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

2. THE AGENT OF FOWL PLAGUE—A MODEL VIRUS?

by

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FOWL PLAGUE is a devastating disease of poultry which was first described in the form of outbreaks in Italy in the last two decades of the nineteenth century.[1] Until then no clear aetiological distinction had been made between it and chicken cholera, and only after Perroncito[2] in 1878 and Pasteur[3] in 1880 had turned their attention to chicken cholera did the difference in clinical manifestations come to be recognized. In 1901 there was another outbreak of fowl plague in Italy. It spread in the early months of the year from the area around Ferrara to Modena, and was subsequently brought from northern Italy over the Alps with the stock of an itinerant poultry merchant to cause an epizootic in Austria, where it travelled the length of the upper Inn valley.[4] It also made an appearance at the Brunswick poultry show, where the authorities unfortunately panicked, closed the show, and insisted that all birds be returned immediately to their place of origin.[5] The inevitable result was numerous outbreaks throughout the German states, as far apart as Oldenburg[6] and Württemberg.[7] All of the outbreaks were duly recorded, and as there was at the time a great deal of interest in the recently established group of pathogens referred to variously as "invisible"[8] or "filterable" viruses,[9] pathologists working on the epizootic were quick to test the ability of the agent to pass through bacteria-proof filters. Three papers were published in rapid succession, reflecting accurately and chronologically the spread of the disease through Italy and into Austria. The respective authors were Centanni and Savonuzzi,[10] Maggiora and Valenti,[11] and Lode and Gruber.[12] In all three studies was the agent shown to be filterable.

The clinical picture and the obvious neurotropic character of the agent led some pathologists in the early years to compare fowl plague to rabies;[13] initially the severity of the symptoms and the very high mortality gave no indication that the virus might be related to that of influenza in man, as was finally proved to be the case in 1955 when Schäfer[14] demonstrated the close relationship between the viruses of fowl plague and influenza A. It is interesting that more recently morphological similarities have been observed between rabies virus and members of the myxoviruses which include the influenza group.[15]

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Centanni's priority for isolating fowl plague virus and demonstrating its filterability was tenuous but undisputed.[16] He also immediately recognized the eligibility of this pathogen as a model for further studies of the properties and behaviour in general of filterable viruses. He wrote in 1902: "Fowl plague virus is not inferior to these other viruses [the agents of foot-and-mouth disease, bovine pleuro-pneumonia and African horse-sickness] with regard to its wide distribution and its lethal effect; it is far superior with respect to convenience of study, requiring only small, easily available animals, whereas for the other agents investigated so far the choice has been restricted almost exclusively to cattle and horses. This does not permit the exhaustive investigations required on these still mysterious viruses".[17] As the years passed, and the volume of virus research increased it became clear that very many aspects of this ever-expanding field could be conveniently studied in systems of fowl plague virus. Thus the early bibliography of this pathogen provides a broad spectrum of many facets of virology and evokes many names which in one way or another changed the face and pattern of medicine between the two world wars.

Centanni himself, in the extensive paper published in German in 1902,[18] introduced a number of possibilities for further research to be pursued in years to come. They included that most central focus of virus research, the question of culture of the virus. By accident or design, or, as sometimes happens, by a felicitous mixture of both, Centanni hit upon a method which in the end was to prove a very important one, i.e. inoculation of embryonated eggs with virulent fowl plague virus. Centanni may also have been influenced in his choice of eggs as experimental objects by the very considerable volume of work on "Entwicklungsmechanik", i.e. the study of the developing embryo inside the egg, which had been published by Wilhelm Roux in the last two decades of the nineteenth century.[19] The method was not perfect, nor was the theoretical background knowledge of the time, and Centanni discontinued his few experiments with eggs with inconclusive results. Judging by the presentation in his paper, he probably undertook the egg experiments with a different problem in mind, a problem which had grown out of studies on rabies in which Centanni had himself been involved—could acquired immunity be inherited, or was it in any way transmitted to the embryo? The classical study of this problem was published in its final, still inconclusive, form by Konradi in 1908.[20]

