

**THE GENESIS OF RABIES PREVENTION INDUCED BY PASTEUR'S
VACCINES AND THE PROTECTIVE PHENOMENON MEDIATED BY
INTERFERONS.**

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ABSTRACT

Considerations about the development of the anti-rabies vaccine based on the analysis of historical documents and published scientific articles including correlations with the effect mediated by interferon – interferosis, are presented. In this review, an updated philosophical interpretation is presented on the technical report elaborated by Dr. Prof. August F. Dos Santos, professor at the College of Medicine and doctor of the Santa Casa de Misericórdia of Rio de Janeiro, referring to the training undergone in France under Louis Pasteur's orientation, during the period of 06/1886 - 07/1887, as well as other published documents of marked impact up to the mid-XXth century.

INTRODUCTION

After the discovery of Brazil, in 1500, many people, coming from all parts of the world, merged in what became the State of Rio de Janeiro. With them, several infectious diseases were imported, with serious consequences for public health. This sanitary problem was diagnosed mainly after the arrival of the imperial family, in 1808. D. Pedro II, emperor of Brazil, and Louis Pasteur knew each other quite well, and letters between them demonstrated the intention of the former to bring the Frenchman to Rio de Janeiro (Franco, 1969). Because that was impractical, the emperor sent one of his own scientists, Dr. Augusto Ferreira dos Santos, to Paris. This foresight of D. Pedro II gave Rio de Janeiro, then capital of Imperial Brazil, a main role in the early days of virology.

The report that Dr. Santos elaborated after spending 14 months at the laboratory of Pasteur, and working together with other prominent scientists on rabies prevention, constitute a rich source of data and historical commentaries, registered and evaluated

intensively by the author, regarding the methods and techniques used in the Laboratory of the Rue D' Ulm, workplace of Pasteur, whose results brought a striking contribution to contemporary Preventive Medicine. In his report, Dr Santos describes in great detail the elaboration and application of the viral preparation used to interrupt the development of infection, before this compromised the CNS of individuals attacked by unmistakably rabid dogs.

The analysis of the vaccine scheme as conceived by Louis Pasteur in the XIX century for rabies prevention offers an excellent range of information on the physiopathological processes involved in the response of individuals to vaccination. This information has academic-scientific and historical characteristics, therefore permitting virological conceptions to be confronted and revised in the light of current knowledge, thus favoring the clarification of many of the effective dogmas in the scope of anti-rabies vaccination.

Unlike the preparation of Pasteur, the product presented by Fuenzalida and Palacios, almost 50 years later, is not infectious, however, results in similar protection when compared to the prevention of the clinical picture of rabies, that invariably ends in death (Fuenzalida & Palacios, 1955).

The products developed by Pasteur and his disciples demonstrated the ingenuity of this French scientist and still serve to underly the basic concepts for virologists who work hard in the area of epidemiology and the prevention of rabies in human beings or domestic and/or wild animals.

It must be noted that the significance of some terms, such as “vaccination” and “virus” changed, meaning at that time “introduce a substance” and “toxin”.

For a better understanding of the reader, the extractions of facts and narratives from Santos' report presented in this article are identified according to the page of the original document and selected by their pertinence regarding the ideas presented in this article.

At a time of unfamiliarity about viruses and viral diseases, of first essays on the meaning of antibodies, Louis Pasteur passes to history presenting his results of research with dogs that, after receiving a sequence of suspensions with characteristics of gradually increased virulence, became protected and did not contract the historical and lethal disease: rabies (Santos, 1888).

“...aplicação destes factos fornece um methodo de vacinação dos cães contra a raiva.” (Santos, 1888, pág.110). [...the application of these factors supplies a dog vaccination method for rabies].

“Numerosas experiências demonstraram a possibilidade de conferir aos animais a imunidade contra a raiva, ainda mesmo seguida à infecção por trepanação.” (Santos, 1888, pág.279). [Numerous experiences demonstrated the possibility to confer immunity against rabies to the animals, even followed by challenge through intra-cranial infection].

