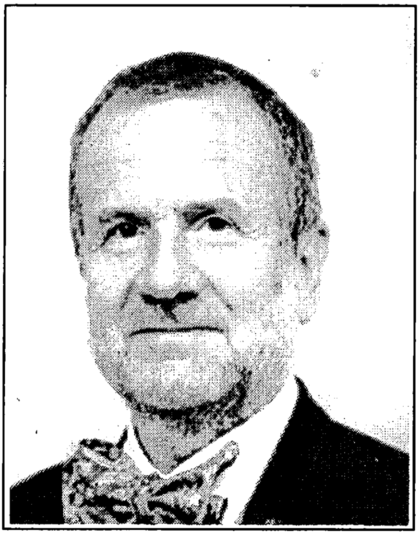

THE HISTORY OF ANIMAL VIROLOGY – PHASES IN THE GROWTH OF A SCIENTIFIC DISCIPLINE

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Motto:
SI QUIS SIT EA IMMANITATAE NATURAE UT CONGRESSUS HOMINUM FUGIT ATQUE ODERIT, TAMEN ID PATI NON POTERIT UT NON ANQUIRAT ALIQUEM APUD QUEM EVOMAT VIRUS ACERBITATIS SUAE
Cicero 'De Amicitia' 23, 87

In 1576, Carolus Clusius (Charles de l'Ecluse, born in Arras 1526, died in Leiden 1606), Professor of Botany at Leiden University, the Netherlands, published a booklet entitled *Rariorum aliquot stirpium per Hispanias observatorium Historia* (The history of strange stripes observed in Spain). He described conspicuous colour changes on the petals of tulips - white streaks and flame patterns - which enormously increased their appeal. The popularity of these varieties is not only reflected by the punctilious plant portraits in still lifes of Flemish and Dutch painters from the beginning of the 17th century, but also by commercial documents: for one bulb of the "Viceroy" tulip a price equivalent to about 30.000 € was paid in Holland. The "bulb madness" had reached its climax around 1635 when the trade finally collapsed. As early as 1637, however, Dutch tulip growers knew that the desired stripe pattern could be transferred to the petals of monochrome tulips by grafting bulbs to those of the streaked variety. Some 250 years later it was shown that the "breaking" of tulip petals is caused by a virus infection. The detailed grafting instructions published in 1675 by Blagrave are probably the first publication of an infection experiment in virology!

This we may regard as the Prehistoric Phase of virology, when the diseases and their contagiousness were known, but superstition and conjecture reigned about their cause and origin. We still have linguistic relics from these times: influenza, probably the most deadly virus disease of man, derives its name from an aetiological theory of the middle ages: the influence of the stars (*influenza delle strelle*) was advocated for an epidemic in Florence. But there are also material relics: the hieroglyphic inscriptions and the mummy of Rameses V (1100 B.C.) that provide evidence of his death due to smallpox; and the funeral relief at the Carlsberg Ny Glyptotek, Denmark, also from Egypt, showing a priest with the unmistakable symptoms of poliomyelitis.

Scientific priority

As in many fields of science, the breakthrough started with a priority fight – a quarrel about who was first – and we enter the Dogmatic Phase of virology. In 1882, Adolf Mayer (1843-1942), a chemist from Heidelberg, Germany, was appointed to a chair at the Agricultural School in Wageningen, the Netherlands (Fig. 1). He reported on a disease in tobacco plants, named it 'tobacco mosaic', and showed that it can be serially transmitted in the apparent absence of micro-organisms (Mayer, 1886). The causative agent of tobacco mosaic was to become the first model virus that revealed many secrets of virion structure. Dmitri Ivanovsky (1864 - 1920) is quoted for his classical filtration experiments in which he demonstrated passage of the causative agent of tobacco mosaic through the pores of a bacteria-proof Chamberland filter (Fig. 2). His paper, read before the Academy of Sciences in St.Petersburg, Russia in 1892, is undoubtedly a landmark in the history of virology. Of special significance for interpreting the author's ideas, however, is his dissertation published in German while he was working in Warsaw (Ivanovsky 1903). In it he reiterated that he was dealing with a microbe which might have passed the pores of the bacteria-proof filter or might have produced a filterable toxin.

The quantum leap towards the modern conception of viruses was taken by Martinus Willem Beijerinck (Fig. 3). Although remembered by virologists for his seminal role in their discipline, Beijerinck was a towering figure in microbiology. He and his successors Albert-Jan Kluyver and Cornelis Bernardus van Niel each addressed basic questions in microbial physiology: how does the intact organism interact with its abiotic and biotic environment? How can fundamental principles be brought to bear on applied problems? What is the place of micro-organisms in the natural world?

