

PROTECTION OF MAN AGAINST RABIES AFTER EXPOSURE PAST HISTORY — PRESENT STATE AND THE FUTURE OUTLOOK

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ABSTRACT

After a period of at least 2000 years that rabies existed on earth and its clinical symptoms were clearly defined, even before Christ, the first basic and precise knowledge about this fatal disease of man and animals was advocated by Zinke in 1804. The discovery of the domesticated rabbit as a convenient laboratory animal for the study and the diagnosis of the disease by Galtier in 1879 and the outstanding finding by Roux in 1881 concerning the neurotropism of the agent of the disease and the intracerebral route of inoculation by which prompt transmission could be established, paved the way for Pasteur and his colleagues for their world-known discovery of protecting animals and man against this disease.

The vaccine produced and administered by Pasteur himself and numerous other types of vaccines always prepared with the same Pasteur's historical and modified virus which he named "fixed virus" were endeavours in the way of improving the safety and the antigenicity of the products to fight a hundred per cent fatal illness.

The evolution of these efforts during the past 90 years, since Pasteur's first attempt to protect an exposed individual against rabies crowned by outstanding and very promising results are discussed.

INTRODUCTION

Among all infectious diseases, rabies has been and still is, to some extent, one of the most complicated and challenging public health problems of

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used extensively for immunization of cattle and even man with encouraging results.

As can be easily gathered, endeavour to promote the antigenicity of antirabies vaccine and to produce safer products aiming at a prompt and dependable protection of the exposed individuals started right from the very day when Pasteur immunized the first exposed human being in 1885. It must be mentioned here that Pasteur himself, after observing several failures in his attempt to protect the exposed persons during a few years that he administered his procedure before his death, must have accepted that his method was not ideal and therefore he urged his colleagues to investigate and improve the procedure initiated by him. Accumulated data resulting from the application of Pasteur's classical procedure or other modified methods with various dosage and types of vaccines, mostly brain tissue preparations, over a period of sixty years with about 40 million cases receiving such treatment created doubts as to the safety and the protective value of the vaccines currently used. Post vaccinal paralytic accidents due to the use of nerve tissue vaccines ranging from one in a thousand to one in ten thousand treated persons was a serious problem and could not be overlooked. Treatment failures among exposed and promptly vaccinated individuals was the second but the most seriously considered problem.

Unsuccessful attempts to protect exposed individuals by the conventional post-exposure treatment with vaccines were sporadically reported from all over the world. The extent and the ratio of treatment failure was unfortunately overshadowed with misleading statistics issued from the treatment centers. Thus a correct assessment of the protective value of the post-exposure treatment in man was not available.

Finally Baltazard and Ghodssi⁽¹⁸⁾ (1953) disregarding the usual statistical evaluation of the efficacy of antirabies vaccination, whereby all of the treated cases were erroneously considered as exposed, assessed the protective value of the post-exposure treatment only in those cases who were definitely exposed to rabies virus (bitten by a confirmed rabid wolf and not any biting wolf). According to their findings 25 per cent of the total number of *exposed* and treated individuals developed rabies. When only the face and head exposures to rabid wolf bite was considered the fatality rate among the treated cases reached 42 per cent. Comparing these mortality rates (the so called treatment failure with 47 per cent fatality in groups of individuals with similar severity of exposure but untreated) the authors announced that the result of the post-exposure immunization with vaccine in severely *exposed* individuals was disastrous. Dependable and prompt remedy to cope with such a discouraging situation was sought. The WHO Expert Committee⁽¹⁹⁾ on Rabies, during its first session (April 1950), already aware of the inadequacy of the available means to protect severe exposures, proposed the use of a combined serum-vaccine treatment in such cases. A well planned schedule for a field trial, reflected in the report of the same Committee was suggested and its execution was entrusted to the Pas-

man. Complicated because in contrast to all other known infectious diseases, attempts to provide protection against it is usually initiated after an individual is already exposed, expecting solid protection against a hundred per cent fatal illness. Challenging, because with no specific cure once the disease is established, it almost invariably ends in death.

Past history. — There is no way to determine for sure as at what era the causative agent of rabies was born on earth. There is also no doubt that the disease in man and animals existed and was clearly defined by Egyptians, Greeks and Romans some 2500 years ago. Democritus (1) (500 B.C.), Aristotle (2) (320 B.C.) described the disease in dogs. Celsus (3) (100 A.D.) as a means to protect man against this fatal illness, recommended cauterization of wounds inflicted by mad dogs. Galen preferred amputation of the wound zones. These or other similar attempts to protect man clearly demonstrate that the relation of the bite wounds and the infectivity of the saliva were suspected about 2000 years ago. On the other hand the overwhelming majority of people during that era could only accept supernatural causes for the establishment of a disease which struck, both animals and man suddenly and without understandable reason. It is very interesting and discouraging at the same time to know that similar concept still reigns among many people in the world until this day and constitutes one of the major public health problems in providing adequate and prompt protection to many exposed individuals. People with this belief reject modern and dependable means of protection and accept elaborations by professional sorcerers and magicians who claim to avert the disease.

