REPORT

ON

M. PASTEUR'S RESEARCHES ON RABIES
AND THE TREATMENT OF HYDRO-
PHOBIA BY PREVENTIVE
INOCULATION.By M. WILLIAM VIGNAL,
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PART IV.

PREPARATION OF THE INOCULATING FLUID.—*Rabbits Inoculated.*—*Antiseptic Precautions.*—NUMBER OF PREVENTIVE INOCULATIONS MADE BY M. PASTEUR.—*Classification.*—*The Interval of Time since Bites.*—*Statistics of Hydrophobia by Leblanc and others.*—*One Death only among M. Pasteur's Patients: Cause of Death.*—*Summary of Facts concerning the People Bitten.*—PEOPLE BITTEN BY MAD WOLVES.—*Statistics of Wolf Bites.*—*Difference in Effects of Hydrophobia: a Dog's Bite and a Wolf's Bite.*—CREATION OF INOCULATION ESTABLISHMENT FOR HYDROPHOBIA.—*Appendix.*—*Facts Concerning Bites from Mad Dogs: Cases Cited.*—*Former and Recent Experiments concerning the Virulence of the Nervous System in Rabid Animals.*—*Hydrophobia Produced by Swallowing Virus.*—*Inoculation with Blood.*—*Micro-organisms of Hydrophobia.*—*The Virulence of Rabid Virus Preserved in Pure Carbolic Acid.*

PREPARATION OF THE INOCULATING FLUID.—The inoculating fluid used by M. Pasteur is prepared as follows. The bottles containing dry air, in which are preserved the rabid spinal cords taken from rabbits, are arranged, according to date, on a table in a room where the air is not disturbed. The assistant, whose duty it is to prepare the inoculating fluid and fill the syringes, takes the cords, one by one, from the bottles, by drawing up the strings by which they are suspended. The cords and the strings are passed through the flame of a spirit lamp, in order to destroy any germs that might have been deposited on them whilst the bottle was being opened. The threads are then cut, and the cords fall into a glass receptacle. These are then pounded by using a glass rod, and veal-broth is added in quantity equal to four or five times the volume of the cord before it was dried. The fluid, thus prepared, is turbid. The glass receptacle containing this fluid is covered by a paper which has been sterilised by heat. The receptacle, the glass rod, and the broth, used in preparing the inoculation-fluid, are previously carefully sterilised.

Rabbits Inoculated.—Rabbits are inoculated in the following manner, and their spinal cords are subsequently used to prepare the fluid above described. The animal is secured on a board, its back upwards. A portion of its fur is cut away from its head; the skin is then incised, and one of the temporal bones is pierced by means of a trephine about six millimètres in diameter, with very fine teeth. This instrument is used so as to prevent the centre-pin from entering the brain, or the crown from injuring it or the dura mater. When the piece of bone has been removed, some drops of the rabid virus are injected into the arachnoid membrane. For this, a Pravaz's syringe is used, the needle of which is bent at a right angle. The dura mater is penetrated by the needle, and it reaches the arachnoid. The incision in the skin of the head is closed by a few sutures.

M. Pasteur inoculated the first rabbit with virus taken from a mad dog. This virus was passed from rabbit to rabbit, and has been used, without interruption, for inoculations, up to the present time. It is estimated that the virus has been passed through 130 rabbits. The incubation-period is rather less than seven days, as has been previously stated.

Antiseptic Precautions.—In trephining the rabbit's cranium, every antiseptic precaution is observed, and the instruments are sterilised. If any blood escape, it is immediately soaked up with sterilised paper. These precautions are eminently necessary, inasmuch as antiseptic fluids cannot be used, and yet the operation must be effected aseptically.

When the animal dies, the same antiseptic precautions are observed in removing its spinal cord, from which are taken fragments, to be suspended from a string in a bottle of the form here shown. Through-

A, Bottle containing dry air; B, thread attached to fragment of rabid marrow; C, caustic potash, to dry the air; D, sterilised wool; H, lower tap for allowing fluid to run away when necessary.

out these manipulations, every antiseptic precaution is observed, and caustic potash is placed in the bottle to dry the air it contains.

