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THE DEVELOPMENT OF THE VIRUS CONCEPT AS REFLECTED IN CORPORA OF STUDIES ON INDIVIDUAL PATHOGENS*

4. RABIES—TWO MILLENNIA OF IDEAS AND CONJECTURE ON THE AETIOLOGY OF A VIRUS DISEASE

by

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WHEN WRITERS of medical texts wish to remind their readers that virus disease is nothing new, they turn to the records of smallpox and of rabies. From antiquity, and through the centuries, the impact of smallpox on social and political history through the extent and severity of epidemics, and the high rate of mortality, has been considerable and equalled among the infectious diseases only by bubonic plague.[1] Rabies on the other hand never claimed large numbers of victims even in epidemic situations. In one outbreak which caused concern in nineteenth-century Lancashire, the total number of deaths in the year 1866 was reported to be thirty-six, and of this Fleming wrote: ". . . West Lancashire appeared to have become a centre from which it spread in various directions, until it became disseminated, and had attained the dimensions of a serious epizooty—formidable alike to mankind, as well as to the other domesticated animals".[2] In a contemporary outbreak in Denmark, the number of cases totalled 227 dogs, nine cattle, six horses, five sheep, and a few cats. There were four human fatalities.[3]

Compared to the numerous shorter and sharper outbreaks of bubonic plague and smallpox in Europe during the centuries when they held sway, and when there were frequently tens, and sometimes hundreds, of thousands of deaths in a few months,[4] the figures for rabies appear almost insignificantly low. Nevertheless, rabies has captured the imagination of writers and thinkers from the time of antiquity. The alarming symptoms in man and dog alike, the prolonged suffering of the victims and the inevitable fatality of the established clinical disease: the distressing syndrome as a whole has meant that outbreaks of the disease have been meticulously recorded out of all proportion to the slight numbers of victims claimed in comparison with the major scourges of mankind. The manner of its transmission has ensured for rabies a unique position in the annals of infectious disease. The detailed descriptions

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[1] For details through the centuries, see B. M. Lersch, *Geschichte der Volksseuchen*, Berlin, S. Karger, 1896.

[2] G. Fleming, *Rabies and hydrophobia: their history, nature, causes, symptoms and prevention*, London, Chapman & Hall, 1872, p. vii.

[3] J. Müller, Danish State Veterinary Serum Laboratories, personal communication, 1975.

[4] Lersch, op. cit., note 1 above.

of the syndrome which have been preserved in earlier writings give evidence that rabies is one—perhaps the only one—virus disease whose clinical manifestations have changed little if at all over a period of more than 2,000 years.

Some of the very early mentions which have been claimed to refer to rabies may be somewhat ambiguous.[5] Pre-Socratic philosophers, notably Democritus, have been included in references to early descriptions of the syndrome.[6] Högyes' claim, in 1897, that Democritus "considered the disease an inflammation of the nerves"[7] is at first sight impressive and encouraging to the historian, but an examination of the extant fragments of his writings[8] reveals no evidence for such a claim. It seems likely that Högyes' remarks were based on Caelius Aurelianus' Latin rendering of the Greek texts of Soranus of Ephesus.[9] Soranus may well have had access to Democritus' original texts, as he flourished before the final destruction of the Great Library of Alexandria.[10] The relevant passage in the chapter on hydrophobia in *On acute diseases and on chronic diseases* reads: "ait enim hydrophobiam esse incendium nervorum" which Drabkin translates as: "he says that hydrophobia is a kindling of the sinews".[11] Most modern authorities would agree with Drabkin that nothing more significant than "sinews" should be read into the use of "nervus", or Greek "neuron", at this time, and that an elementary understanding of the anatomy of the brain and the nervous system only began to develop with Herophilus' dissections of human cadavers.[12] According to Caelius Aurelianus (or Soranus) Democritus did perhaps not draw any very clear distinction between hydrophobia and tetanus.[13] If we rely on this same source of information (and we do not have much choice for this period), it was left to one Gaius, a follower of Herophilus, to be the first to state, in a book *On hydrophobia,* that ". . . the brain and its membrane are the parts affected" in this disease.[14]

[5] The oft-quoted passage from the pre-Mosaic Eshunna Code (*c.* 2300 B.C.), (see for example F. Rosner, 'Rabies in the Talmud', *Med. Hist.*, 1974, **18**: 198–200, and also E. S. Tierkel's historical chapter in Y. Nagano and F. M. Davenport (editors), *Rabies. Proceedings of a conference,* Tokyo, October 1970, Baltimore, University Park Press, 1972, p. 3), refers to the bites of "vicious" dogs. It would seem that viciousness in dogs, and death following savaging by such dogs, could conceivably have had causes other than rabies under the conditions of hygiene likely to have prevailed in ancient Mesopotamia.

[6] See for example H. N. Johnson, 'Rabies virus', in F. L. Horsfall and I. Tamm (editors), *Viral and rickettsial diseases of man,* Philadelphia, J. B. Lippincott, 4th ed., 1965, p. 815.

[7] A. Högyes, 'Lyssa', in H. Nothnagel (editor), *Specielle Pathologie und Therapie,* vol. 5. part 5, II, Vienna, Alfred Hölder, 1897, p. 4.

[8] Kathleen Freeman, *Ancilla to the pre-Socratic philosophers,* Cambridge, Mass., Harvard University Press, 1957, pp. 91–120, and K. Freeman, *The pre-Socratic philosophers, a companion to Diels. Fragmente der Vorsokratiker,* Oxford, Basil Blackwell, 1959, pp. 289–326.

[9] Caelius Aurelianus (Soranus of Ephesus), *On acute diseases and on chronic diseases,* edited and translated by I. E. Drabkin, Chicago, University of Chicago Press, 1950.

[10] Soranus of Ephesus was educated in Alexandria and practised in Rome at the time of Trajan and Hadrian, i.e., first to second century A.D. He was considered the greatest of the Methodist physicians. The best-known Latin version of his texts was prepared by Caelius Aurelianus, who is thought (on stylistic grounds) to have lived in the fifth century. The library of Alexandria was destroyed by Christians in A.D. 391 and finally, by Muslims in 642.

[11] Caelius Aurelianus, op. cit., note 9 above, p. 385, 133.

[12] See Edwin Clarke and Kenneth Dewhurst, *An illustrated history of brain function,* Oxford, Sandford Publications, 1972, (Herophilus flourished *c.* 300 B.C., Democritus more than a century earlier.)

[13] Caelius Aurelianus, op. cit., note 9 above, p. 371, 112, and p. 377, 120.

[14] Ibid., p. 373, 114.

