what, according to Chalmers, had been one of the first random trials in medical history [29].

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25. See HÜTTELMAN, Axel. Diphtheria serum and serotherapy. Development, Production and Regulation in fin de siècle Germany, *Dynamis*, present issue.
26. *Revue d'hygiène et de police sanitaire*, 1894, *16*, 784-798.
27. LANDOUZY, Louis. *Sérothérapie. Leçons de thérapeutique et matière médicale,* Paris, Georges Carré &C. Naud Editeurs, 1896.
28. FIBIGER, Joannes. Om serumbehandling af difteria. *Hospitalstidende,* 1898, *6*, 309-325 and 337-350. The English translation of the lecture delivered by Fibiger at the Congress of Medicine at Moscow (30/11/1897)has been published in *Bmjjournals.com/content/317/issue 7167*. Fibiger's paper is discussed by HROBJARTSSON, A.; GOTZSCHE, C.; GLUUD, C. The controlled clinical trial turns 100 years: Fibiger's trial of serum treatment of diphteria. *British Medical Journal*, 1998, *317*, 1243.
29. CHALMERS, Ian. 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.

3. The designing of French standards and norms in accordance with German ones

In contrast with earlier articles on anthrax or rabies vaccines, the protocols concerning the isolation and inactivation of diphtheria toxin were extensively described, making them easily reproducible elsewhere. The protocols of bacterial growth were progressively improved for better secretion of toxin in the supernatant of long-term cultures. A strain of *Corynebacterium diphteriae* (strain nr 261) was selected as a high producer of toxin under specific growth conditions [30]. This indicates an important move towards the use of reference strains for production instead of bacterial samples freshly isolated from patients and selected on the basis of their *in vivo* virulence. Accordingly, the methods used to control the sequential steps of the production and selection of serum by using *in vivo* assays on guinea pigs and rabbits were fully described. The French procedure was designed by Roux, but its basis had been established previously in Germany. The toxicity unit of diphtheria toxin was defined *in vivo* as the volume of supernatant which kills a 500-g guinea pig in 48 hours. This in turn enabled the definition of protective units. The description of that 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 500-g guinea pig in 48 hours) was preceded by a one-page description of the three successive definitions of units used by Behring and Paul Ehrlich (1854-1915). The important point for the present analysis is the fact that protective units, although defined differently in France and in Germany, are linked by a simple proportionality factor, which facilitates the comparison of reagents. The need for international units is emphasized: Roux speculates that standardization would be of more general use if comparative studies could be carried out using Behring's toxin and sera [31]. Ehrlich and Roux first used a standardization based on the neutralization of the activity of the toxin (the amount of serum needed to neutralize 100 lethal doses of toxin). Because of the variability in toxicity of *C. diptheriae* culture supernatants, Ehrlich, and immediately after him, the Institut Pasteur,

30. DELAUNAY, Albert. *L'Institut Pasteur des origines à aujourd'hui,* Paris, Editions France-Empire, 1962, p. 326.
31. ROUX, CHAILLOU, MARTIN, note 22.

rapidly turned to the use of serum standards, more stable than toxin standards. The procedure was described in detail in the lectures on diphtheria given by Roux and Martin to physicians [32]. Finally, the quantity of serum to be administered to children was defined on a weight- (guinea pig) to-weight (child) basis, which implies that researchers admit that the human response to the serum was qualitatively and quantitatively identical to that of guinea pigs. The entire production and test process thus obeyed a defined and public protocol. Standardization of biological reagents had become an integral part of Pasteurian scientific culture as early as the beginning of 1894. The anti-diphtheria serum was a drug in standardized use in France at the beginning of 1895 [33]. The standardization of anti-diphtheria serum in France is definitively associated with the fact that German scientists had already worked out their own standards. Roux writes, «*A vrai dire, nous n'attachons pas beaucoup d'importance à toutes ces definitions compliquées* (...) *Cependant il était nécessaire de parler de ces unités de mesure, puisqu'elles sont employées à chaque instant dans les travaux allemands*» [34]. Such a sentence is unambiguous concerning the compliance with German methods (*in vivo* assay on guinea pigs) and the approach to norms (definition of comparative units).

Up to that point, the table-tennis research on serotherapy has led to nearly identical results in the two countries. Later, divergence on the serotherapy issue between France and Germany is found in the choice between a state-controlled standardization and production of sera in Germany following Ehrlich's proposals, and endorsement by state agencies of privately defined standardization and production of sera in France —private and placed under the auspices of the Institut Pasteur and its subsidiaries. This point is discussed elsewhere [35].