In his youth, Beijerinck had been an awkward boy with a keen interest in botany. He began his studies in chemistry at the Delft Polytechnical School, where he met the future Nobel laureate J.H. van 't Hoff, and the two boys supplemented their laboratory training with experiments performed on their own. After having received a doctorate in 1877 and a few years of teaching and research in botany, Beijerinck accepted the position of microbiologist at the "Nederlandsche Gist en Spiritus Fabriek" in Delft and was appointed professor at his alma mater in 1895. With his two sisters he moved into quarters built for him (next to his laboratory), where he lived until his retirement in 1921. Beijerinck has been described as a difficult person to get along with, subject to attacks of depression. He did not think that a scientist should marry, and he frowned upon any sign of friendship between students of opposite sex. He died ten years later, after having lived peacefully and surrounded by plants - having chosen in the last years of his life to go back to his first love, botany.

The genius of Beijerinck shows when he reconciled the two conceptions of the tobacco mosaic agent as, on the one hand, a molecule in solution, and, on the other, a pathogenic agent which multiplied: "There is another explanation to be considered, namely that the contagion, to reproduce itself, must be incorporated into the living cytoplasm of the cell, into whose multiplication, it is, as it were, passively drawn". This is the language of modern virology.

In the heated glass house provided to him in Delft, Beijerinck performed a series of experiments that lead him to the following conclusions:

1."The infection is not caused by microbes but by a living liquid virus"; this statement was based not only on the widely quoted porcelain filter experiments but also on observations of diffusion through thick layers of agar gel.

2."Only growing plant organs, where cellular division takes place are susceptible to infection. There only does the virus multiply". Here he concludes that "outside of the plant no multiplication can be observed" and adds that "the mode of multiplication of the virus reminds one, in many ways, of that of ... chromoplasts which also grow only within

cellular protoplasm, even though they have an independent existence and function separately ..."
3."The virus can be dried without loosing its infectious property".
4."The virus can spend the winter in soil outside of the plant and in a dry state".
5."The virus is inactivated by boiling temperature"; here he excludes the possibility of dealing with sporulating anaerobic organisms.

In analyzing priority claims one should appreciate conceptual originality rather than comparing publication dates. The polemics surrounding such claims reflect the Olympic spirit in science - *citior, altior, fortior* - giving the illusion that fame can be quantitated. Beijerinck's achievements for virology are sometimes disputed in this trivial sense, and Ivanovsky is quoted as his competitor, as having been the first. Beijerinck himself was more gracious than later historiographers in acknowledging that he did not know about Ivanovski's earlier publication, and he gave him credit. Ivanovski, however, related that he had "succeeded in evoking the disease by inoculation of a bacterial culture, which strengthened my hope that the entire problem will be solved without such bold hypotheses" (Ivanovsky 1899). In 1903, when further criticizing Beijerinck's conclusion about the *contagium vivum fluidum*, Ivanovsky claimed it to be a *contagium vivum fixum*. He wrote: "...the persistence of infectivity of the filtered sap can only be explained by the assumption that the microbe produces resting forms..." (spores). All these quotes demonstrate that Ivanovsky did not grasp the scope of his observations, that in his mind Koch's Postulates had fossilized into dogma (Bos 1981).

When assessing achievements of the early workers, which we would call virologists today, one should steer clear of the trap of anachronism; it is a semantic trap. Thus "virus" meant something quite different to Ivanovsky and Beijerinck, to Loeffler and Frosch, to Reed and Carrol than it means to us, and "fluid" at the turn of the century was synonymous with "non-corpuscular" only down to the dimensions that particles could be visualized at that time. It took another forty years to demonstrate the particulate nature of virions.

The beginnings of animal virology

At the same time, filtration experiments were also performed with an animal pathogen in Germany, which lead to the identification of the cause of foot and mouth disease (FMD) as a "filterable" or "invisible" virus, also in 1898. The finding resulted from a close collaboration between Friedrich Loeffler, professor and director of the Institute of Hygiene in Greifswald (Fig. 4), and Paul Frosch, then employed at Robert Koch's Institute of Infectious Diseases in Berlin. There Loeffler had been Koch's assistant until his appointment to the Greifswald chair in 1888. Already in 1890 Robert Koch had deplored the fact that many infectious diseases were still etiologically undefined; at the occasion of the 10th International Congress of Medicine in Berlin he proclaimed "... I tend to believe that the diseases mentioned (he referred to influenza, pertussis, trachoma, yellow fever, rinderpest, pleuropneumonia) are not caused by bacteria but by structured disease agents that belong to quite different groups of micro-organisms".