The first recognizable extant description of rabies is found in the writings of Aristotle, who in a brief paragraph on diseases in dogs wrote: "Rabies drives the animal mad, and any animal whatever, excepting man, will take the disease if bitten by a mad dog so afflicted; the disease is fatal to the dog itself and to any animal it may bite, man excepted".[15] The reference to man has not surprisingly left later commentators puzzled. Reluctant to question Aristotle's credibility, some have sought other explanations; Moseley[16] and Rossi[17] in the early part of the nineteenth century suggested, not very convincingly, that the syndrome might have changed over the centuries. Fracastoro's interpretation seems more likely; he believed that Aristotle was merely drawing a distinction between animals which inevitably develop the disease when bitten by a rabid dog, and man who may or may not develop clinical symptoms.[18] John Hunter saw the remark as proof that rabies was then a disease which had only recently established itself and was as yet incompletely known.[19]

By the time Celsus wrote on rabies in the first century A.D., he achieved a remarkable degree of accuracy in the oft-quoted passage: "Especially if the dog was rabid, the virus must be drawn out with a cupping glass".[20] The aptness of the quotation is spoilt a little by the fact that he had, in a preceding paragraph, written of "[the wounds] which are caused by the bite, sometimes of a man, sometimes of an ape, often of a dog, not infrequently of wild animals or snakes. For almost every bite has in it virus of some sort";[21] but in subsequent paragraphs he distinguished consistently between the use of "virus" to denote the agent transmitted by the bite of a rabid dog, and of "venenum" representing that of the bites of poisonous snakes.

Lwoff has pointed out that this hardly makes Celsus the father of virology,[22] and Pirie was inclined to consider the distinction in usage inadvertent.[23] However, there could be an alternative explanation. The dictionary informs us that one meaning of "virus" in Latin was "slime" or "slimy liquid"; Celsus' usage may well have been deliberate, and may have quite simply reflected his awareness that whatever agent was responsible for the transmission of rabies was to be found in the frothy, or slimy, saliva of the rabid individual, whereas snake venom was administered comparatively cleanly through the fangs. Whichever conclusion we decide to draw, at 2,000 years' remove such attempts at interpretation can be nothing more than exercises in semantics.

Pliny, whose *Natural history* appeared almost simultaneously with Celsus' *De*

[15] Aristotle, *Historia animalium*, vol. IV, book VIII, 604a.

[16] Benjamin Moseley, *On hydrophobia, its prevention and cure, with a dissertation on canine madness: illustrated with cases*, 5th ed., London, Longman, 1809, p. 5.

[17] G. R. Rossi, 'Osservazioni anat.-patologiche sopra l'idrofobia', *Ann. universali Med.*, 1825, **33**: 5–42, see footnote p. 40.

[18] Hieronymi Fracastorii, *De contagione et contagiosis morbis et eorum curatione*, translation and notes by Wilmer Cave Wright, New York and London, P. G. Putnam's Sons, 1930, p. 128–129.

[19] John Hunter, 'Observations and heads of inquiry on canine madness', *Transactions of a Society for the improvement of medical and chirurgical knowledge*, vol. 1, London, 1793, pp. 294–329, pp. 328–329.

[20] Celsus, *De medicina*, with an English translation by W. G. Spencer, London, W. Heinemann, 1938, p. 112.

[21] Ibid., p. 110.

[22] A. Lwoff, 'The concept of virus', *J. gen. Microbiol.*, 1957, **17**: 239–253, p. 240.

[23] N. W. Pirie, 'The viruses', in R. Harre (editor), *Scientific thought 1900–1960*, Oxford, Clarendon Press, 1969, p. 227.

medicina, recommended the prophylactic removal of the "worm" in a dog's tongue, a measure which with its underlying curious idea of the aetiology of the disease has been remarkably persistent.[24] Pliny's main concern was with prophylaxis and treatment, and his ideas were rather more primitive than those of Celsus. A number of the remedies he recommended as antidotes to be administered to persons bitten by mad dogs were based on the use of ash from burnt organic material, another example of a popular and long-lived superstition in the realm of *materia medica*.[25]

In medicine as elsewhere there was little if any progress during the Dark Ages, and the next detailed description of the rabies syndrome is found in Fracastoro's writings, so rewarding to the historian of microbiology and infectious disease. Fracastoro gives a complete account of the syndrome, and relates the clinical symptoms from his own observations; the long incubation period he can document by the case history of a boy, seen by him, who showed no signs of the disease until eight months after being bitten by a rabid dog.[26] His descriptions of the terminal stages of the disease are as accurate and as disturbing as many much later ones. Where Celsus was a Roman philosopher with an interest in medicine, Fracastoro was very much the sixteenth-century physician, although "Syphilus sive morbus gallicus", did something to earn him a reputation as a poet. His description of rabies is a much more finished professional account of the syndrome than the one left us by Celsus.

Fracastoro's fame rests primarily, and deservedly, on his formulation of the concept of contagion. With no recourse to the microscopic observations on which are based the reputations of Athanasius Kircher and of Leeuwenhoek, who lived and worked more than a century later, Fracastoro was able, by means of clinical observations allied to the power of logical thought, to construct a frame of reference within which he could use his concept of germs and contagion in a manner which was not to be vindicated finally until Pasteur and Koch laid the foundations of modern microbiology by their discoveries in the latter half of the nineteenth century.

The temptation to read too much into the manuscripts of earlier periods applies to the writings of Fracastoro as much as to those of Democritus and Celsus. The point is well illustrated by consideration of his chapters on rabies. He introduced his account by stating that "it cannot be contracted by every sort of contact, or by fomes, or at a distance, but only when the outer skin is so torn by the bite of a dog that blood is drawn; as though the contagion takes place in the blood itself through the contact with the teeth and foam from the mouth of the rabid animal. Its incubation is so stealthy, slow and gradual, that the infection is very rarely manifest before the twentieth day, in most cases after the thirtieth day, and in many cases not till four or six months have elapsed. . . ."

[24] Pliny, *Natural history*, book 29, XXXII; 98–102. The belief in the prophylactic value of removal of the "worm" from the dog's tongue persisted long enough to merit consideration in nineteenth-century textbooks, as did the perennial notion of spontaneous occurrence of rabies in dogs due to conditions of stress such as extreme heat ("dog days"), or sexual frustration (see E. H. Ackerknecht, 'Zur Geschichte der Tollwut', note 117 below).

[25] When Zinke published the results of his study on rabies in 1804 (note 33 below) he recommended for treatment of patients suffering from hydrophobia a powder which in addition to arsenic, cinnabar and dragonblood (a resin) contained as an essential ingredient the ash of old, burnt-out shoe-soles.

[26] Fracastorii, op. cit., note 18 above, pp. 124–133.

And later in the account he wrote: "Since, then, this contagion is not communicated by fomes, and is not produced in the skin by simple contact, but requires laceration of the skin, we must suppose that its germs are not very viscous [*lenta*], and that they are perhaps too thick [*crassiora*] to be able to establish themselves in pores . . ."[27]. Even allowing for difficulties in interpretation of Fracastoro's medieval Latin, and taking into account his terms of reference (or perhaps lack of terms would be more accurate), we are left with a great deal of admiration for the power of his thinking. On the other hand it may be stretching admiration to the point of eulogy to claim that he assumed these germs, or "seeds" of disease, to be "of the nature of colloidal systems", as one commentator in the early years of the present century interpreted his use of the adjectives "*lentus*" and "*glutinosus*",[28] when the concept of colloids came to the fore in bacteriological thinking.