The effectiveness of the process of rabies prevention transposed to human beings bitten by rabid animals is the consecration of the method.

“Nesta memorável sessão (da Academia Francesa de Ciencias), o grande sábio comunicou os factos...registrando as primeiras aplicações do tratamento preventivo da raiva na espécie humana...” (Santos, 1888, pág.127). [In this memorable session, (at the French Science Academy) the great wise man

communicated the facts ... registering the first applications of the preventive treatment of rabies in human beings].

“A raiva, esta afecção terrível, contra a qual todas as tentativas terapêuticas tinham até o presente naufragado, encontrou finalmente o seu remédio! O Sr. Pasteur, que não teve, nesta senda, precursor algum, foi conduzido, por uma série de investigações prosseguidas sem interrupção durante annos, a crear um novo methodo de tratamento por meio do qual pôde-se impedir, com mão segura, o desenvolvimento da raiva no homem recentemente mordido por um cão enraivado...” (Santos, 1888, pág.127). [Rabies, this terrible disease, against which all the therapeutic attempts had foundered up to now, finally found its medicine! Mr. Pasteur, who did not have, in this pathway, any precursor, was driven, by a series of investigations continued without interruption for years, to create a new method of treatment through which it was possible to interrupt, safely, the development of rabies in a human recently bitten by a rabid dog].

“Em sessão de 01 de março de 1886, apresentando à Academia das Sciencias os resultados da applicação do methodo que descobrira para prevenir a raiva após mordedura, comunicou o illustre Sr. Pasteur...” (Santos, 1888, pág.130). [In the session on March 1st 1886, the illustrious Mr. Pasteur presented to the Academy of Sciences the results of the application of the method he had discovered to prevent rabies after bite,...].

“As estatísticas, feitas com o maior rigor, confirmam as vantagens da applicação á espécie humana da admirável descoberta a que conduziram taes experiências” (Santos, 1888, pág.279). [Statistics strictly carried out, confirm the advantages of

the application to humans of the admirable discovery to which those experiences led].

“A victoria do tratamento preventivo da raiva está definitivamente alcançada. Os algarismos o demonstram á evidencia; a sua eloqüência é superior à eloqüência das palavras.” (Santos, 1888, pág.279). [The victory of the rabies preventive treatment is definitely achieved. The numerals speak for themselves; their eloquence is superior to the eloquence of the words].

The report of Dr. Santos also places emphasis on the premise that, according to Pasteur, it is necessary to inoculate an infectious suspension of virus, obtained by sequential propagation in rabbits (fixed virus), later being inoculated in dogs or human beings.

“... terminar o tratamento por uma última inoculação muito virulenta, inoculação d’um vírus de verificação, a fim de confirmar e de reforçar o estado refratário” (Santos, 1888, pág.279). [...to finish the treatment the last inoculation should be a very virulent challenge virus, in order to confirm and to reinforce the resistant state].

“Em janeiro de 1887, começou-se novamente a empregar um vírus menos forte e a inocular uma única vez a medula de dous dias; esta modificação no tratamento não produziu bons resultados. Voltou-se então ás vacinações anteriores, e o sucesso do methodo não tardou a mostrar-se” (Santos, 1888, pág.279). [In January 1887, a weaker infectious virus preparation started to be used again as well as to inoculate the brain of two days; this modification in the treatment did not produce good results. One then went back to previous vaccinations, and the success of the method did not take long to be shown].

With the better knowledge about antigen-antibody reactions and antibody genesis, from the beginning of XXth century on, the correlation between resistance against viruses and the presence of reactive immunoglobulins in the serum was established. The available bibliography from this period suggests that the methodologies used for obtaining this immunobiological against rabies were very distinct, adaptations being introduced in agreement with the conditions and the available animal species. Examples are the descriptions of the elaboration and the application of these products by Souza (1927, 1929) and Lima (1932), also the use of infected bovine brain for the preparation of viral suspensions intended for immunization, taking care to procure the inactivation of the infectious principles of the sample. For this, one applied, and still applies, chemical products and/or the use of heat (Nagano & Kojima, 1954; 1958). The follow-up of the process is made by the quantification of the antibodies produced, which, when interacting with the viral elements, inactivate the infectivity of the preparation.