32. Archives de l'Institut Pasteur, fonds Ramon, box RAM-42.
33. Anti-diphtheria serum is registered in the *Codex medicamentarius gallicus* 1908; there was no *Codex* edition between 1884 and 1908.
34. ROUX, CHAILLOU, MARTIN, note 22.
35. GACHELIN, Gabriel. The development of anti-diphtheria serum-therapy at the Institut Pasteur: construction of a culture of standardization. *In: Wertbestimmungen-Evaluations. Standardising therapeutic agents 1890-1930* [Christoph Gradmann, ed., in press]. See also the papers by Simon and Hüntelmann in the present issue of *Dynamis*.

4. The rapid decline of strong anti-German feelings at the Institut Pasteur

The last open conflict between Pasteur's and Koch's followers about scientific issues appears to have occurred in 1887, still concerning the anthrax issue [36]. In a few years after 1882, the attitude of French bacteriologists towards science produced in Germany is transformed into a scientific and probably, but insufficiently documented, economic race, at least as far as diphtheria is concerned.

It appears from the diphtheria case that after 1887/1888, science around Pasteur becomes structured in such a way that it answers most of Koch's criticisms. This may have been due in part to the fact that the two leaders have been rather left aside in the work on diphtheria, the leaders of which are now Roux in France and von Behring in Germany. In France, the Institut Pasteur is inaugurated in 1888. Its plans were supervised by Roux [37]. The institute is designed so that all matters concerning the different aspects of microbiology could be dealt with within the same building. It was initially thought of as an anti-rabies institute and indeed possesses the proper space for consultation and preparation as well as a kennel outside of the main building. It was in fact, from the beginning thought out much more as an institute of general microbiology than as a rabies institute. Before that, research was carried out in Pasteur's own laboratories and facilities at the Ecole Normale Supérieure under the close supervision of Pasteur. From 1888 onwards, laboratory space is clearly allocated to heads of laboratories rather autonomously dealing with various aspects of microbiology. The scientists in charge of the different laboratories were nearly all in their 40s or younger: Roux 35, Chamberland 36, Metchnikoff 42, Grancher 44; Yersin, the youngest, was 24 and Duclaux, the oldest, was 47. The dispensary for rabies is placed under the supervision of Jacques Joseph Grancher, paediatrician associated with Pasteur from the beginning of the work on rabies. Thus, medical applications are physically and administratively set apart from research. Other highly significant changes concern the scientific and medical staff working at the new institute. Pasteur's declining health forces him to gradually resign after 1887 from his official positions and

36. Conflicts related to commercial interests and distribution will not be discussed in the present paper.
37. L'Institut Pasteur. *Annales de l'Institut Pasteur,* 1889, *3*, 1-17.

pushes Roux, at the age of 35 in 1888, towards leading intellectual and administrative positions within the Institut Pasteur [38]. Duclaux is nominated Director in 1895, after Pasteur's death; but evidence indicates that Roux is, along with Metchnikoff, the scientific leader of the Institute. The sudden taking over of research responsibilities at the Institut Pasteur by a rather young and primarily civilian staff (by comparison to Koch's nearly exclusively army physician environment) is associated with an open attitude towards European, particularly German, science. The association of their respective training makes the Institut Pasteur a kind of multidisciplinary research centre. Roux, a former army physician who had left the army for insubordination, brings medical thinking into research that he conducts with a more rigorous approach to science than that of Pasteur. 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) and was very familiar with German laboratory life. He brings in a wealth of knowledge on diverse biological systems, including the defense of invertebrates against microbes. Yersin had first studied medicine in Marburg and attended Koch's lessons in Berlin in 1889. Emile Duclaux (1840-1904) is a chemist and a biologist, a specialist of fermentations, and teaches at the university. Chamberland, a physician and biologist, was a close associate of Pasteur on the anthrax issue, and is sufficiently aware of German approaches to the issue to be sent to Germany in 1887 to discuss results with Koch and Loeffler [39].