For all their percipience, the ideas on contagion and germs advanced by Fracastoro can perhaps best be described as prophetic. Work with early microscopes did something to corroborate his postulates, but there was little real further progress until vastly improved microscopes and associated techniques became available during the nineteenth century.

Understandably, Fracastoro still carried with him some of the less advanced ideas and superstitions of his day, and he did not entirely discount the influence wielded by unfortunate constellations of stars and planets and of other natural and supernatural phenomena.[29] Nevertheless, the basic soundness of his theory of transmission of infectious disease was well ahead of his time, and his tentative attempt to explain the aetiology of rabies at least has a more professional ring to it than the one offered in London in 1613 by Spackman, who wrote: "Rabies, That is to say, Doggish madnesse, is an effect beside nature, ingendred or bred in a living creature, of some certaine peculiar poyson, and communicated or imported to man . . .".[30]

The eighteenth century saw a spate of publications dealing with rabies and hydrophobia.[31] Little that was new was added to the knowledge of the disease, and nothing at all that could be of any help to the sufferer, in spite of widespread efforts to test a multitude of more or less exotic remedies.[32] However, in the field of rabies, as in so many other fields, the beginning of the nineteenth century marked the beginning of a more scientific approach. In the year 1804, G. G. Zinke published in Jena a modest volume. Its German title was very long;[33] its claim to provide an infallible

[27] Ibid., p. 126–127. Fracastoro used "fomes" to denote clothes or household articles which might harbour germs.

[28] F. H. Garrison, 'Fracastorius, Athanasius Kircher and the germ theory of disease', *Science, N.Y.*, 1910, **31**: 500–502.

[29] Fracastoro also seriously discussed the possible reasons why lying under a sorb tree should induce recurrence of the illness.

[30] For an account of Spackman's work, see J. W. Barber-Lomax, 'The biting of madde dogges', *J. small anim. Pract.*, 1960, **1**: ii: 101–108.

[31] For the literature in the English language, see Charles F. Mullett, 'Hydrophobia: Its history in England to 1800', *Bull. Hist. Med.*, 1945, **18**: 44–65 (p. 47: ". . . . toward the end of the 17th century the promptings of the Royal Society inaugurated a voluminous literature which after 1730 reached extraordinary proportions . . . .").

[32] Even Frederick the Great found time to take an interest in the matter, see Hanns O. Münsterer, 'Die Tollwutbehandlung unter Friedrich dem Grossen', *Medsche Mschr., Stuttg.*, 1956, **10**: 191–195.

[33] G. G. Zinke, *Neue Ansichten der Hundswuth, ihrer Ursachen und Folgen, nebst einer sichern*

remedy against the bites of rabid animals was no more justified than all other such claims which had been made since antiquity. Yet this little volume contained epoch-making material; it is the first record of rational experiments designed to prove the transmission of the infective agent of rabies from the rabid dog, through infected saliva, to other dogs, cats, rabbits, and even fowl. Zinke's experiments are remarkable for their elegant simplicity and completeness,[34] and they form a fitting introduction to the nineteenth century. They combine in one slender volume the first recorded rational transmission experiments with a viral disease (Jenner's work on vaccination belongs both in its conception and in its execution in rather a different category) and serious considerations regarding centuries-old *materia medica* most of which are at best harmless, if useless.[35] Zinke's experimentation commands our admiration all the more when we consider that it was conceived and carried out at a time when Schelling's *Naturphilosophie* flourished in Germany,[36] and irresistibly affected even the thinking of men of the calibre of Johannes Müller.[37]

However, it would appear that just possibly Zinke's experiments were conceived before the appearance of Schelling's work, and not by Zinke. In 1793 was published in London the first volume of a journal which was to be discontinued only two volumes later, the *Transactions of a society for medical and chirurgical knowledge*. It contained an article by John Hunter on canine madness.[38] Having reported and discussed the known facts of the disease, Hunter went on to outline experiments which might contribute to a more thorough knowledge, and hence to eventual control of the disease. Hunter pointed out the desirability of determining whether rabies was transmissible between different species of animals, and the transmission by incision and transfer of infected saliva on the point of a lancet should be feasible. These were in all essentials the experiments carried out by Zinke, who used a small brush for transfer of the saliva. Hunter also suggested that "an experiment with the saliva of a hydrophobic patient might easily be made upon a dog"—the experiment which Magendie and Breschet carried out twenty years later.[39] He also advocated experiments in which what he called "counter poisons" might be tested by being applied in addition to the infected material to the incision made, an idea which was put into painstaking practice by Zinke.[40]

Zinke had read Hunter's observations on hydrophodia. He referred to the paper directly in two places in his book,[41] and indirectly once or twice more, but he does not tell us whether or not Hunter's remarks on potential experiments were his original

Behandlungsart der von tollen Tieren gebissenen Menschen, Für Ärzte und Nichtärzte bestimmt, Jena, C. E. Gabler, 1804.

[34] Ibid., pp. 184–194.

[35] Of the powder described in note 25 above, Zinke warns his lay readers that "because of its content of arsenic, the utmost caution is called for", ibid., p. 198.

[36] F. W. J. von Schelling published *Ideen zu einer Philosophie der Natur* at Leipzig, 1797, and *Erster Entwurf eines System der Naturphilosophie*, Jena and Leipzig, 1799.

[37] According to R. H. Major, *A history of medicine*, Oxford, Blackwell, 1954, vol. 2, pp. 788–789, Müller was greatly affected by Schelling's *Naturphilosophie* while a student at Bonn; later he was instrumental in replacing speculative attitudes with strictly experimental methods.

[38] Hunter, op. cit., note 19 above.

[39] F. Magendie, 'Expérience sur la rage', *J. physiol. exp.*, 1821, **1**: 40–46, p. 42.

[40] Zinke, op. cit., note 33 above, pp. 184–194.

[41] Ibid., p. 63 and p. 144.

inspiration.[42] There is no evidence at all that Magendie in Paris had read either Zinke or Hunter when he reported in 1821, in the first volume of his own *Journal de physiologie experimentale,* the transmission of rabies to a dog by inoculation of saliva from a human case,[43] the experiment referred to above.[44]

The very involvement of Magendie, a pioneer neuro-physiologist,[45] in rabies experimentation at this stage could be seen as an indication of a growing awareness of the neurotropic character of the disease agent. During the 1820s and 1830s, further attempts were made to obtain proof of the involvement of the nervous system as the seat of what was referred to occasionally as the "virus", but more often as the "contagious material". In Berlin in the 1820s Hertwig made several attempts to transmit rabies by the implantation of nervous tissue from rabid animals into healthy ones, but was unsuccessful.[46] Experimentation continued to be very difficult, and even dangerous, since in spite of Zinke's results, the animals used were still exclusively cats and dogs. The reintroduction of domesticated rabbits into rabies research by Galtier in 1879[47] was almost immediately taken up by Pasteur, and has become inextricably linked with the French school.