The optimistic atmosphere at the turn of the century, the enthusiasm about discovering more - perhaps even all - human and animal pathogens is reflected in the minutes of the 7th International Veterinary Congress, Baden-Baden, 7-12 August 1899. It was held under the protectorate of His Royal Highness the Grand-Duke Frederick of Baden, and this is how Friedrich Loeffler's report (in its original translation) reads for Tuesday, August 8th:

"The necessary funds were granted by the German Empire and the Prussian State, and I was charged with the execution of the work, which at first I carried on in the Institute for Infectious Diseases in Berlin, afterwards, in that of Hygiene at Greifswald, with the assistance of Professor Frosch, and later, from January 1898, of Dr. Uhlenhuth.

When I undertook the work, the aetiology of foot and mouth disease was little studied. It was known that the disease was transmitted to cattle, pigs, sheep and goats, and that its germs might be carried by diseased animals and also by persons who had been in contact with them. The mode of action of the germ, and the ways of infection were unknown.

The great results obtained in struggling with some infectious diseases of man by the discovery of the virus, and the scientific study of the biological character of those diseases, indicated the road to be followed. Many learned men had already found microorganisms, which they considered as the virus of foot and mouth disease. It was necessary in the first place to establish which of those organisms causes the affection; but all our researches remained without results and absolutely negative.

The microscopical examination of coloured and not coloured preparations, the various methods of cultures did not permit us to discover the virus in the fluid, where it ought to have been found, namely, in the contents of the aphthae.

However, an entirely new and very interesting fact could be established. In order to see, whether the contents of the aphthous vesicles, when filtered and attenuated with water, would grant immunity, they were passed through filters, which would with certainty hold back the most minute micro-organisms, for instance the bacilli of influenza. Still, the germ of aphthous fever did pass. In this way we were able to obtain a pure virus and to obviate any accidents that might arise from the presence of other organisms in the fluid that we used".

In view of the semantic trap mentioned above it should be noted that Loeffler used the word 'virus' in the generic sense. Since antiquity the term has been applied to denote slime, animal semen, foul odour, acrid and salty taste, poison in general, snake and scorpion venom; an early quote can be found in Cicero's 'De amicitia' (On friendship, written about 45 B.C.) where "...*virus acerbitatis suae*..." may be translated as "... the venom of one's bitterness" (Klotz, 1857).

Thus animal virology originated at the same time as plant virology, and it took only four more years before the viral aetiology of yellow fever, a mosquito-borne infection was solved (Reed and Carol, 1902). Animal virology arose from the need to control a disease of veterinary importance, as exemplified above; Friedrich Loeffler was less concerned with the properties of the agent than with its elimination from the German cattle population.

Before commenting on the importance and impact of animal virology, some definition is required. This is where ambiguity starts. Loeffler had a medical education, as had Frosch, though he held the chair for Hygiene at the Berlin Veterinary School during the last twenty years of his life. Is animal virology that branch of the discipline to which persons with a veterinary education have contributed? Then the fundamental studies at the Max-Planck Institute for Virus Research in Tübingen by Werner Schäfer – a vet by training - on murine retroviruses would fulfil the criterion. Or is animal virology rather for the sake of companion and farm animals, as medical virology is aimed at human health? Then Erich Traub's studies at the Federal Research Institute for Animal Virus Diseases in Tübingen (FRIAVD) on murine lymphocytic choriomeningitis virus should be excluded – they have no applied character; however, some 30 years later, a Nobel Prize was awarded to Rolf Zinkernagel and Peter Doherty for the elucidation of cellular immunity, using this infection model. Today, we speak of animal virology as the branch of the science that studies viruses replicating in cells of animal and human origin.

As every historian will confirm, chronological distance is a prerequisite for a fair assessment of the past. Thus we may mock the nature philosophers of the 1940ies that asked the question – considered moot today - whether viruses are dead or alive. They were led to this problem after having seen the crystals of tobacco mosaic virus, tomato bushy stunt virus, tobacco necrosis virus forming in highly purified suspensions. After all, crystals are characteristic of the inorganic, mineral domain, was the general feeling. But everybody could have performed the experiment contradicting this assertion: when shaking garden peas in a shallow pan, these will arrange to form hexagonal rosette patterns, which may be called paracrystalline, by filling the available space. Thus regular arrangements of bodies in space are by no means indicative of their inert nature.