NUMBER OF PREVENTIVE INOCULATIONS MADE BY M. PASTEUR.—Since July 6th, 1885, M. Pasteur has applied his prophylactic treatment for hydrophobia to 688 people who have been bitten by dogs.

Classification.—We classify them as follows: 1 bitten on July 4th, 1885; 1 on October 14th, 1885; 98 before December 15th, 1885; 100 between December 15th, 1885, and February 15th, 1886; 488 from February 15th, 1886, to April 15th, 1886.

The Interval of Time since Bites.—Thus, since the period when the bites were inflicted, the following intervals of time have elapsed in the several cases: in 1, more than nine months; in 1, more than six months; in 98, more than four months; in 100, two months. The 488 remaining cases are in the period called dangerous.¹

Statistics of Hydrophobia by Leblanc and others.—In order to estimate the importance of these statistics, it must be borne in mind that 95 out of the 100 people inoculated by M. Pasteur² had been bitten by dogs declared mad, either by a medical man or by a veterinary surgeon, or ascertained to be so at M. Pasteur's laboratory. According to statistics drawn up by M. Leblanc, in Paris, there occurs one death in six. This proportion was observed during six years. Thus, there are 16.66 per cent. of deaths. Other statistics state that the proportion of deaths is 15 to 25 per cent.

It is ascertained, by the statistics, that the incubation-period varies from forty to sixty days. Longer incubation-periods are rare.

One Death only among M. Pasteur's Patients: Cause of Death.—Among all the people inoculated, one has died, Louise Pelletier, a child 10 years of age, on the seventeenth day after the prophylactic treatment had terminated. She was bitten, on October 3rd, by a big mountain-bred dog. On November 9th, thirty-seven days after being bitten, she was brought to M. Pasteur. The wounds inflicted by the bite were very serious, in the axilla and on the head. The latter wound, notwithstanding most careful and skilful treatment, was in a purulent condition when she arrived at M. Pasteur's laboratory. It covered an area of twelve to fifteen centimètres, and the scalp was detached from the cranium.

M. Pasteur was desirous to determine the cause of the child's death, whether it was the virus from the mad dog, or the virus used for inoculation. He, therefore, removed a small quantity of the child's cerebral matter, with which he inoculated some rabbits. These rabbits were seized with hydrophobia, of the paralytic form, eighteen days later; other rabbits were inoculated from the spinal cord of the first rabbit inoculated from Louise Pelletier. These animals died from hydrophobia after fifteen days' incubation. Therefore, it is evident that Louise Pelletier died from the virus of the mad dog which had bitten her. If the virus of the inoculations had caused death, the incubation-period in the second series of rabbits would have lasted only seven days.

¹ The investigation made by the Comité d'Hygiène of France shows that, of 176 cases of hydrophobia, 123 die before the seventieth day subsequent to the bite; forty-seven between the seventieth day and the 130th; six after the 130th and before the 240th day.

² We have already stated that M. Pasteur only treats those people who have been bitten on the hands or face, or whose clothes have been torn and lacerated by the dog's teeth, so that the virus has passed into the wound.

Summary of Facts concerning the People Bitten.—M. Pasteur's experiments, detailed above, demonstrate that, among 688 persons inoculated, M. Pasteur's prophylactic treatment had proved inefficacious for one person only; 200 among these have reached either the ninth, sixth, fourth, or third months after inoculation. The remaining 488 are in different degrees of the incubation-period; M. Pasteur considers that, up to the time of writing this report (April 14th, 1886), there is every reason for looking forward to the most happy results.

These statistics, compared with those stated above, concerning the average number of deaths after bites from mad dogs, render any remarks from us unnecessary. It is probable that the death of Louise Pélletier can be explained by the serious nature of her wounds, and the long time that was allowed to elapse between the infliction of the bite and the application of the prophylactic treatment. But even if this probability be not admitted by some, no one will deny that every prophylactic treatment sometimes fails. Is the value of Jenner's discovery questioned because all who are vaccinated do not escape contracting small-pox?