One concluded then that the measurable elements responsible for the phenomenon of immunization of individuals against rabies should be the antibodies.

More than 20 years after the presentation of Pasteur, Semple developed a product without infectious characteristics, with high potential for antibody genesis in mice. In the responding animals, protection against the development of rabies was observed (Vodopija & Clark 1991).

In 1955, in Chile, Fuenzalida and Palacios presented the solution for a large part of the problem of side effects, when offering an inactivated vaccine produced from the brain of new born mice, infected with fixed virus of the Pasteur strain (PV) or of the Challenge Virus Standard (CVS) (Fuenzalida & Palacios 1955). This viral preparation, containing

about 2% of nervous tissue, and rabies virions, is inactivated previously by a treatment with beta-propiolactone and subsequently added antimicrobial agents (0,01% of Thimerosal and 0.1% of phenol) in order to prevent possible microbiological contamination of the product to be inoculated. The potential of inducing immune response to all types of vaccine preparations is evaluated in accordance with the protocol adopted by the controlling State health agencies (NIH - National Institutes of Health, USA). The final product to be injected must have the antigenic reactivity of, at least, 1,0 international unit (IU)/dose.

Afterwards, this vaccinal protocol became routine in all public health programs in Brazil.

Those that are vaccinated and express an amount of anti-rabies reactive antibodies equal to or higher than 0,5 IU/ml, are considered in the category of “immunized”; while those with values below this index or without detectable response, are considered susceptible (Rabies Surveillance Standards 2008).

Nowadays, groups that are exposed routinely to contamination by rabies virus are still formed, exclusively, by individuals that present a protective response to the vaccination scheme. In the service norms of activities with rabies, the principle, is adopted that individuals who do not present reactive antibodies for the Challenge Virus Standard - CVS - antigen after three series of five vaccine doses are considered non-responders and should not be admitted to the service (WHO 2002).

Technically comparing the details described in the report of Santos about Pasteur virus preparation, and the product offered by Fuenzalida and Palacios, the differences between the two types of vaccinal products involve their animal origin (Pasteur = rabbit; F & P = mouse) and their potential for infectivity (Pasteur = active; F & P = inactive).

Pasteur was conscientious of his premise about the need to include some doses of infectious virus suspension in the vaccinal schedule to be injected.

“...o methodo repousa no emprego de vírus, a princípio desprovidos de actividade apreciável, em seguida dotados de actividade fraca e, enfim, de mais em mais forte. Na applicação do tratamento, as medullas inócuas são succedidas pelas medullas de virulência progressiva.” (Santos, 1888, pág.332).

[..the approach rests on the use of virus, deprived of infectious activity in the beginning, next endowed with weak activity and, finally, stronger and stronger. In the application of the treatment, the harmless medullas are followed by medullas with progressively increased infectivity].

Pasteur considered that, beyond the viral suspension, another factor, inherent in the immunological system of the infected individual, participated in the containing of rabies development.

“... afirma o sabio Pasteur que estão elles em mais perfeito accôrdo com a idéa de uma matéria vaccinal que estaria associada ao micróbio da raiva, conservando este intacta a sua virulência especial em toda a serie de medullas em desecção, porém sofrendo nestas uma destruição progressiva mais rápida do que a matéria vaccinal. ...cita o seu auctor diferentes factos experimentaes do maior valor, que demonstram que a immuidade subsequente à inoculação subcutanea, alcançada por uma quantidade elevada de qualquer vírus rabido, é explicada antes pela existência de uma matéria vaccinal que acompanha o micróbio do que pela acção deste microbio.” (Santos, 1888, pág.333). [... The sage Pasteur affirms that they [the facts about] rabies vaccine are in the most

perfect harmony with the idea of a vaccinal matter that would be associated to the rabies microbe, preserving its special virulence intact in the whole series of desiccated medullas, however suffering in this process a quicker progressive destruction than the vaccinal matter. ... the author quotes different experimental facts of the highest value, which demonstrate that the subsequent immunity to the subcutaneous inoculation, reached by a great quantity of any rabies virus, is better explained by the existence of a vaccinal matter that accompanies the microbe than by the action of this microbe].