Table 1

Nobel Prizes in Physiology or Medicine

1951	Max Theiler	yellow fever discoveries
1954	Enders, Weller, Robbins	growth of poliovirus in cell culture
1966	Huggins/Rous	sarcoma virus
1969	Delbrück, Hershey, Luria	viruses & viral diseases
1975	Dulbecco, Temin, Baltimore	tumour viruses, reverse transcription
1976	Blumberg, Gajdusek	hepatitis, kuru
1978	Nathans, Smith, Arber	restriction endonucleases
1993	Roberts, Sharp	gene splicing (adenovirus)

The Pioneer Phase

Starting in the 1930ies, the discipline of virology matured and provided a host of new insights – not only into the nature of the infectious agents but equally into the workings of the host cell. If one analyses only the achievements that were awarded Nobel Prizes (Table 1), the tendency is obvious: while initially the virus, its structure, replication, pathogenicity and immunogenicity was the object of study, the findings by Nathans, Smith and Arber (1978) of restriction endonucleases and by Roberts and Sharp (1993) of gene splicing (employing bacteriophages and adenoviruses, respectively) herald a different trend: viruses being used as tools to dissect the intricate networks of the cell.
In Table 2, some salient findings in the Pioneer Phase of virology are listed, together with events in world history.

Table 2 : The Pioneer Phase of virology

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YEAR	STRUCTURAL VIROLOGY	ANIMAL & PLANT VIROLOGY	WORLD HISTORY
1933-1935	Crystallization of TMV	Isolation of human influenza virus, 1933	Franklin D. Roosevelt and the New Deal
1937-1939	Crystallization of tomato bushy stunt virus	Yellow fever vaccine tests prove successful bacteriophage one step growth curve	Spanish Civil War
1941	Tomato bushy stunt and tobacco mosaic virus X-ray diffraction patterns	Influenza virus hemagglutinates red blood cells	Pearl Harbor
1945	An X-ray diffraction pattern obtained on a single crystal of tobacco necrosis virus		Death of President Roosevelt; end of second world war; atomic bomb explosion
1946-1949		Poliovirus cultured in human embryonic tissues	India achieves independence from Great Britain; creation of Pakistan (1947); the first digital computer ENIAC is put to use
1952-1953	Determination of the structure of DNA by X-ray diffraction (Watson and Crick)	Hershey-Chase experiment showed that DNA enters the bacterial cell.	First test of Hydrogen bomb. (1952); Stalin dies (1953)
1954-1955	Crystallization of polio virus (Schwerdt and Schaffer)	Jonas Salk's vaccination studies using killed polio virus successful	Brown vs Board of Education (1954), the Supreme Court decision leading to school desegregation
1956-1957	Crick and Watson propose the subunit structure of spherical viruses	Infectivity of TMV RNA reported Interferon discovered	Soviet Union launches Sputnik (1957)

1959-1960	First high resolution structure of a protein (myoglobin); negative staining of viruses	Bacteriophage infection leads to synthesis of new enzyme in bactaria	Castro overthrows Batista in Cuba (1959)
1961-1962	Principles of icosahedral virus structure	First deciphering of the genetic code	Berlin wall (1961). First man to orbit earth in space – Gagarin (1961)
1968-1969		Polio RNA shown to be translated from a single initiation site and proteolytically processed	Martin Luther King assassinated (1968); NASA puts a man on the moon (1969)
1970		Reverse transcriptase discovered	
1975-1976		Recombinant DNA Asilomar meeting	
1978-1979	Structure of tomato bushy stunt virus at 2.8 angstroms resolution	Elimination of the disease of smallpox	
1980-1981	Structure of southern bean mosaic virus at 2.8 angstroms Structure of satellite tobacco necrosis virus at 4 angstroms	Founding of the American Society for Virology, 1981 First reports of what was to become the AIDS epidemic	
1981-1982	Structure of the influenza hemagglutinin	Conformational change in HA observed at low pH	
1983		Identification of HIV as the cause of AIDS	
1985	Structure of the common cold virus (rhinovirus); structure of poliovirus		