In fact, the English veterinary surgeon William Youatt[48] appears to have recognized, although not exploited, the potential of the rabbit as an experimental animal in this area at least thirty years earlier. In a volume published posthumously he wrote: "I very much regret that I never instituted a course of experiments on the production and treatment of rabies in this animal. It would have been attended with little expense or danger, and some important discoveries might have been made".[49]

Throughout the nineteenth century waves of rabies moved back and forth over the continent of Europe, and the many medical and veterinary journals initiated during the same period provide us with records reflecting the changing epidemic situations.[50] But while until 1880 epidemic situations changed frequently, rabies prognosis had changed not at all since the time of Celsus. There was still no hope for victims

[42] There is no reason to suppose that Hunter himself had any plans for experimentation in this field, and in any case he died in the same year. None of his English pupils appears to have taken up the challenge. On the contrary, in the early years of the nineteenth century Benjamin Moseley warned: 'The source of rabid poison, in all animals, is unquestionably in the mouth. I have no doubt but deadly inoculation might be performed in a way, which I do not think prudence would justify the mentioning— There is mischief enough already in the world." (op. cit., note 16 above, p. 42).

[43] Magendie, op, cit., note 39 above.

[44] Hunter had warned that such experiments would be "both difficult and dangerous" (op. cit. note 19 above, p. 324); Magendie informs us at one point that when called to inspect an obviously rabid mastiff at an establishment for fighting dogs in Paris he could do nothing alone, but that he was able to return the following morning "accompanied by a number of students known to me for their courage, sang-froid and dexterity, all necessary qualities on this occasion. . . ." (op. cit. note 39 above, p. 43).

[45] See for example Paul F. Cranefield, *The way in and the way out: Francois Magendie, Charles Bell and the roots of the spinal nerves,* Mount Kisco, N.Y., Futura Publishing Co., 1974.

[46] K. H. Hertwig, 'Beiträge zur nähern Kenntniss der Wuthkrankheit oder Tollheit der Hunde', *Hufeland's Journal der practischen Arzneykunde und Wundarzneykunst,* 1828, **67**: 3–173.

[47] V. Galtier, 'Étude sur la rage', *C. r. hebd. Séanc. Acad. Sci., Paris,* 1879, **89**: 444–446.

[48] William Youatt, *The dog,* London, 1851. Youatt (1776–1847), destined for the nonconformist ministry, followed his inclination to become a veterinary surgeon, lecturing to veterinary students at University College, London, and founding the journal *Veterinarian* 1828 (*Dictionary of national biography*).

[49] Youatt, op. cit., note 48 above, p. 148.

[50] Cf. the historical sections in M. F. Röll, *Die Thierseuchen,* Vienna, W. Braumüller, 1881.

of the established clinical disease, and short of immediate cauterization of bite wounds there were no preventive measures either which could offer the least hope of results. The nineteenth century saw many celebrated discoveries in medical and veterinary pathology, but discoveries are rarely made without much painstaking and time-consuming preliminary work. Rabies was no exception.

The year 1880 was to prove a critical date in the fight against infectious diseases. Pasteur developed his first chicken cholera vaccine,[51] and shortly afterwards an anthrax vaccine.[52] The previous year Galtier had realized the convenience of using rabbits with their predominantly paralytic and convulsive response rather than the "furious rage";[53] he recognized the potential importance of his findings, and in addition to publishing his results in a veterinary journal,[54] he submitted a note to the Academy of Sciences in Paris. He had proved that rabies was transmissible from dog to rabbit, and from rabbit to rabbit in series. He explained the motives for his experiments by declaring that his "necroscopic" results had convinced him that the only hope for the future lay in finding "an agent capable of neutralizing the rabies virus after it has been absorbed and thus to prevent the clinical illness."[55]

At about the time Galtier's observations were read to the Academy of Sciences, Pasteur became interested in the problem. Between 1879 and 1881 he established the principle of prophylactic inoculation with attenuated material. It began with the chance observation of the attenuation of a forgotten flask of chicken cholera culture left undisturbed for a few weeks instead of the routine twenty-four hours; it culminated in the triumphantly successful anthrax vaccination experiment at Pouilly-le-Fort in May and June 1881.[56] A few weeks later Pasteur delivered his famous address on "Vaccination in relation to chicken cholera and splenic fever" to the International Medical Congress in London, in which he explained his use of the terms "vaccine" and "vaccination", with a final bow to Jenner.[57]

Throughout this period, Pasteur had pursued a programme of experiments with rabies. The development of a rabies vaccine was to be the last, and perhaps the most spectacular, of all his discoveries. For the first and only time, he was up against an infectious agent he could neither see nor cultivate. He referred to it indiscriminately as "virus" or "microbe", but the terms were in no way significant. "Virus" to Pasteur denoted any infectious agent he happened to be concerned with at any particular moment; likewise "microbe", a term adopted with enthusiasm by the French school after it had been proposed by Sédillot in 1878.[58] The development of a rabies vaccine was the final example of Pasteur's persevering ingenuity; unable to grow the elusive

[51] L. Pasteur, 'Sur le cholera des poules. Études des conditions de la non-récidive de la maladie et de quelques autres de ses caractères', *Bull. Acad. Méd.*, 1880, 2e ser., **9**: 390–401.

[52] L. Pasteur, Chamberland and Roux, 'Le vaccin du charbon', *C. r. hebd. Séanc. Acad. Sci., Paris*, 1881, **92**: 666–668.

[53] Galtier, op. cit., note 47 above.

[54] V. Galtier, 'Études sur la rage', *Annls Méd. vét.*, 1879, **28**: 627–639.

[55] Galtier, op. cit., note 47 above.

[56] For a dramatic description of this episode, see René Vallery-Radot, *La vie de Pasteur*, Paris, Librairie Hachette, 1900, p. 446.

[57] L. Pasteur, 'Vaccination in relation to chicken cholera and splenic fever', *Lancet*, 1881, **ii**: 271–272.

[58] C. Sédillot, 'De l'influence des découvertes de M. Pasteur sur le progrès de la chirurgie', *C. r. hebd. Séanc. Acad. Sci., Paris*, 1878, **86**: 634–640, p. 634.

Figure 1.
Doctor treating with vervain a patient bitten by a mad dog. From Pseudo-Apuleius, *Herbarium,* III 8–9, in the Biblioteca Medicea Laurenziana, Florence (MS. plut. no. 73. 16). (*Reproduced by courtesy of the Director*).

Figure 2.
Slaying a mad dog. From Dioscorides, *Acerca de la materia medicinal y de los venenos mortiferos . . .*, Salamanca, M. Gast, 1566. (*Reproduced by courtesy of the Wellcome Trustees*).

agent in culture he grew it, and attenuated it, in its natural habitat, in the spinal cords of his laboratory rabbits.