The present state. — The first step in basic and correct understanding of rabies was taken by Zinke (4), who as early as 1804 demonstrated the infectivity of the saliva of a rabid dog by establishing the disease in a healthy dog inoculated with the suspect saliva. The introduction of the domesticated rabbit as a convenient laboratory animal, for both diagnosis and study of rabies by Galtier (5, 6) (1879) and the discovery by Roux (7) (1881) that the infective agent of the disease could be readily recovered from the brain of rabid animals followed by the outstanding finding of the intracerebral transmission by Roux (8) paved the way for L. Pasteur for a detailed study of the behaviour of the specific agent of the disease which he could maintain in the laboratory by serial passages from brain to brain in rabbits.

Continuous efforts of Pasteur and his colleagues to visualize the causative agent of rabies or to propagate it in artificial media remained unsuccessful. Once they were determined that the agent was not a bacteria they called it "virus", meaning poison in Latin. As a confirmation of this concept it was

demonstrated later that the agent could pass through Berkfeld filter.

The maintenance of the rabies virus by serial passages in rabbits resulted in modifications in the behaviour of the agent. Pasteur and his colleagues became aware of these changes. According to their prompt observations the incubation period of the disease in experimental rabbits had become shorter and it had lost its pathogenicity for large animals when inoculated by routes other than the brain. A third and the most outstanding characteristic of the modified virus elucidated by Pasteur et al. in 1884 (8) was the fact that the strain of virus maintained in the laboratory though non-pathogenic by the muscular or subcutaneous routes was immunogenic. Dogs and other animals previously inoculated with this modified strain of virus demonstrated protection against a subsequent challenge with the pathogenic natural virus, the so-called "street virus".

With the encouraging results in animals and his previous experiences in transforming pathogenic chicken-cholera agent into immunizing material against the disease by simply aging the cultures of the organism at room temperature, Pasteur seized the idea of protecting exposed individuals against rabies, by the administration of his modified strain which he called "fixed virus" to distinguish it from the natural or the street virus. To further minimize the possible danger of the administration of this modified virus in man he attenuated it by aging and dessication of the harvested spinal cords of rabbits inoculated with the fixed virus

Finally the day came when in July 1885 (almost 90 years ago), the first human being severely wounded by a suspected rabid dog, received the preparation of Pasteur (9), consisting of the emulsified 14 days old rabbit spinal cord affected with his modified virus. The exposed individual was inoculated with 13 more daily inoculations of gradually less attenuated material, so that on the final day of the treatment, almost fresh and non-attenuated fixed virus was used. This was the first attempt in protecting man against rabies by immunizing him with gradually increasing amounts of a modified but live virus. Joseph Meister, the young boy, who was thus treated by Pasteur, survived. Thus the procedure of Pasteur was universally accepted and many individuals exposed to suspect bite wounds were taken to Pasteur or to other treatment centers, which were established later in other countries, to save their lives by Pasteur's procedure.

Joseph Meister was indeed severely wounded by a suspect dog. I use the word suspect instead of rabid; because in documents left from Pasteur and his colleague, concerning the biting dog, the clinical symptoms related to rabies disease in carnivores were observed but paradoxally, attempts for laboratory confirmation of the disease, in that animal, was not made. Thus, there is no way to elucidate whether the dog which wounded Joseph Meister was really rabid or not. But we can tell, for sure, that not every biting dog is rabid, also not every rabid dog has virus in its saliva, and more important than all,

not every individual bitten by a rabbit animal having rabies virus in its saliva, will develop rabies, if left without any specific antirabies treatment whatsoever. These are the facts that made the assessment of the protective value of Pasteur's vaccine almost impossible. One must also recall that, free from any prejudice in his scientific career, Pasteur himself observed and declared several unsuccessful attempts in protecting exposed individuals by his procedure during the few years, that he lived after his discovery. Therefore, being aware of the unreliable protective value of Pasteur's vaccine, attempts to improve the antigenicity of and the availability of adequate quantities of a potent vaccine to treat any number of exposed individuals, arriving at any time, were initiated.

Two years after the first attempt to immunize man with the modified virus, Roux (10) (1887) demonstrated, that the infectivity of tissues containing rabies virus, could be maintained by preservation in glycerol. In a first step to render Pasteur's procedure more practical, Calmette (1891)(11) employing Roux's finding, maintained, ready for administration in man, rabbit cords of varied infectivity by preserving them in glycerol. Fermi(12) (1908) was the first to employ phenol in the production of vaccine. Suspensions of fixed virus, treated with phenol were kept at room temperature (22°C) for a few days, then kept at lower temperature prior to administration. Fermi's finding provided time and possibility for bacteriological control of the material to be used in man. This was a partially inactivated and partially live virus vaccine.