PEOPLE BITTEN BY MAD WOLVES.—Besides the 688 people bitten by mad dogs, M. Pasteur has applied his prophylactic method to a number of Russians, sent to him to be treated for bites from mad wolves, and has now nineteen more under treatment. The first arrival consisted of nineteen persons who had been bitten on March 1st, in the environs of Smolensk. They were all severely bitten. Six among them were obliged to be surgically treated for their wounds, at the Hôtel Dieu. One of the six was bitten in fifty-four different places; others had part of their faces torn away; all had deep wounds on the head or hands, and also had their clothes torn.

Three of the nineteen Russians have died from hydrophobia. One death occurred at about the middle of the prophylactic treatment. At the necropsy, half of one of the wolf's teeth was found in a wound on the head; it had remained between the skin and the temporal bone. Part of the man's face was torn away. Two other patients had been submitted to a complete prophylactic treatment, and M. Pasteur had commenced on them a second period of treatment. One of these had deep wounds on the neck, reaching from one side to the other. The wolf had seized him by the neck. The other had a severe wound on the head; part of the cranium was fractured and depressed; he was also bitten on both arms and the left thigh. The last arrival of nineteen Russians have also been badly bitten by a mad wolf on the head and hands.

Statistics of Wolf Bites.—According to statistics sent to M. Pasteur, among forty-seven people bitten by wolves, there were thirty-six deaths.

M. Brouardel, in his article on Hydrophobia ("Rage") published in the *Dictionnaire Encyclopédique*, makes the following statements. Fifty-eight persons were bitten by a mad wolf in the village of Eiranguiez en vic (Russia). M. Resen, who owned nearly the whole village, received the people who were bitten into his country-house, and cauterised their wounds with caustic potash. Among them, thirty-nine died from hydrophobia, which gives a percentage of deaths amounting to 67.24. In May, 1784, seventeen persons were bitten by a wolf, caught at Brive, in France. Ten died from hydrophobia, which gives a percentage mortality of 58.82.

Difference in Effects of Hydrophobia: a Dog's Bite and a Wolf's Bite.—The preceding facts show that the mortality resulting from the bite of a mad wolf is much more considerable than from the bite of a dog. The incubation-period is often much shorter. According to M. Pasteur, these different results are explained by the number of wounds inflicted, the depth of tissue injured, and the regions attacked by the mad wolf which seizes his victim, and bites his face and head. The necropsies of the three Russians, who died at the Hôtel Dieu, also the inoculation made with the spinal cord, taken from the first who died, show that the rabid virus of wolves and that of dogs have the same degree of virulence. The difference between hydrophobia consequent on the bite from a mad dog, and that of a mad wolf, results from the number and nature of the bites. Dogs, rabbits, and guinea-pigs were inoculated from the spinal cord of the Russian. These facts induced M. Pasteur to ascertain if, for wolf-bites, his prophylactic method might be modified, and more inoculations be made in a shorter space of time. He will communicate to the Academy, at a future date, the results of his investigation.

In all cases, and especially for wolf-bites, it is advisable to begin the prophylactic treatment as soon as possible. The Russians from Smolensk were six days travelling to Paris, and arrived at M. Pasteur's laboratory fourteen or fifteen days after they were attacked by the

wolf. Thus their treatment might have been commenced eight days sooner than it was, and it is impossible to say how this might have influenced the course of events.