“... não poderá succeder, continua o Sr. Pasteur, que o vírus rabido seja formado de duas substâncias distintas, e que, ao lado da que tem vida e é capaz de pullular no systema nervoso, haja uma outra, não viva, que tenha a faculdade, quando existe em proporção adequada, de obstar o desenvolvimento da primeira?”. (Santos, 1888, pág.333). [couldn't it be possible, continues Pasteur, that the rabies virus consists of two distinct substances: one that has life and is able to proliferate in the nervous system, and another one, not alive, that has the faculty, when it exists in appropriate proportion, to oppose the proliferation of the first one?].

”As inoculações anti-rabidas dão a prova incontestável da actividade vaccinal dos produtos de evolução dos agentes virulentos. Nos primeiros líquidos injetados, estes agentes não existem; foram todos mortos pela desecção em presença do ar. Estes líquidos não são, porém, menos activos, pois preparam o organismo para receber impunemente novos líquidos, cada vez mais ricos em agentes pathogenicos, até os líquidos cuja injeção, feita de chofre, sem prévia

preparação do organismo, provocaria quase infalivelmente a raiva mortal.”

(Santos, 1888, pág.334-335). [The anti-rabies inoculations offer indisputable proof of the vaccinal activity of the evolution products of virulent agents. In the first injected liquids, these agents do not exist; they were all killed by desiccation in the presence of air. These liquids are not, however, less active, since they prepare the organism to receive new liquids with impunity, richer and richer in pathogenic agents, up to liquids which, injected all of a sudden, without prior preparation of the organism, would provoke almost without failure, mortal rabies].

Continuing the reading of this same Chapter of the report by Dr Santos, the following is found:

“Bardach formula a hypothese de que o virus rabido, tendo passado por muitas gerações de coelhos e tendo-se tornado fixo, adquiriu a faculdade de elaborar, cultivando-se no systema nervoso de outros animaes, certos productos resultantes de sua actividade vital, que augmentam a energia dos phagocytos em digerir os micróbios rabidos e conferem assim uma imunidade duradoura. Póde-se algumas vezes obter a immunidade pela introdução sob a pelle de uma única seringa de virus vivo, e esta possibilidade augmenta com a quantidade de virus de passagem introduzida; só o virus de passagem póde concorrer para o desenvolvimento da immunidade, não sendo este resultado obtido pelo virus da raiva das ruas reforçado.” (Santos, 1888, pág.337). [*Bardach formulates the hypothesis that the rabies virus, after several passages in rabbits, became fixed, acquiring the faculty of elaborating certain products resulting from their vital activity, when they are being propagated in the nervous system of other animals. These products*

augment the energy of the phagocytes in digesting the rabies microbes and so developing a lasting immunity... Sometimes it is possible to obtain immunity by the subcutaneous introduction of a single syringe dose of fixed virus, and this possibility is higher when introducing a larger amount of passaged virus; only passaged virus can contribute to the development of immunity, this result not being obtained by the inoculation of street rabies virus.].

“A proposito das vaccinas chemicas, disse o Sr. Pasteur na sessão de 20 de Agosto deste anno (1888) na Academia das sciencias,, que a vaccina chimica da raiva não póde tardar a ser conhecida e utilizada,...A medulla aquecida, tornada não virulenta, era, pois, vaccinal por uma vaccina chimica.” (Santos, 1888, pág.338). [With regard to chemical vaccines, Mr. Pasteur said in the session on August 20th (1888) in the Academy of sciences,that the chemical vaccine of rabies would not take long to be known and used... The heated medulla, made not virulent, was, in this way, vaccinal for a chemical vaccine].