In the early years of the century, several authors pointed out the suitability of fowl plague virus as a working model for virus research; von Prowazek wrote in 1908: "Because of the surprisingly swift course of the disease and convenience of the experimental work involved, the study of the aetiology of fowl plague is especially important for the whole field of investigation of the so-called ultramicroscopic disease agents, which, judged by their general biology, appear not to belong to the group of bacterial disease agents".[21] Later it was used on two separate occasions for whole series of papers in attempts at comprehensive studies of the different aspects of one individual virus as a representative model. The latest of these came out of the Max Planck Institute for Virus Research in Tübingen, and culminated in the paper by Schäfer[22] in 1955 on comparative seroimmunological studies of the viruses of influenza A and fowl plague. The main points of both series are discussed later.

In view of the great volume of experimental work done with fowl plague virus as a

model, it seems reasonable to consider it also as a model for the development of the concept of the virus, for the thinking and the conjecture concerning the nature of viruses which were only just emerging at the beginning of the present century. Today many of the puzzles are resolved; we have accumulated a reasonable fund of knowledge of the nature and mode of action of a number of the more important pathogenic agents classified as viruses, and some taxonomic arrangement is becoming possible. Nevertheless, there are still areas of uncertainty, where one may be struck by a sense of *déjà-vu*. The hunt for the agent of viral serum hepatitis is a case in point. What is the role of Australia antigen?[23] Is the Dane particle, or its inner component[24] the infective unit? Or is the former a product of the infected cell, formed in response to infection? There is a distinct analogy with papers written in the first decade of the present century, when precisely this type of speculation was applied to the role of inclusion bodies, whether of tobacco mosaic disease[25] or of trachoma, or even of fowl plague.[26] We know now that they were indeed one form of the infective principle; it took more than thirty years to establish that knowledge unequivocally from the time that they were first observed. It is some measure of the progress of medical methodology that at the moment the solution to the problem of the identity of the hepatitis agent seems almost within grasp, and very likely we shall soon know the answer; whereas in the early years of the century there was no immediate hope of unravelling the identity of or the role played by inclusion bodies in the aetiologies of the diseases caused by "filterable viruses", whether in plants, animals or man.

To return to fowl plague virus, let us then follow its progression in clinical and theoretical terms up through the years of the present century. It was first recorded as a threat to Italian chicken farmers and through itinerant poultry merchants to the rest of Europe; now recent implications of recombination between influenza types of human and avian origin threaten man in several continents with new and different types of virus developing at a rate which makes it difficult for even the highly efficient vaccine centres around the world to keep the necessary step ahead of them.[27] Like the virus of swine vesicular disease which has only recently been isolated[28] and even more recently been linked with an aetiological agent for disease in man, i.e. Coxsackie B5 virus,[29,30] fowl plague was first recognized as a disease with a specific aetiology of its own in Italy. The three papers which initially established the filterability of fowl plague virus in 1901 were discussed in detail previously.[31] Centanni, in a concluding paragraph of great perspicacity,[32] indicated his willingness to believe that the virus of fowl plague might indeed belong in a hitherto unknown category, somehow straddling the realms of the living organisms and of essentially non-living, complex organic molecules, when he wrote: "In view of the inadequate methods currently at our disposal, we must of course defer the question of whether, in the reproduction of this virus, we are dealing with living organisms or complex chemical molecules, or even with entities belonging in some transitional area between the two".