Before he could attempt this, he and his staff had had to establish, by painstaking experimentation and analysis of results, two basic characteristics of the rabies agent. One was the neurotropic character of the virus, suspected by many throughout the nineteenth century; Pasteur delivered unequivocal proof, and also showed that the infectious principle was present in rabid animals not only in the saliva, but throughout the central nervous system. Second, and equally important, he arrived at a "virus fixe", a standardized form of the virus with a fixed incubation period. The first step was inoculation of street virus directly under the *dura mater* of dogs; this shortened the incubation period to no more than two weeks.[59] The same virus passed through rabbits became greatly exalted, resulting in a progressive shortening of the incubation period until a limit was reached of six to seven days. The virus had become stabilised, or "fixed". It was with this fixed virus that a vaccine was developed[60] which after years of intensive work, animal experiments, and agonizing ethical considerations was put to the test as a last resort on the badly bitten Joseph Meister.[61] The boy did not develop clinical rabies despite the severity of his injuries.

Many more cases of successful post–exposure prophylaxis followed, and put a fitting seal on the career of Louis Pasteur. Still rabies vaccination was in one particular essentially different from those previously developed by Pasteur. The microbe, if such it was, remained unknown, unseen, uncultivated on artificial media.

After Pasteur's death, and after "invisible" and "filterable" viruses had become accepted generic terms, nearly every one of Pasteur's former associates[62] claimed that he had said, in 1881, that perhaps the pathogen of rabies was too small to be seen. There is no incontrovertible proof of this in his writings; he did talk of an agent "*infiniment petit*", but this is a fairly vague term and not very significant when we remember that since the time of Leeuwenhoek micro-organisms had frequently been referred to collectively as the "infinitely small". The closest approach to a written statement of his views came in a reply to a question from Bouley, posed in 1883: "Is there then no rabies microbe?" To this Pasteur replied: "All I can assure you is that if you show me two brains, one rabid and the other healthy, then I will be able to tell, on the basis of a microscopic examination of the two, which is rabid and which is not. They both show an immense number of molecular granulations, but those of the rabid bulb are much finer and far more numerous. One is tempted to think of a microbe of infinitesimal dimensions, formed neither as a bacillus nor as a micrococcus: it is as though it consisted of mere points".[63] It is worth remembering

[59] L. Pasteur, Chamberland and Roux, 'Sur la rage', *Bull. Acad. Méd.*, 1884, 2e ser., **13**: 661–664.

[60] L. Pasteur, 'Méthode pour prévenir la rage après morsure', *C. r. hebd. Séanc. Acad. Sci., Paris*, 1885, **101**: 765–774.

[61] The previous year Pasteur had written to the Emperor of Brazil: ". . . But however much I multiply my cases of protection in dogs I know that my hand will shake when I have to go on to man" (S. Paget, *Pasteur and after Pasteur*, London, Adam and Charles Black, 1914, p. 79).

[62] See for example E. Roux, 'Sur les microbes dits "invisibles"', *Bull. Inst. Pasteur, Paris*, 1903, **1**: 7–12, 49–56, p.7; Remlinger, op. cit., note 65 below, p. 849; and A. Philibert, 'Virus cytotropes (virus filtrants-virus filtrables)', *Annls. Méd.*, 1924, **16**: 283–308, p. 284.

[63] L. Pasteur, Chamberland and Roux, 'Nouvelle communication sur la rage', *Bull. Acad. Méd.* 2e ser., 1884, **13**: 337–344, p. 339.

in this connexion that Pasteur's friends and biographers agreed that he was not interested in theory as such, only as it suggested new experiments to be done.[64] He certainly never speculated in print on what the nature might be of the points, were they the pathogen. On the other hand, there is some evidence, largely circumstantial, to suggest that the notion that rabies virus might be so small an entity that it might even pass through bacteria-proof filters had been broached in their circle at this time. When Remlinger finally succeeded in showing that the agent of rabies could be made to pass through filter candles in 1903,[65] he mentioned that Nocard and Paul Bert,[66] as early as 1880 and 1882, respectively, had failed to pass rabies virus through what were at that time very primitive filters of porous clay.[67]

However, even if we accept that but for technical difficulties the pathogen of rabies might have been the first filterable virus on record, earlier than either the mosaic disease virus of the tobacco plant or foot-and-mouth disease virus, we are forced also to accept by inference that Pasteur and his associates no more than Koch's associates, Loeffler and Frosch who discovered foot-and-mouth disease virus, were inclined to interpret an agent of invisible and filterable proportions as anything more or less than a very small microbe. The simultaneous study by a team led by Nocard and Roux of the mycoplasma agent of bovine pleuropneumonia confirmed this attitude.[68] Having fought hard to establish the principle of a living, cellular microbe as a specific agent for every infectious disease, neither Pasteur nor Koch was yet ready to consider other possibilities.

Although in the first review article on the new group of pathogens, "Sur les microbes dits 'invisible',"[69] Roux in 1903 discussed the revolutionary concept put forward by Beijerinck concerning tobacco mosaic virus, it does not seem to have had much impact in Paris. It certainly did not influence research into the nature of rabies virus in the early years of the twentieth century to any great extent. When Remlinger first proved the pathogen of rabies to be a filterable entity in 1903, he did in fact consider Beijerinck's arguments concerning the virus of tobacco mosaic at some length. He even tried to repeat Beijerinck's diffusion experiments with rabies virus; not surprisingly, his result did not confirm Beijerinck's. He decided that rabies was most likely caused, "like foot-and-mouth disease, pleuropneumonia, cattle plague and yellow fever, by an ultra-microscopic organism". In attempting to clarify this statement, Remlinger provides us with a perfect illustration of the sort of confusion caused by the work on pleuropneumonia. He wrote: This "latter microorganism [bovine pleuropneumonia] passes through Chamberland F. The pathogen of rabies

[64] E. Duclaux, *Pasteur: Historie d'un esprit*, Sceaux, Imprimerie Charavie, 1896, p. 391.

[65] P. Remlinger, 'Le passage du virus rabique a travèrs les filtres', *Annls. Inst. Past., Paris.*, 1903, **17**: 834–849.

[66] Paul Bert, 'Contribution à l'ètude de la rage', *C. r. hebd. Séanc. Acad. Sci., Paris*, 1882, **95**: 1253–1254; Bert states that he made the experiments in 1878 and 1879.

[67] Roux' tale of the development of the first Chamberland filter from the stem of a clay pipe (*Bull. Assoc. anc. élèves Inst. Past.*, 1971, No. 49), seems paradoxically to combine apocryphal qualities with a ring of truth; Chamberland himself did not mention it in his first report on the filters to the Academy of Sciences in 1884 (*C. r. hebd. Séanc. Acad. Aci., Paris*, 1884, **99**: 247–248).

[68] Nocard, Roux, Borrel, Salimbeni and Dujardin-Beaumetz, 'Le microbe de la péripneumonie', *Annls Inst. Past., Paris*, 1889, **12**: 240–262.

[69] Roux, op. cit., note 62 above, pp. 7–12, 49–56.

must be of larger dimensions since it is arrested by this same filter. However, the microbe of Roux and Nocard is—albeit very imperfectly—visible in the microscope, and the rabies microbe is invisible both in the filtrate and under all other conditions. Here is a contradiction whose explanation must be sought in the fact that the pleuro-pneumonia virus is 'mobile' (Roux and Nocard), 'very mobile' (Cotton and Mouton), whereas the virus of rabies is immobile or at least shows very little mobility . . .".[70]

Another source of confusion, as with many other virus diseases, was the observations made on inclusion bodies. Negri's first paper on the bodies named after him had appeared earlier in the same year;[71] he had identified them as protozoa, and the causal organisms of the disease. Remlinger disagreed; his filtration and also centrifugation experiments did not support the likelihood of a pathogen of the dimensions assumed for the protozoon.