Another priority issue

While Robert Koch and Louis Pasteur have become household names, so to speak, in microbiology, another figure in the virology, immunology, vaccinology triangle has been almost completely forgotten. I should like to draw the animal virologists' attention to a self-taught Dutchman, a miller and farmer, who is still remembered in his birthplace (Fig. 5). A monument was recently erected in Winsum/Friesland (Fig. 6) to honor Geert Reinders (1737-1815), the 'inoculator' and savior of the country from rinderpest. After the 1768 epidemic in the Netherlands he concluded that

* cattle which had experienced the natural illness were protected from disease after another infection,
* the same was true for animals with only light symptoms e.g. after vaccination, and
* the mode of inoculation and supportive therapy had no influence on the outcome of infection. He also discovered what we today would call "maternal immunity", the protection transferred from an immune cow to its calf.

The principle of vaccination had been known for over 2000 years. The ancient Greeks were aware that individuals who recovered from the plague had immunity, or diminished susceptibility, when exposed to the disease for a second time. However, it was not until the end of the 18th century that Edward Jenner provided the first scientific evidence of the vaccination principle (Fig. 8). Jenner, a country doctor, inoculated an 8 year-old boy with pustules of cowpox and protected him against an intentional smallpox infection. Benjamin Jesty, an English cattle breeder, had previously observed this phenomenon, but had not investigated it.

The term vaccination thus comes from the cowpox virus, *vaccinia*, which derived its name from the Latin *vacca*, meaning cow. It was only after Louis Pasteur's successful immunization attempts, in 1885, that the tremendous potential of prophylactic immunization was fully realized by the public and the scientific community. The vaccine inoculates he used were accidentally weakened forms of chicken cholera and intentionally attenuated rabies virus, but the mechanisms responsible for immunity were not understood at this time. Most vaccination attempts were based on trial and error. The worldwide application of vaccines in the last century has accomplished an almost complete elimination, or at least control, of many of the life threatening infectious diseases to affect man, e.g. poliomyelitis, diphtheria, measles, mumps, rubella and pertussis. Similarly, routine vaccinations used in veterinary practice have had a tremendous impact on the health and welfare of livestock and companion animals. There is no doubt that prophylactic immunization has a long history of successes and represents the most effective approach to immune modulation – irrespective of the present anti-vaccination tendencies. However, despite the increase in our knowledge of immunological pathways, there remains much to be clarified before the outcome of immune interventions can be predicted.

Geert Reinders published his observations in 1776 - Edward Jenner's vaccinia protection experiments appeared in press 22 years later (Fig. 9). At that time, however, Jenner was already a public figure, known as a skilful surgeon, eventually becoming a member of the Royal Society due to his discovery of the nesting parasitism of the European cuckoo. Reinders' findings were published in Dutch and had a small readership, Jenner's in English, which was to become the language of science. Historically, it would appear that veterinary vaccinology indeed predated medical vaccinology – as veterinary virology preceded medical virology. The speed of progress, however, was quite different.

The universal nature of the new agents defined in phytopathology and veterinary medicine became apparent when the US Army surgeon Walter Reed and James Carroll reported their findings on the cause of yellow fever. The authors nobly express their "sincere thanks to Dr. William H. Welch of the Johns Hopkins University, who during the past summer, kindly called our attention to the important observations which have been carried out in late years by Loeffler and Frosch, relative to the etiology and prevention of foot-and-mouth disease in cattle". Again filtration through porcelain candles was used for ascertaining the novel nature of the infectious agents.

If the history of virology is to convey anything more than nostalgic sentiments it should teach present-day scientists some lessons. About a century ago, the discovery of novel disease agents was "in the air". Or rather, the tools were in the literature: methods and techniques developed in one domain were available to be utilized in another field, with spectacular results. What we perceive as modern science management, to cross the barriers between disciplines, to listen to scientists from other provinces has been a fertile attitude through the ages - it also stands at the origins of virology. At the time, formulating the virus concept was a bold act which - had it turned out wrong - would have brought ridicule to its author. It is the difference between folly and visionary insight that determines whether footprints are left in the cultural landscape, in the pursuit of immortality.

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Figure legends

Fig. 1
Adolf Mayer, as seen by his students. This cartoon shows the German professor as the sorcerer, looking at the homunculus in the alembic. The drawing is an allusion to Goethe's Faust drama, the scene in the witch kitchen, with Mephisto looking over his shoulder.