CREATION OF AN INOCULATION ESTABLISHMENT FOR HYDROPHOBIA.—M. Pasteur wishes an inoculation establishment to be created at Paris, whither patients from all parts of Europe could be treated, as the delay involved by the journey to Paris would not be dangerous. The reasons which lead M. Pasteur to wish, at the present moment, for only one vaccine establishment, are well founded. He fears that, if these establishments were multiplied, errors, and perhaps fatal errors, might be made by persons in preparing the rabid virus; and these errors would be attributed to his method, before it be sufficiently tested to be universally held blameless. Professor Vulpian, who is best acquainted with the question of the preventive treatment of hydrophobia, said, a few days ago, at the Academy of Sciences: "It is necessary, and it will be so for a long time, that M. Pasteur's preventive treatment for hydrophobia should be applied in Paris, under his personal superintendence." In order to understand this, it is necessary to consider how delicate are the operations necessary for keeping up the stock of virus and preparing the inoculation-fluids. They require the constant and careful attention of a skilful manipulator, sufficiently patient to repeat every day the same manipulations, without once missing. Every day, fresh rabbits are inoculated; every day, fragments of rabid spinal cord are placed in dry air; every day, the fragments of spinal cord which have been too long prepared must be rejected; and all the operations, which precede those enumerated, have to be effected. Therefore, it is evident that each establishment must have two principals identical in skill, in case either one, from absence or ill health, be unable to perform the daily duties. The expense of such an establishment would far exceed that incurred by travelling to Paris, and remaining there the time required for treatment. Scientific men can qualify themselves, according to M. Pasteur's suggestion, in the vaccine establishment at Paris, in order to go to South America, some parts of North America, and Australia, in order to teach there the preventive method of inoculation for hydrophobia.

APPENDIX.—In this part of our report, besides giving the details of bites from mad dogs, which have been treated by M. Pasteur, we propose rapidly exposing certain facts in connection with hydrophobia, but which have not a direct bearing on preventive inoculation, and have been omitted in the body of our report.

Facts Concerning Bites from Mad Dogs: Cases Cited.—Jupille, from de Villers Parlay, aged 15, was seriously bitten, on October 12th, 1885, on both hands, by a mad dog, which he had seized to kill. This is the second patient treated by M. Pasteur. Subsequently, the following were treated.

Etienne Roumier, aged 48, was bitten on both hands, on November 4th, 1885, by a dog, ascertained to be mad by M. Moreau, a veterinary surgeon. Twenty-four hours after the accident, the bite was cauterised and dressed.

Chapot, aged 43, and his daughter, aged 14, were both bitten on the left hand, on November 6th, 1885. The daughter was more seriously bitten than her father. The wounds were washed out with strong solution of ammonia. The dog was declared to be mad, at the Lyons Veterinary School.

François St. Martin, aged 10, was bitten on the right thumb, on November 7th. The wound was washed with ammonia. The dog was declared to be mad, by M. Dupont, the principal of the Service Sanitaire des Epizooties.

Marguerite Luzier, aged 13, was bitten on the leg by a mad cat, on November 11th, 1885. The wound was cauterised with carbolic acid. The child was so badly bitten, that it was necessary to send her to the Hospital for Children, in order to have her wounds treated.

Corbillon, aged 27, was bitten on November 12th, 1885. The dog was recognised to be mad by M. Chautereau, veterinary surgeon at Clermont. The wound was cauterised by hot irons, eight hours after the accident.

Bouchet, aged 5, was bitten on November 12th, on the left hand and thigh; the clothes were torn. The dog was declared mad by M. Corot, veterinary surgeon at Aubervilliers. The wounds were burned with hot iron three-quarters of an hour after the accident.

Madame Delcroit was bitten on November 6th, on the right foot. The wound was cauterised with hot iron, nine hours after the accident. The dog was recognised to be mad by M. Frélier, veterinary surgeon.

Plantin was bitten at the beginning of November, 1885, on the right hand. The wound was cauterised forty-eight hours after the accident. The dog was declared to be mad by M. Eloire.

Jeanne Pazal, aged 7, was bitten, on November 12th, by a dog, de-

clared by Dr. de Tindray to be mad. The child was taken to Dr. de Tindray forty-eight hours after the accident; he wisely decided cauterisation to be useless.

Mme. Achard was bitten on November 9th on the right foot. On November 12th, she was bitten on the right hand by the same dog, which was declared by M. Charloy, veterinary surgeon, to be mad. The wounds were not cauterised.

Mme. Alphonsine Legrand was bitten on the chin on November 6th, 1885, by a dog, declared by M. Decarme, a veterinary surgeon, to be mad.