Sample(13) (1919) demonstrated that Fermi type vaccine could be totally inactivated but still retain its antigenicity by incubating the phenol treated suspensions at 37°C for 48-72 hours. This was supposed to be a totally killed vaccine.

The Ultraviolet light treated rabies fixed virus suspensions for the production of a killed vaccine was a distinct advance made by Hodes et al.(14) (1940). Klinger and Bernkof(15) introduced the multiplication of rabies virus in the chick embryo. Later on, Johnson(16) produced a variety of rabies fixed, virus, by serial passage of a street virus strain, in one day old chicks. He demonstrated that after 100 passages, this strain of virus, which he named Flury after the name of a rabies victim from whom the virus was originally isolated, its pathogenicity for mammals and specially its capacity to invade the central nervous system and the calivary glands was markedly reduced, when inoculated peripherally.

Koprowski et al.,(17) further attenuated Johnson's Flury strain, by serial passages in chick embryos and demonstrated that dogs could be solidly protected against rabies with the administration of one single dose of this virus at its lower egg passage level (LEP). At higher egg passage level (180 passages in eggs) the Flury strain was no more pathogenic for large mammals. However it retained slight pathogenicity for new born mice, hamster and guinea-pig by intracerebral route, but not for adult mice. The virus at this stage has been

teur Institute of Iran.

We had to wait a few years until during the summer of 1954 an exceptionally favourable and unique event availed. 29 individuals were bitten, the majority on the face and head, during a few hours by one rabid wolf invading a sleeping village at night. The combined serovaccination trial recommended by the WHO was executed in this group. The outcome of this trial reported by Baltazard and Bahmanyar⁽²⁰⁾ (1955) was so conclusive and encouraging that it was recommended as the best available protection against rabies by the WHO Expert Committee at its third session 1956. The procedure was adopted all over the world and many human lives, otherwise condemned to death, were saved. In Iran alone, following the adoption of the combined serum-vaccine administration, the treatment-failure rate for the severely exposed cases fell from 40 per cent to almost zero (Bahmanyar 1966)⁽²¹⁾. It seemed that the problem of post-exposure protection of man against rabies was solved once for ever. True though it is to a great extent, the procedure was far from being perfect for the following reasons. Though very rare, but still occasional treatment failures were still observed after serum-vaccine treatment. The vaccination schedule reduced to 14 daily doses instead of 21 or more was not only a long and troublesome procedure but caused side effects including post-vaccination paralysis, sometimes ending in death, that could not be overlooked. The efforts of many workers, in the field of rabies was therefore oriented, during the last two decades, to overcome those deficiencies. A brief account of these efforts crowned with successful achievements are as follows.

Since many years the immuno-allergic accidents were attributed to the presence of encephalitogenic factors in the nervous tissue of adult animals used for the preparation of various types of vaccines. To avoid this hazard, brain tissue of new born animals considered to be free from those factors were substituted for adult animals. Thus Fuenzalida⁽²²⁾ (1955) derived his vaccine from new born mice. Karakujumcan⁽²³⁾ used 4-7 day old rats for this purpose. Lipton (1953) and Gispen (1965) employed on day old suckling rabbit for the preparation of non-allergic vaccine. The suckling mouse brain vaccine and the new born rat brain vaccine has been extensively used in South America and the USSR. Though too early for a correct assessment, observations up to date indicate reduction but not elimination of post-vaccination paralytic accidents in humans treated with vaccines derived from suckling animals. It has also been claimed that these vaccines are more antigenic than preparations from adult animals.

Aiming at a complete elimination of post-vaccination accidents in man, other investigators adapted the fixed virus strains to avian embryos and produced vaccines from them for human immunization. The Flury strain of rabies virus modified by Johnson by serial passages, in one day old chicks, was adapted to chick embryo by Koprowski⁽²⁴⁾ and at its 50th passage level was extensively used for immunization of dogs with excellent results. This was called the Flury

low egg passage (LEP). The same virus strain at 180th egg passage level, called HEP (high egg passage), has been used both in man and cattle as live vaccines, providing adequate protection in animals but with questionable results in man. Powell and Culbertson (1956) were the leaders in the adaptation and sufficient multiplication of rabies virus in duck-embryo. Using the Pitman-Moore strain of fixed rabies virus, propagated in duck-embryo and inactivated by beta-propiolactone, the as called DEV vaccine was produced and extensively used in the United States for both pre and post-exposure protection of man. Based on the results of laboratory experiments and field trials the avianized vaccines, live or inactivated, were not free from encephalitogenic factors. They were also found to be less immunogenic in man as compared with the nervous tissue vaccines. The production and the administration of those vaccines although an important progress in the immunization of animals could not be considered as a definite resolution to the problems related to the protection of man after exposure to rabies virus. Efforts to cope with these deficiencies were therefore initiated with promising achievements.