The familiarity of Pasteurian scientists with German scientific life is also shown by the titles of the books in the institute's library and by the coverage of foreign publications in the scientific journal the *Annales de l'Institut Pasteur*, launched in 1887 by Duclaux [40]. Although the rationale for creating a new journal clearly was the promotion of the results obtained

38. In 1889, Roux, in addition to being the acting director of the Institute, was also 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. DEA of LEGOUT, Sandra. *La famille pasteurienne: le personnel scientifique permanent de l'Institut Pasteur de Paris entre 1889 et 1914,* Paris, EHESS, sept. 1999.
39. CHAMBERLAND, Charles. Résultats pratiques de la vaccination charbonneuse. *Annales de l'Institut Pasteur*, 1887, *1*, 301.
40. The editorial board grouped Roux, Chamberland , Duclaux, Grancher, Nocard and Straus.

at the Institut Pasteur and related institutions [41], the style of the papers was not polemical, and the *Annales* reported summaries of all works carried out elsewhere, particularly in Germany, that had appeared important enough to the editors. Other medical and scientific journals obviously have contributed for long to the diffusion of scientific information: the interesting point here is that the process stems from the inside of the Institut Pasteur and thus reflects a local attitude [42]. Indeed, in their papers concerning diphtheria, Roux and his colleagues discuss positively the successive and decisive contributions of their German colleagues. As already mentioned, the 1894, best-known paper written by Roux and Louis Martin (1864-1946) dealing with the preparation and testing of anti-diphtheria sera, is preceded by an extensive bibliography including papers published abroad and a detailed analysis of the papers by Behring, Kitasato and co-workers on the production of sera able to neutralize bacterial toxins. Roux openly admits that Institut Pasteur's team had greatly benefited from earlier work on the inactivation of toxins and selection of the most suitable animals for vaccine production. He acknowledges German contributions by writing «*Nous pouvons declarer que nos résultats confirment, dans ce qu'ils ont d'essentiel, ceux de M. Behring et de ses collaborateurs*» [43]. The existence, at the same moment, of close and collaborative contacts (exchange of bacterial strains, sera and various reagents) between German and French microbiologists extends beyond the diphtheria case, as shown by a recent study on the endotoxin-anti-endotoxin issue [44]. Over a period extending from 1885 to 1905, Metchnikoff, first in Odessa, then in Paris, and A. Besredka (1870-1940, a Ukrainian-born scientist who moved with Metchnikoff to Paris and succeeded him in 1916), on one side and R. Pfeiffer (1858-1945) in Koch's laboratory, on the other side, though conflicting on the issue, nevertheless

41. The first article of the first issue of the *Annales* is a letter by L. 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.
42. That trend is further developed later. The *Bulletin de l'Institut Pasteur,* launched in 1903, exclusively contains reviews of scientific papers published elsewhere. It is an informative source of what was considered important for the medical and biological communities. The German contributions are largely quoted.
43. ROUX, Emile; MARTIN, Louis. Contribution à l'étude de la diphterie (serum-thérapie). *Annales de l'Institut Pasteur,* 1894, *8*, 609.
44. RIETSCHEL, Ernst; CAVAILLON, Jean-Marc.Endotoxin and anti-endotoxin: The contribution of the schools of Koch and Pasteur: Life, milestone-experiments and concepts of Richard Pfeiffer (Berlin) and Alexandre Besredka (Paris). *J. Endotoxin Res.*, 2002, *8*, 71-82.

cooperated over the existence of anti-endotoxin antibodies able to neutralize endotoxins, an important issue in the emerging field of innate immunity. Institut Pasteur scientists did not hesitate to attend meetings in Germany, an attitude contrasting with that of Pasteur. French microbiology was thus inserted differently into the European landscape of the discipline, which to a certain extent means the sharing of methods and concepts, not to say the ability to use the same language.

Part of the change in attitude towards German science is also likely to have been associated with the political and social convictions of some of the main scientific leaders of the Institut Pasteur. Pasteur had been deeply engaged in political circles of the Second Empire, and was politically conservative and strongly anti-German. By contrast, as mentioned, Roux's statements did not show chauvinistic attitudes. Roux's letters to Metchnikoff are explicit on that point, and Roux expresses his opposition to anti-German campaigns concerning the attendance at meetings in Germany. Also in his letters, over a period of 15 years, Roux several times expresses his admiration and confidence in Koch [45]. Roux, Metchnikoff and Behring were friends to the point that Roux and Metchnikoff were the godfathers of Behring's sons [46]. Behring and Metchnikoff freely exchanged scientific information on various subjects including work in progress [47]. On the political side, Roux defended Alfred Dreyfus at least at the moment of the revision trial of 1899 [48]. 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 et du citoyen*», of which he became vice-president. Chamberland had been elected Deputy to the parliament in 1885 and belonged to the *républicain radical* group and proved active in defending laws on hygiene. Metchnikoff was a convinced socialist and pacifist and experienced serious problems with the tsarist administration in Odessa.