Antoine Cattier, aged 43, was bitten on the hand on November 16th. The wound was cauterised with hot iron twenty hours after the accident. The dog was recognised by its master to be mad; it had the characteristic bark, it refused food, and gnawed and swallowed wood and other things.

On November 15th, M. and Mme. Ternat, Mme. Delzors, and Mme. d'Alibard, were all four bitten by a mad dog, recognised to be mad whilst alive, and also after death, by a veterinary surgeon. They were cauterised some time after the bite.

Dr. John H. was bitten on November 13th, 1885; he had two serious wounds on the lower lip. No cauterisation was made. The dog was recognised by Dr. H. to be mad.

Mme. Faure, a widow, was bitten on the leg on September 1st, 1885, at Alma, in Algeria, by the same dog that had bitten four children, one of which died at the Hospital Mustapha, in Algiers, two months after the bite. Dr. Moreau, of Algiers, described very minutely the symptoms of hydrophobia exhibited by the child. The other three children were sent to M. Pasteur, and their treatment was commenced in the middle of November.

Mme. Gréteau was bitten on November 14th, in two places, on the third finger, the pulp and the nail, which was cut in two. The dog was ascertained by Dr. Douand to be mad. The wounds were washed out with ammonia, and slightly cauterised.

M. Voisenet, aged 50, was bitten on November 16th in both legs, by a bitch, which was declared mad by M. Colas, veterinary surgeon. The wounds were slightly cauterised four hours after the accident.

M. Guichen, aged 67, was bitten on November 15th, on the left hand, by the same dog which had bitten Mme. Gréteau, mentioned above.

Walter H. (London) was sent to M. Pasteur by Sir James Paget. The wounds had been slightly cauterised. He was bitten on the hand on November 15th. His brother died, five years ago, from the bite of a dog, which was believed to be quite unimportant.

Calmeau was bitten, on November 15th or 16th, on the abdomen, thigh, and knee; his clothes were torn to shreds. M. Cola declared the dog mad. It was the same animal that bit Voisenet.

Jean Lorda, aged 36, was bitten on October 25th. He arrived at M. Pasteur's Laboratory on November 21st, twenty-seven days after the bite. The same dog bit two cows and seven pigs the day Lorda was bitten. These nine animals died mad; the pigs after a short incubation of fifteen days to three weeks. After the death of the pigs, Lorda became frightened, and went to Paris to M. Pasteur. One cow died thirty-four days after it was bitten, the other fifty-two days. The cows were thoroughly cauterised with hot irons immediately after the bites were inflicted. Lorda is in excellent health; he finished the course of treatment on November 28th, 1885.

Jullion was bitten on November 30th. The child, perceiving the dog approach him, began to cry. The dog bit him inside the mouth, on the palate, the upper lip, the right eye, and nose. It was impossible to cauterise. The dog was declared mad by M. Guillemard, veterinary surgeon.

Former and Recent Experiments concerning the Virulence of the Nervous System in Rabid Animals.—At the commencement of our report, we mentioned that M. Desboué had theoretically concluded the nervous system in rabid animals to be rabid; also that M. Galtier's experiments tended to negative this belief; but that, finally, the experiments of M. Pasteur and of his assistants, MM. Chamberland and Roux, absolutely proved the virulence of both the central and peripheral nervous system in rabid animals.

It is interesting to recall to memory Rossi's experiment, made in 1808. He inoculated a dog with rabies, by placing in a wound a piece of crural nerve which he had removed from a living mad cat. This experiment was quite overlooked, and probably, but for the experiments made by M. Pasteur and his assistants, would never have come to light.

Hydrophobia Produced by Swallowing Virus.—We have, in another part of our report, given a few words to Professor Galtier's experiments relative to the immunity from hydrophobia conferred by intravenous injections of rabid virus; and we also stated that the results of M. Pasteur's experiments did not confirm those of M. Galtier

(Part I, p. 672). It may, nevertheless, be useful to state M. Galtier's conclusions, published with his note. They contain an important fact concerning the ingestion of rabid matter, and its relation to the transmissibility of rabid virus, hitherto little studied. "Rabid virus, injected into sheep's veins, does not produce rabies in them, but appears to render them refractory to the disease. Hydrophobia can be contracted by swallowing the virus. The actual seat of inoculation by this method is not ascertained, but it is nevertheless certain that any person or animal receiving into the digestive tract the virus of hydrophobia runs the risk of contracting the disease."