The effect of interferons associated with animal viroses was discovered in 1957 (Isaacs & Lindenmann, 1957; Ho, 1966). Interferons are exported proteins produced by cells, in the beginning of their anabolism state, to signal to other cells. The action of these cytokines can be verified by the blocking effect on cellular proteomic synthesis, which presents itself as a drug with anti-viral and anti-neoplasm potentials (Langer, 2007). Innumerable studies showing the application of these interferon properties have been developed, including its efficient role in the prevention of rabies (Rutz-Goremz & Isaacs, 1963; Hilfenhaus et al. 1975, Moreno et al. 1979).

Now it is known that interferon is not only produced by infected cells during viral infections but also in response to the presence of microbes and their products, as well as RNA and viral glycoproteins (Sen, 2003). It activates macrophages and natural killer lymphocytes (NK) (Rutz-Gorems & Isaacs, 1963). It can be concluded, thus, that individuals infected by rabies virus, if receiving preparations that are good interferon inducers, have less risk of succumbing to the disease (Delhay et al. 2006).

“A imunidade deve ser adquirida antes do acumulo do vírus rabido nos centros nervosos. Póde-se dizer, em geral, que todos quantos excedem o intervalo de 15 dias, contados da terminação do tratamento, sobrevivem.

Qual é o mecanismo da imunidade adquirida? Como a vacina, introduzida em uma parte do corpo, determina o estado refratário, que faz com que o microbio rabido, que pullula em outro lugar, pare em seu desenvolvimento e desapareça? A esta pergunta responde Gamaleia que, graças às inoculações vacinaes, produzem-se modificações no systema lymphatico do organismo vaccinado; estas modificações conduzem à destruição do vírus rabido ou diminuem a sua quantidade nos casos de raiva retardada.”.

(Santos, 1888, pág.336-337). [The immunity must be acquired before the accumulation of rabies virus in the nervous centers. One could say that, in general, all that exceed the interval of 15 days, counted from the ending of treatment, survive.

What is the mechanism of the acquired immunity? How does the introduction of vaccine material in a part of the body mediate the state of resistance, which

makes the rabies microbes, abundant somewhere in the body, stop their development and disappear? To answer this question Gamaleia said that vaccine inoculations produce modifications in the lymphatic system of the vaccinated organism; these modifications lead to the destruction of the rabies virus or reduce its quantity as demonstrated in the cases of delayed rabies.].

Analyzing the above, it can be considered that the experiments carried out by Louis Pasteur and his followers already outlined rabies prophylaxis in a quite precise manner.

Studies on vaccines about their role in the stimulation of interferon, carried out mainly during the 70's and 80's, confirmed the better induction of protection for those with this capacity (Rutz-Goremz & Isaacs, 1963; Hilfenhaus et al. 1975, Moreno et al. 1979).

Using the results of Hilfenhaus', Rutz-Goremz & Isaacs' and Moreno's work in the construction of a premise, an hypothetic-deductive thought could be elaborated, in order to explain the efficiency of the vaccinal products of Pasteur and Fuenzalida-Palacios, according to the following hypothesis:

Considering that interferon alone induces protection against fatal rabies, if Pasteur's inactivated vaccine alone does not induce the state of protection against rabies; but Pasteur's infectious fixed virus vaccine, as well as Fuenzalida-Palacios inactivated vaccine preparation did, the state of rabies protection mediated by those infectious and inactive vaccine preparations should be interpreted as a consequence of an induced expression of endogenous interferons.

Immunologically, endogenous interferons are liberated by CD8+T lymphocytes when they are responding against cells presenting viral infections (Pasteur fixed virus). CD4+T lymphocytes response depends on antigen concentration. This dependence can be

used to explain the difference between Pasteur and Fuenzalida-Palacios inactivated vaccine preparations (Luger & Schwarz, 1974, Almeida et al., 1997, Oliveira et al 2000).

Another argument that supports the presented hypothesis was already demonstrated by Pasteur's team utilizing a heated virus preparation obtained from medulla collected from dead rabbits that were inoculated with a street virus preparation obtained from a dog.

It must be mentioned that the development of anti-rabies vaccines did not then. New technologies of vaccine preparation, such as the use of genetic engineering and virus production in cell cultures, have contributed enormously to minimize some of its non-desired side effects.

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