Research facilities and access to teaching at the Institut Pasteur were largely open to foreigners. Many foreign physicians attended the *Cours de*

45. Archives de l'Institut Pasteur (AIP), Fund MTC2, letter of Roux to Metchnikoff 19 August 1890.
46. Correspondence between Metchnikoff and Behring, and Roux to Metchnikoff, AIP, Fund MTC2.
47. AIP, Fund MTC2 correspondence Behring-Metchnikoff.
48. AIP, Fund Metchnikoff, file 2, letters of Roux to Metchnikoff dated August 16, September 7 and 12, 1899, concerning the Rennes trial.

microbie technique created by Roux in 1888. Metchnikoff, who spoke several foreign languages, opened his laboratory to researchers of many countries (at least 20 different countries of origin were listed from 1888, date of the founding of the laboratory, until Metchnikoff's death in 1916 [49]). During the period of time considered (ca 1890 to ca 1900), a minimum of 8 German scientists spent a year or more at the Institut Pasteur, predominantly in Metchnikoff's and Roux' laboratories. That number, though small compared to the number of Russians, is nevertheless equivalent to the number of British scientists (about 9). Among the German scientists having worked in Roux's laboratory, the presence of Hermann Duenschmann is worthy of note. He spent 2 years (1893-1894) working on black-leg disease and published a paper in *The Annales de l'Institut Pasteur* reporting attempts to prepare a curative antiserum against the disease [50]. It is also worth noting that he developed a technique of immunization using organ filtrates, which remained in use by Roux for years [51] . Dueschmann also worked on megacytes and reported discussions with Metchnikoff and people from his laboratory. Thus, in contrast to what had been the Pasteur laboratory at the rue d'Ulm, the Institut Pasteur quite rapidly moved towards being a kind of international institution in which Metchnikoff and Roux most probably played a dominant role. In other words, the years 1885-1890 constituted a kind of rapid transition period from the «heroic age» towards an open and international approach of microbiology and science in general. Foreign visiting scientists worked at the bench along with their French colleagues.

As a consequence, research in microbiology at the Institut Pasteur had adopted some of the important issues of Koch's and coworkers' laboratory, namely methods, quantification of reagents and norms, while keeping to its original «host-microbe relationship» approach to infectious diseases, which led to the subsequent development of immunology. The same motives that had made German microbiology «palatable» and had to some extent transformed the Pasteurian approach to microbiology, had converted the Institut Pasteur into a genuinely international structure, on the one hand

49. LEGOUT, note 38.
50. DUENSCHMANN, Hermann. Étude expérimentale sur le charbon asymptomatique. *Annales de l'Institut Pasteur*, 1894, *8*, 403-434.
51. Quoted and discussed in *Blackleg vaccines: their production and use*, Kansas State Agricultural Experiment Station, Technical Bulletin 10, June 1923, Topeka , Kansas, 1923.

strengthening its influence abroad, on the other hand benefiting from a flow of foreign experience, models and diseases.

The place given by the Institut Pasteur to German science as a model and as a competitor extended to other fields of research such as chemistry and biological chemistry. Duclaux, who shared with Roux the opinion that microbiology and fermentations had to be placed in close contact with research in organic chemistry, sent a chemist, Gabriel Bertrand (1867-1962), to study that very problem in Germany. The conclusion of the mission of Bertrand [52], who stayed in Germany in 1897, resulted in the construction of the biological chemistry building dedicated in 1901. Concerning chemistry, a recent analysis of the history of therapeutic chemistry in France made by Debue-Barazer extends the conclusions reached in microbiology to therapeutic chemistry at the Institut Pasteur [53]. Ernest Fourneau (1872-1949) a pharmacist, escaped the tradition of Berthelot's chemistry by being trained in Germany, first in Heidelberg in 1899 with L. Gattermann (1860-1921), a specialist in the synthesis of organic molecules, then in Berlin in the laboratory of Emil Fisher (1852-1919), a former student of A. von Bayer, also a specialist in the synthesis of organic molecules including peptides, and finally in Munich in Richard Willstätter's laboratory in 1901, where he studied the structure and synthesis of alkaloids. Fourneau came back to France in 1902 and moved to the Institut Pasteur soon after, at Roux's request. He then created the laboratory of therapeutic chemistry, largely inspired in its methods and research themes by his German experience. As for anti-diphtheria serotherapy, the syntheses of organic molecules by Fourneau and co-workers paralleled syntheses carried out in German laboratories and showed a similar convergence and rivalry between the Institut Pasteur associated with Rhone-Poulenc, and the German laboratories and firms, concerning arsenic derivatives used against malaria, sleeping sickness and syphilis, and derivatives of alkaloïds used for anaesthesia, to keep to the period of time we have considered [54]. Debue-Barazer concludes that this was not mimicry or copying of German chemistry on the part of Fourneau but rather the rational use of what had been learned to develop one's own