Inoculation with Blood.—Besides the prophylaxis for Hydrophobia, at which M. Pasteur has arrived by his experiments already described, he has indicated another, which he appears to have abandoned, as he does not mention it subsequently. He says, "By means of inoculations, under certain given conditions, made with blood taken from rabid animals, I have succeeded in greatly simplifying the process, and yet at the same time rendering dogs completely refractory to hydrophobia."

Micro-organism of Hydrophobia.—The existence of a specific microbe of hydrophobia is not yet positively demonstrated. Nevertheless, certain facts are established, which apparently indicate that one exists. M. Pasteur has stated that, if two preparations be made, one with a normal medulla oblongata, and another with one that is rabid, they both are found to contain a considerable number of molecular granules; but that the rabid medulla contains a larger number of granules, and they are much smaller. One is tempted to believe in the presence of an infinitely small microbe, neither presenting the form of a bacillus nor that of a constricted micrococcus, but that of small specks.

Since that communication was made, M. H. Fol has stated that, in preparations of medulla oblongata, hardened under Ehrlich's fluid, and coloured according to Weigert's method, he has detected micro-organisms, presenting the aspect of perfectly spherical granules of a blue colour, not arranged in any definite order, 2 μ in diameter. Furthermore, M. Fol cultivated some rabid cerebral matter, in broth, made by triturating a sheep's brain, mixing it with water and carbonate of potash. A sediment was deposited after an interval of four days, and the same micro-organisms were present in it. This cultivation fluid, when less than six days old, often produces hydrophobia in animals, but not if of older date. M. Pasteur has examined M. Fol's preparations, and has told us that he is quite inclined to believe that the micro-organism detected by him will be proved to be the specific organism of hydrophobia.

M. Gibier, in 1883, stated that he had seen, in the medulla oblongata of a rabid animal, examined under a magnifying power of 500 to 600 diameters, a micro-organism twenty times smaller than a red blood-corpuscle. Later, a plate of it appeared in his doctoral thesis. Judging from these plates, it appears to us to be granules of myelin; it is certainly not the micro-organism that M. Fol has described, which, unless coloured, is invisible under a magnifying power of 600 diameters.

The Virulence of Rabid Virus Preserved in Pure Carbonic Acid.—The virulence of rabid virus, which, when preserved in dry air, becomes perfectly inert between seven to ten days, is preserved during several months, if kept in pure carbonic acid, and thus kept from contact with the air, and from the influence of foreign microbes (*loc. cit.*, October 26th, 1885, p. 767, note).

* *Comptes Rendus de l'Académie des Sciences*, 19 Mai, 1884, p. 1230.

⁵ *Ibid.*, 25 Février, 1884, p. 458.

⁶ *Ibid.*, December 14th, 1885, p. 1277.

PRESENTATION.—Dr. L. A. Weatherly, being about to leave Portishead, has been publicly presented with a very handsome timepiece, and the following address, with more than two hundred signatures of working men and women: "To Lionel Alexander Weatherly, M.D., M.R.C.S. Sir,—We, the undermentioned, members of the working classes of Portishead, Portbury, and Clifton, beg leave to offer you this timepiece, as an humble mark of our heartfelt gratitude and sincere thanks for your invariable kindness and deeply-valued services to ourselves and families. We feel that no words of ours could adequately convey our regret at losing so faithful and kind a gentleman and friend as you have always been to the working classes of each place. We beg to wish you every success in the new sphere of usefulness you have chosen, and humbly pray that health and every other blessing may attend you; and, although we are losing you, pray believe that your honoured name will ever live in the hearts of ourselves and our children." (Signed) Robert Edbrooke, chairman; William H. Edbrooke, Francis Simpkins, John Reed, Henry Charles Barrington, committee; and followed by the names of 212 working men and women.