Further centrifugation experiments in subsequent years, by J. O. W. Barratt[72] and by Remlinger himself[73] confirmed him in his view that he was dealing with an organism of ultra-microscopic dimensions. Negri was equally convinced of the accuracy of his protozoal theory.[74] Enjoying exceptional longevity,[75] and being totally dedicated to rabies research, Remlinger wrote and published on the subject for more than fifty years. In 1918, when war had occasioned one of his few visits to his native country, he spoke at the Paris Academy of Medicine on the nature of rabies virus.[76] As in the case of fowl plague virus, the new ideas of colloid chemistry were having an impact during this time. To Remlinger they suggested a possible explanation of the paradoxical behaviour of the pathogen he was studying, an explanation which would allow him to place it, as Centanni had attempted to do with fowl plague virus,[77] and Sanfelice with fowl-pox virus,[78] in an intermediate category where some characteristics were those of the very smallest microbes, bordering on invisibility, while others belonged to such colloids as the diastases,[79] representing the upper limit of non-living entities. Remlinger, slightly apologetic for the audacity of his theory, tentatively suggested that the stress placed on the pathogen by forcing

[70] Remlinger, op. cit., note 65 above, p. 849.

[71] A. Negri, 'Beitrag zum Studium der Aetiologie der Tollwuth', *Z. Hyg. InfektKrankh.*, 1903, **43**: 507–528.

[72] J. O. W. Barratt, 'Centrifugation and disintegration in relation to the virus of rabies', *Zentbl. Bakt. ParasitKde*, Abt. I, Orig., 1904, **35**: 633–640, 769–775.

[73] P. Remlinger, 'Action de la centrifugation sur le virus rabique', *C. r. Séanc. Soc. Biol.*, 1905, **58**: 27–28.

[74] A. Negri, 'Uber die Morphologie und der Entwicklungszyklus des Parasiten der Tollwut (Neurocytes hydrophobiae Calkins)', *Z. Hyg. InfektKrankh.*, 1909, **63**: 421–440.

[75] Pierre Remlinger was born in Alsace in 1870, and survived until 1964. According to his obituary (*Annls Inst. Pasteur, Paris*, 1965, **108**: 689–694), his life was characterized by the strict discipline of the dedicated scientist: "Without family and pupils he lived a solitary life in the Institute [the Institut Pasteur] at Tangiers, devoting all his energies to his work, eschewing academic reunions and even the excitement of scientific conferences."

[76] P. Remlinger, 'Contribution a l'étude de la nature du virus rabique', *Bull. Acad. Méd.*, 3e ser., 1918, **79**: 137–139.

[77] E. Centanni, 'Die Vogelpest', *Zentbl. Bakt. ParasitKde*, 1902, Abt. I, **31**: 145–152, 182–201.

[78] F. Sanfelice, 'Untersuchungen über das Epithelioma Contagiosum der Tauben', *Z. Hyg. InfektKrankh*,. 1914, **76**: 257–281.

[79] Remlinger, op. cit., note 76 above, p. 139.

it through such fine filters might somehow serve to modify its physical and chemical properties and "transform the tenuous ultra-microscopic organism of rabies into a veritable colloid, thus affecting in some way the transition between two realms". He went on to point out that in such a case there would be no need to postulate the existence of a separate toxin as in the case of diphtheria or tetanus, but that the pathogenic character might be inherent, "representing a sort of allotropic state which might be induced by physical or chemical forces".[80]

With this hypothesis, Remlinger admitted, he was approaching Beijerinck's concept of a "*contagium vivum fluidum*": but he emphasized that whereas the latter would pass through any filter regardless of pore size, the passage of rabies virus depended upon the degree of porosity of the filter used; there remained unexplained differences between the various pathogens classified as "filterable viruses".[81]

A common characteristic of all the filterable viruses was still the inability to grow *in vitro*. Noguchi, encouraged by success with certain spirochaetes,[82] made an attempt to grow rabies virus as well; it is probably the first recorded use of rabbit kidney for this purpose.[83] He reported a measure of success; but attempts by others to repeat his experiments failed,[84] and the kidney, ultimately so useful in tissue culture, was not again successfully used until the Maitlands reintroduced it in their classic experiments with foot-and-mouth disease virus in 1928.[85]

Negri had written at length on the morphology and supposed cycle of development of what he held to be the causative organism of rabies;[86] it had even been named *Neurocytes hydrophobiae*.[87] In the 1920s, Wright and Craighead,[88] Doerr and Zdansky,[89] and Levaditi and co-workers[90] found an organism associated with an infectious encephalitis in rabbits; it was named *Encephalitozoon cuniculi*. At the same time, Manouelian and Viala observed, in sections of hippocampus, structures which were smaller than Negri bodies, but which, they claimed, eventually merged to form the larger bodies; they proposed for this parasite the name *Encephalitozoon rabiei*.[91] Levaditi's group disagreed; there were similarities between the two parasites, but not

[80] Ibid.

[81] Ibid.

[82] Hideyo Noguchi, 'The pure cultivation of Spirochaeta duttoni, Spirochaeta kochi, Spirochaeta obermeieri, and spirochaeta novyi', *J. exp. Med.*, 1912, **16**; 199–210.

[83] H. Noguchi, 'Contribution to the cultivation of the parasite of rabies', ibid., 1913, **18**: 314–316

[84] F. Lucksch, 'Die filtrierbaren Infektionserreger', *Prag. tierärztl. Arch.* (*A*), 1925, **5**: 83–140, p. 121.

[85] H. B. Maitland and M. C. Maitland, 'Cultivation of vaccinia virus without tissue culture', *Lancet*, 1928, **ii**: 596–597.

[86] Negri, op. cit. note 71 above, and 'Zur Aetiologie der Tollwuth. Die Diagnose der Tollwuth auf Grund der neuen Befunde', *Z. Hyg. InfektKrankh.*, 1903, **44**: 519–540.

[87] Negri, op. cit., note 74 above.

[88] J. Homer Wright and Eugene M. Craighead, 'Infectious motor paralysis in young rabbits', *J. exp. Med.*, 1922, **36**: 135–140.

[89] R. Doerr and E. Zdansky, 'Zur Aetiologie der Encephalitis epidemica', *Schweiz. med. Wschr.*, 1923, **4**: 349–351; and 'Parasitologische Befunde im Gehirne von Kaninchen, welche zu Encephalitisversuchen gedient hatten', *Z. Hyg. InfektKrankh.*, 1923, **101**: 239–244.

[90] C. Levaditi, S. Nicolau and R. Schoen, 'L'étiologie de l'encéphalite épizootique du lapin, dans ses rapports avec l'étude expérimentale de l'encéphalite léthargique. *Encephalitozoon cuniculi* (nov. spec.)', *Annls. Inst Pasteur, Paris*, 1924, **38**: 651–712.