52. Gabriel Bertrand (1867-1962), biologist and chemist, created the laboratory of biological chemistry at the Institut Pasteur. He is the discoverer of oligo-elements.
53. DEBUE-BARAZER, Christine. Quand l'admiration d'une culture conduit à l'innovation thérapeutique. Ernest Fourneau et les Allemands, *Gesnerus,* 2007 (in press).
54. DEBUE-BARAZER, note 53.

way of research. The biological chemistry building was a combination of laboratories organized in a manner reminiscent of that suggested by L. Hugounenq in his 1898 report on the teaching of medical chemistry in Germany [55]. The «Pasteurian touch» to that construction was brought by the addition of a hospital devoted to infectious diseases according to Roux's general plan for the Institute [56].

Classical historiography presents the attitude of France towards Germany after the Franco-Prussian war of 1870 as dominated by an obsession for revenge. Keeping to the scientific domain, to a certain extent the Koch-Pasteur dispute can be viewed in that way. Concerning science and universities in general, the reality appears somewhat different, and the history of serum-therapy, like that of anti-endotoxin sera and organic syntheses later, are to be placed in the more general context of their relations during the last third of the 19th century [57]. The opinion that France had been defeated by Prussian science, industry and technology was used as a strong argument for reforming French universities in 1885 largely following the German model [58]. The place of the German model in the organization of universities, teaching and research in France has recently been extensively analysed by Charle [59]. This was not anything new at the end of the 19th century. The relative decline of the place occupied by French research in general among European universities had been observed since 1860, particularly by chemists [60]. The creativity of French chemistry was declining since the middle of the 19th century and, as Wurtz noted before the Franco-Prussian war, Germany was the place organic syntheses were getting shaped [61]. The situation was preoccupying enough to send missions to Germany as early as 1866 in order to study the organization of German universities assumed to be at the origin of German military, scientific and

55. HUGOUNENQ, Léon. L'enseignement de la chimie médicale en Allemagne et en France. *Revue internationale de l'enseignement,* 1897, *33,* 97-105.
56. OPINEL, note 12.
57. PAUL, Harry W. *The sorcerer's apprentice: the French scientist's image of German Science, 1840-1919,* Gainesville, University of Florida Press, 1972 and DIGEON, Claude. *La crise allemande de la pensée française, 1870-1914,* Paris, Presses Universitaires de France, 1992.
58. WEISZ, Georges *The emergence of modern universities in France*, Princeton, Princeton University Press, 1983.
59. CHARLE, Christophe. *La république des universitaires 1870-1940,* Paris, Editions du Seuil, 1994.
60. PAUL, note 57.
61. PAUL, Harry W. *From knowledge to power: the rise of the science empire in France 1860-1939,* Cambridge, Cambridge University Press, 1985.

economic successes. The shock of defeat and the causes attributed to it prompted the resumption of missions to Germany, as early as 1877, all of them followed by the writing of reports largely publicized in issues of the *Revue internationale de l'enseignement*. Nearly all of the reports were enthusiastic about the quality of research and teaching in German universities, as well as about the quality of the relations between professors and students. This was not limited to biology and chemistry. A very similar reciprocal transfer of knowledge between Germany and France has recently been noted in experimental psychology [62]. German universities were indeed used as a starting point to reform the higher education and research system in France. Microbiology, experimental biology and chemistry are merely mentioned in Charle's studies, with the exception of some aspects of Caullery's report on zoology [63]. The case of the Institut Pasteur partly fills in the gap. It indicates that German science has certainly acted as a model and the attitude of Pasteurian scientists was not any different from that of other university professors. The German way of doing science was known of Pasteurian scientists and was adapted to their own specific goals and approaches. Analysis of laboratory practices, rather, shows a reciprocal use of the data obtained on both sides in a kind of epistemology «in progress» aimed at designing specific biological and chemical reagents. The harsh discussions between German and French scientists contributed significantly and positively to improving emerging concepts and practices as well as to setting up norms and standards for biological reagents. ▮

62. CARROY, Jacqueline; SCHMIDGEN, H. Reaktionsversuche in Leipzig, Paris und Würzburg: Die deutsch-französische Geschichte eines psychologischen Experiments, 1890-1910. *Medizinhistorisches Journal*, 2004, *39*, 27-55 (English preprint available from the Max Planck Institut für Wissenschaftgeschichte, Berlin).
63. CAULLERY, Maurice. La zoologie dans les universités allemandes. *Revue internationale de l'enseignement*, 1894, *27*, 398-420.