The new era in the production of non-encephalitogenic, safe and highly antigenic rabies vaccines was opened by the advent of the growth of rabies virus in cell and tissue cultures. The first attempts in this field made by Levaditi as early as 1913 remained unsuccessful and soon abandoned, very probably because of unavailability of antibiotics at that time. 45 years later (1958), Kissling⁽²⁵⁾ succeeded in the growth, with reasonable titers of the CVS strain of rabies virus, in primary hamster kidney explants. Soon after this discovery other investigators attempting the propagation of rabies virus, in different primary explants or cell lines, found out that this virus could multiply in almost all of the non-nervous tissues they tried. The aim thereafter was to find a tissue or a cell line in which the virus would multiply and produce adequately high titers, suitable for the production of potent vaccines. Fenje⁽²⁶⁾ (1960) using hamster kidney cells as host for the SAD strain produced an encouragingly antigenic vaccine. Kissling himself with Reesen produced likewise a potent vaccine with CVS strain in the same cells. Rueggegger and Sharpless prepared a vaccine with Flury HEP strain in chicken fibroblast. This vaccine was demonstrated to be significantly antigenic in human volunteers. Abelseth⁽²⁷⁾ propagated the kidney cell adapted SAD strain obtained from Fenje in porcine kidney cells. This virus called ERA with very high antigenic value has been extensively used as a live virus vaccine for the immunization of both dogs and cattle. Wiktor, Fernandes and Koprowski⁽²⁸⁾ (1964) succeeded to grow various modified rabies viruses (The CUS, Flury HEP and Pitman-Moore strains) in human diploid cell (HDC), Wistar-33 line. The most immunogenic of those vaccines was the one produced with the PM virus and inactivated with beta-propiolactone⁽²⁹⁾. A single dose of this vaccine administered in primates protected seven out of eight of them against a subsequent challenge with street rabies virus. Trials in 8 previously vaccinated and 8 non vaccinated

volunteers by the same authors demonstrated very high booster effect and reliable antigenicity for that vaccine (1965). A batch of the same type of the HDC vaccine produced by Lang, Institut Merieux (1971) was entrusted to Pasteur Institute of Iran, for the evaluation of its antigenicity in non-vaccinated human volunteers. The results of this trial performed by Bahmanyar⁽³⁰⁾ (1972, paper under publication), clearly indicate that with only 4 doses of this safe and completely free from encephalitogenic factors, prompt and dependable immunity, reflected by very high antibody titers can be induced in man.

The future outlook. - Thanks to the outstanding achievements resulting from the continuous efforts of many investigators during the last two decades and specially the demonstration of the reliable protective value of the combined serum-vaccine treatment, it can be stated with confidence that almost all of the exposed individuals arriving for treatment in time are saved. Heterologous antirabies serum produced in various animals such as horses, mules, sheep, rabbits and so forth has been and is being used at present. Although these immune sera are carefully purified and concentrated, allergic and anaphylactic reactions due to them are not rare. Therefore efforts are directed towards the production of rabies immune globulin from human origin as a safe substitute. The availability and use of the human immune globulin will doubtless eliminate all of the side effects of the heterologous serum.

At present the procedure for the immunization of man with vaccines currently in use call for 14 daily doses plus 3 boosters (duck embryo or brain tissue vaccines). Scientific grounds towards the reduction of the number of doses from 14 to 7 is already established by the workers of Pasteur Institute, Paris, using a highly potent suckling mouse brain vaccine. With the advent of the tissue culture vaccines and the outstanding results of the field trials with the HDC vaccine demonstrating that prompt and very high antibody levels can be induced in man by the administration of only 4 doses of that vaccine, it can be expected that the pre or post-exposure protection of man will fall in line with the procedure of immunization against other viral diseases.

The use of the homologous antirabies globulin in man is considered to be totally safe. One can expect that it will not only be a remarkably more efficient and safer substitute for the heterologous serum but, it may also be sufficient in itself in protecting man against rabies. Experiments in this respect are underway.

Taking into consideration the available biologicals, the protection of man against rabies in the near future may consist of either the administration of a combined treatment with one dose of human immune globulin plus 4 doses and a booster of the HDC or a similar vaccine or the employment of only the homologous immune globulin in several doses.

It seems that we are not too far from the availability of safe, dependable and humane procedure to protect man against rabies.

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