[91] H. Manouelian and J. Viala, '"Encephalitozoon rabiei" parasite de la rage', ibid., 1924, **38**: 258–267.

sufficient to justify classifying them as belonging to the same genus. They suggested yet another name for the species, and declared: "*Glugea lyssae* undergoes a complex cycle of development, only certain phases of which are so far known to us . . .".[92]

Even Remlinger was impressed by their histological evidence, and the idea of a parasite with different stages of development appealed to him the more because of a fact he had been unable to reconcile with his other findings for many years. Inoculating series of rabbits with fixed virus under the *dura mater,* sacrificing individuals at twenty-four hour intervals and subsequently inoculating other rabbits with this brain material, he found it to be still infective after twenty-four hours. With animals sacrificed two or three days after inoculation the transmission experiments were invariably negative; subsequently they became again positive. If the causative organism were a microsporidium, as had been claimed, then this interval might represent a stage in its development when it was unable to transmit the disease.[93]

In the spring of 1927, an international conference on rabies took place at the Institut Pasteur in Paris. A. C. Marie spoke on the nature of the virus of rabies. He quoted Högyes' remarks, thirty years before, of the possible enzymic qualities of the virus,[94] but discounted this view and any notions of spontaneous occurrence of the disease under environmental stress, concluding that the known facts were consistent only with the presence of a micro-organism in the tissues affected.[95]

Having weighed the evidence for the *Medical Research Council's System of Bacteriology,* 1930, Harvey and McKendrick concluded that "The properties and mode of action of rabies virus were very thoroughly worked out originally by Pasteur himself, and knowledge of this subject, except for the discovery of the Negri Body, and the alignment of the virus with other neurotropic filterable viruses, has not been greatly added to since his time".[96]

While the French school continued to be preoccupied with aspects of vaccination, and with the morphology and possible taxonomic position of the rabies agent, others tried to place it within the framework of the filterable viruses in general. In 1928 A. E. Boycott discussed "The transition from live to dead; the nature of filtrable [*sic*] viruses" in a balanced essay,[97] presenting the case as it must have looked at that time to a majority of pathologists. Boycott's conclusion that "A good many people are willing to believe that the bacteriophage is generated by its bacillus—which is probably the truth"[98] serves to remind us that before work on bacteriophage delivered the crucial pieces of evidence needed to complete the conceptual picture for molecular genetics and virology, it had managed to contribute a very great deal to the general confusion prevalent in the 1920s and early 1930s.[99]

[92] C. Levaditi, S. Nicolau and R. Schoen, 'Recherches sur la rage', ibid., 1926, **40**: 973–1068.

[93] P. Remlinger and J. Bailly, 'L'évolution du parasite de la rage comporte-t-elle un cycle?' ibid., 1929, **43**: 1396–1407.

[94] Högyes, op. cit., note 7 above, p. 62.

[95] A. C. Marie, 'Sur la nature du virus rabique', *Annls. Inst. Pasteur,* Paris, 1928, **42**, Suppl.: 12–35.

[96] W. F. Harvey and A. G. McKendrick, 'Rabies', in Medical Research Council: *A system of bacteriology in relation to medicine,* 1930, vol. 7, p. 203.

[97] A. E. Boycott, 'The transition from live to dead; the nature of filterable viruses', *Proc. R. Soc. Med.,* 1928, **22**: 55–69.

[98] Ibid., p. 68.

[99] The phenomenon of lysogeny (see A. Lwoff, 'Lysogeny', *Bact. Rev.,* 1953, **17**: 269–337), for so long imperfectly understood, gave weight to the idea of spontaneous production of bacteriophage.

Boycott was willing to concede the absurdity of claiming that diseases such as smallpox, measles and rabies should occur spontaneously when so obviously something was transmitted from one case to the next;[100] but he had an uneasy suspicion that certain other virus diseases such as for example herpes might well originate in the cells of the host, induced by external stimuli. Such dualism had been the inevitable corollary since the very first attempts to explain the obligate intracellular mode of replication of viruses and their lack of independent metabolic activities, by suggesting that they might be using existing cellular mechanisms, and perhaps might even be identical with certain discrete cellular constituents.[101]

If Boycott found it difficult to co-ordinate his own incompatible readings of different individual virus diseases, he could derive some comfort from the often repeated assertion by various authorities that so far the common characteristics of known filterable viruses were negative ones, which did not necessarily indicate a close biological relationship.[102] He concluded somewhat uneasily; "Taking one thing with another, I am inclined to think that they are both the cause and the result of their diseases as Sanfelice suggested for epithelioma contagiosum. Somehow or other a virus arises in an animal or plant and by its action on the tissues causes them to produce more of itself. Some viruses (e.g., smallpox) acquire a considerable capacity of spreading from infected to normal individuals and the majority of cases of the disease are so caused; the virus is on its way towards independence. Others (e.g. herpes) have little or no power of dispersion and most cases are due to the virus arising *de novo* under the appropriate stimulus (whatever that may be) . . .".

Three years later, H. H. Dale took up the theme and wrote of " . . . the central difficulty in dealing with the group of agents at present classed together as viruses. They seem to form a series; but we do not know whether the series is real and continuous, or whether it is formed merely by the accidental association, through a certain similarity in effects, and through common characteristics of a largely negative kind, of agents of at least two fundamentally different kinds . . .".[103]

In this paper Dale also made an attempt to come to terms with the question of possible autogenous origin of viruses, stating that "It is difficult, again, to imagine that a virus like rabies could be permanently excluded from a country if it had such an autogenous origin". Even at this late date of October 1931, this brought a reply in the *Lancet* from a correspondent who wrote: "I should not myself regard a spontaneous origin of rabies as out of the question, under certain circumstances", making it clear that in his view such circumstances might include ". . . extreme environmental changes, such as temperature or fasting . . .".[104] And so this notion, refuted at regular intervals throughout the centuries, was still abroad in 1931, when the preparation of crystalline tobacco mosaic virus was only a few years away.[105]

[100] Boycott, op. cit., note 97 above, p. 66.

[101] See for example Sanfelice, op. cit., note 78 above.

[102] See for example introductory chapters of T. M. Rivers (editor), *Filterable viruses*, Baltimore, Williams & Wilkins, 1928; and Medical Research Council, op. cit., note 96 above, vol. 7.

[103] H. H. Dale, 'The biological nature of the viruses', *Nature, Lond.*, 1931, **128**: 599–602, p. 601.

[104] H. M. Woodcock, 'The nature of viruses', *Lancet*, 1931, **ii**: 936.

[105] W. M. Stanley, 'Isolation of a crystalline protein, possessing the properties of tobacco-mosaic virus', *Science, N.Y.*, 1935, **81**: 644–645; and J. C. Bawden, N. W. Pirie, J. D. Bernal and I. Fankuchen, 'Liquid crystalline substances from virus-infected plants', *Nature, Lond.*, 1936, **138**: 1051–1052.