Fig. 2
The 4-kopek stamp issued by the Russian Post Office in 1964 to honour Dmitri Ivanovski, the "first discoverer of the viruses", as the inscription on the left says. A nice detail is the drawing of the filtration device (lower left) used by Ivanovski

Fig. 3
Martinus Willem Beijerinck (born 1851 in Amsterdam, died 1931 in Gorssel, The Netherlands) as a young man

Fig.4
Geheimrat Prof. Dr. Friedrich Löffler (1852-1915)

Fig.5
Geert Reinders (born 1737 in Bedum, died 1815 in Bellingeweer) – "the lucky vanquisher of rinderpest", as the caption says. His contemporaries called him "the inoculator"

Fig. 6
The Reinders bronze monument in Winsum, Groningen Province, The Netherlands

Fig. 7
Front pages of the publications by Geert Reinders (1776) and Edward Jenner (1798); the Dutch text says: "Observations and experiments, most of them on inoculations of cattle. To prove that we can protect against rinderpest our calves born to recovered cows through inoculations. Also a clear instruction about how to inoculate without danger and in an easy manner. By Geert Reinders, houseman in Garnwert"

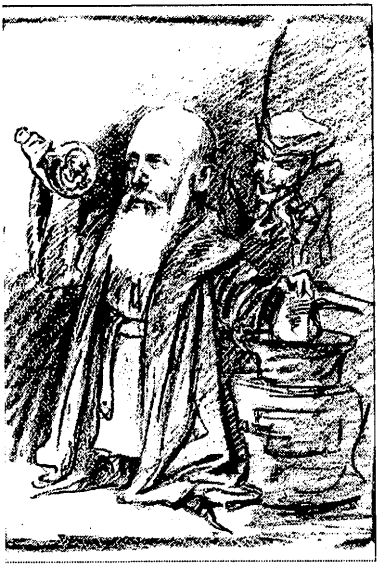


Fig. 1

Fig. 2


Fig. 3


Fig. 4

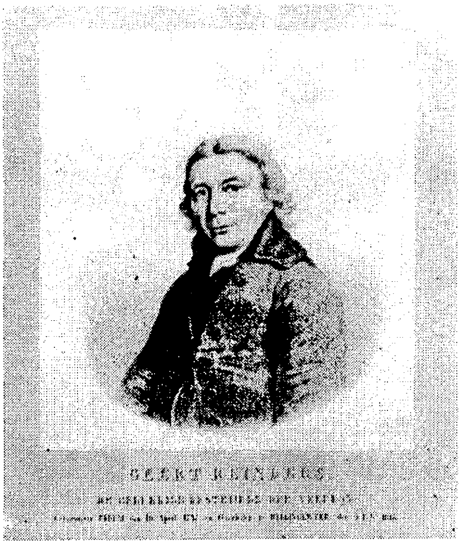


Fig. 5

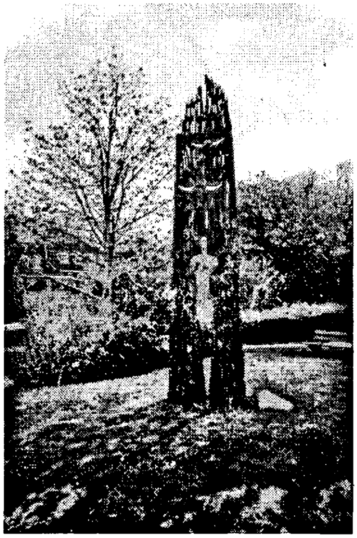
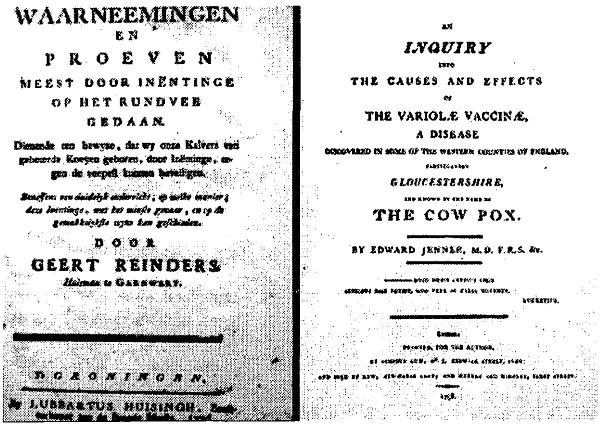


Fig. 6



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THE VARIOLÆ VACCINÆ,
A DISEASE
GLOUCESTERSHIRE,
THE COW POX.
BY EDWARD JENNER, M.D. F.R.S. &c.

Fig. 7