The great spurt of activity in research into the nature of viruses in the early 1930s was not reflected in work on rabies. The celebrations at the Institut Pasteur in Paris in 1935, marking the fiftieth anniversary of the first protective inoculations administered to Joseph Meister and Jean-Baptiste Jupille, also included the publication of a commemorative volume. Thirteen papers by members of Pasteur Institutes at home and abroad represented many aspects of work on rabies; none was concerned with the nature of the virus.[106] For all the viruses at this time, hopes of obtaining concrete information could be sustained only in one particular sphere. The size of rabies virus came up for appraisal when Galloway and Elford included it in their ultrafiltration studies in 1936.[107] They reported for the particle diameter a value of 100–150 mμ, a tolerable approximation of the value accepted today.[108]

Not until the early 1960s did new factual information about the virion of rabies begin to emerge. By then work on tobacco mosaic virus, bacteriophages, influenza viruses, polio virus, and others, had yielded those basic facts which have helped us to an informed concept of the biology of viruses. The year 1963 brought a spate of papers with very considerable contributions to our knowledge of the morphology and chemical composition of the rabies virion.

The previous year, Sokolov and Vanag had published results indicating that the Negri bodies consisted of granules of RNA imbedded in a matrix of DNA.[109] Now, all at once, it was established that rabies belonged to the RNA viruses,[110] electron micrographs were produced which showed for the first time the characteristic bullet shape of the virion,[111] suggesting its close morphological relationship with the virus of vesicular stomatitis, and finally it was shown that lipid formed an essential part of the infective particle.[112] The latter observation confirmed facts reported by Remlinger in 1918 during a study on the effect of ether on the virus of rabies.[113] Just as fowl plague virus had been shown to be inactivated by ether long before the existence of its lipid envelope and its essential function was known,[114] so Remlinger, and before him Roux,[115] tested the effect of ether on the infectivity of rabies virus in the course of experiments designed to improve vaccination procedures.

[106] See *Annls Inst. Pasteur, Paris,* 1935, **55**: Suppl.: Numéro commémoratif sur la rage.

[107] I. A. Galloway and W. J. Elford, 'The size of the virus of rabies ("fixed" strain) by ultrafiltration analysis', *J. Hyg.*, 1936, **36**: 532–535.

[108] F. Fenner, B. R. McAuslan, C. A. Mims, J. Sambrook and David O. White, *The biology of animal viruses,* New York and London, Academic Press, 1974, p. 30, quote an average size of 175 x 75 nm for the bullet-shaped rhabdovirus particle.

[109] N. N. Sokolov and K. A. Vanag, 'The nature of intracellular inclusions in experimental rabies', *Acta virol.*, 1962, **6**: 452–457.

[110] See V. V. Hamparian, M. R. Hilleman and A. Ketler, 'Contributions to characterization and classification of animal viruses', *Proc. Soc. exp. Biol. Med.*, 1963, **112**: 1040–1050.

[111] M. C. Davies, M. E. Englert, G. R. Sharples and V. J. Cabasso, 'The electron microscopy of rabies virus in cultures of chicken embryo tissues', *Virology,* 1963, **21**: 642–651.

[112] R. E. Kissling and D. R. Reese, 'Antirabies vaccine of tissue culture origin', *J. Immun.*, 1963, **91**: 362–368.

[113] P. Remlinger, 'Action de l'éther sur le virus rabique', *C. r. hebd. Séanc. Acad. Sci., Paris,* 1918, **166**: 750–751.

[114] E. Weineck showed this during structural studies (E. Weineck, 'Ueber die Protein-Lipoid-Symplexnatur des Hühnerpestvirus durch Erythrocyten', *Z. ImmunForsch. exp. Ther.*, 1940, **97**: 189–193).

[115] Roux never published these results, but Remlinger knew of them and mentioned them (op. cit., note 113 above, p. 750).

Although the introduction of cell cultures has since led to improvements in the preparation and standard of vaccines,[116] their safety record is not yet sufficiently good to encourage general vaccination programmes even for veterinary and research staff occupationally at risk. At the same time we must accept that the threat of rabies being reintroduced into Britain remains undiminished. In 1966 Ackerknecht commented with some optimism on the epidemiological outlook in Europe;[117] but a current outbreak of rabies on the European continent is spreading westwards as inexorably as any nineteenth-century counterpart. If our means to combat the disease have improved since 1885, we are still far from being able to eradicate it.

When the British Government sent a commission to France to report back on Pasteur's vaccination results in 1887,[118] the young secretary to the commission was Victor Horsley.[119] Years later, when giving evidence before the Royal Commission on Vivisection, Sir Victor Horsley recalled a conversation with the father of rabies vaccination. Pasteur had told the young Horsley then: "Why do you come here to study my method? . . . You do not require it in England at all. I have proved that this is an infectious disease: all you have to do is to establish a brief quarantine covering the incubation period, muzzle all your dogs at the present moment, and in a few years you will be free".[120] Horsley did not ignore Pasteur's advice. It was largely due to his initiative that Britain could be declared free of rabies in 1902,[121] and, after a period of reintroduction, again, and finally, in 1922.[122] Anyone doubtful of the justification of current legislation aimed at preventing reintroduction of rabies should remember the conclusions reached by Louis Pasteur and Sir Victor Horsley before the turn of the century.

SUMMARY

A disease numbering among its animal vectors some of man's closest vertebrate associates, rabies has been chronicled perhaps more exhaustively than any other virus disease. Opinions on its aetiology have been recorded since the time of Aristotle. The difficulties inherent in work with a pathogen as dangerous and elusive as that of rabies have prevented it from assuming much general importance for research on aspects of the virus concept. On the other hand, Pasteur's development of post-

[116] C. Kaplan, 'Rabies vaccine—an assessment', *Proc. R. Soc. Med.*, 1971, **64**: 228–231.

[117] Erwin H. Ackerknecht, 'Zur Geschichte der Tollwut', *Schweiz. med. Weschr.*, 1966, **96**: 746–748, p. 747.

[118] See *Nature, Lond.*, 1887, **36**: 232–235.

[119] V. A. H. Horsley (1857–1916), who became Sir Victor Horsley in 1902, died prematurely of heat-stroke while acting as consulting surgeon to the British Forces in Mesopotamia. After the commission's return from Paris he wrote several papers on rabies, see for example V. Horsley, 'On hydrophobia and its "treatment"', *Br. med. J.*, 1888, **1**: 1207–1211, and 'On rabies: its treatment by M. Pasteur', ibid., 1889, **1**: 342–344.

[120] See ibid., 1908, **1**: 1183–1186.

[121] "Horsley became the authority through whom Walter Long (later Lord Long of Wraxall), to his eternal credit, was enabled to withstand the opposition which included his own fox-hunting friends" (Plarr's *Lives of the fellows of the Royal College of Surgeons of England*, Bristol, John Wright, 1930, p. 563).

[122] B. Bisseru, *Rabies*, London, William Heinemann, 1972, p. 237.

exposure prophylaxis marked the beginning of a new era in the approach to vaccination against infectious diseases, and the certain fatality of the clinical disease provided compelling reasons for the use of a vaccine and for continuing attempts to improve the original product.

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