Lode, on the other hand, after careful filtration experiments, became convinced that he was dealing with an agent similar in nature to bacteria, but of an extreme tenuity,[33] and the question of the microbial or non-microbial nature of the agent remained wide open. By 1907, Lode had added experiments concerned with the resistance of fowl plague virus to various chemicals. He found it to be relatively

resistant to glycerol, and concluded that this might suggest a relationship with the "filterable agents (rabies, vaccinia, syphilis?) whose protozoan nature is probable", and which had been observed to react in a similar way.[34] Besredka, abstracting the paper for the *Bulletin de l'Institut Pasteur*, remarked dryly that "this attempt at classification which seems somewhat brittle today may possibly gain added weight at some future date if and when we shall know the identity of the virus of rabies and of that of vaccinia".[35]

However, it was becoming clear that the merely physical common characteristic of filterability was insufficient to justify the indiscriminate combination in one class of an increasing number of pathogens, unless common biological properties could also be found. In a paper published in 1903[36] and famous not so much for its relevance to virus research as for its clear thinking and conclusion with regard to the aetiologies of the infectious epithelioses and the epitheliomata,[37] Borrel placed a number of viruses in a common group characterized by the ability to induce infectious epithelioses i.e. the agents of sheep-pox, vaccinia, variola, foot-and-mouth disease, rinderpest, and fowl-pox. Soon afterwards another, quite different, attempt was made to warrant the placing of a number of pathogens in a sub-group with a common characteristic. Its author was von Prowazek, originally a botanist, but ultimately a versatile and brilliant microscopist with catholic interests.[38] Because of his intense interest in microscopy, von Prowazek's endeavours naturally focused on those "filterable viruses" which produced visible changes in the host at the cellular level, i.e. those which like vaccinia, sheep-pox, fowl-pox and trachoma formed inclusion bodies visible under the microscope. He placed them in a class which he named "chlamydozoa".[39] In so doing he took the first step in removing a number of viruses from the general indiscriminate accumulation, and hence in acknowledging that "filterable" did not necessarily represent an ultimately viable classification. Prowazek had found that staining of samples containing inclusions from the conjunctiva of patients suffering from trachoma after the method described by Giemsa[40] revealed two distinct components, i.e. tiny well-defined, reddish granules, surrounded by an amorphous blue substance stained blue.[41] Prowazek assumed that the granules were virus elementary bodies, and the surrounding substance a reaction product of the host cell; he coined the term "chlamydozoa", or mantled animals, for this type of organism. It would seem to have been a contradiction in terms even from the beginning, since von Prowazek himself always assumed that the mantle was formed by the host cell in response to the foreign invasion, and hence that it was not an integral part of the virus.

In his article of 1907 Prowazek introduced the chlamydozoa concept as follows: "We propose to deal here with a number of pathogenic micro-organisms whose taxonomic position has not yet been entirely clarified, but which according to their biological properties, their partly intracellular development (multiplication, resting phases ["Ruhestadien"] of vaccinia), and their reaction to certain substances such as bile and sodium taurocholate (with the exception of fowl plague) appear to be related more closely to the protozoa than to the bacteria. These micro-organisms exist in a state of very characteristic and highly adapted parasitism: in contrast to bacterial invasion, the host cell responds to the viral invasion by the formation of very characteristic, specific reaction products. . . ".[42] It is interesting that this paper on the

chlamydozoa accords considerable space to fowl plague. Prowazek observed, as had Kleine[43] and Schiffman[44] before him, what he referred to as "nucleus-like inclusions in fowl plague bodies (possibly disease agents)", in the brain of a goose dead from fowl plague. Prowazek illustrated his findings with diagrams (Fig. 1). It might seem unlikely that with the means at his disposal in 1907 he should have obtained pictures comparable to those seen with modern techniques of phase-contrast and electron microscopes; yet there is a distinct resemblance between von Prowazek's diagram and electron micrographs of intracellular inclusions of fowl plague virus published by Flewett and Challice in 1951.[45]

The chlamydozoa theory was a false trail which ultimately had no positive effect on the development of the virus concept, except for the attempt to create a group with common biological, in addition to the merely physical, characteristics. In the paragraph quoted above, Prowazek also appears to take for granted the intracellular replication of the agents, although he apparently did not ultimately regard them as obligate intracellular parasites.[46]

The question of whether filterable viruses could be grown on artificial media continued to occupy the minds of other workers. Centanni had tried out several organic media, including whole fowl blood, back in 1901,[47] without success. Marchoux, who is otherwise known mainly for his work on dysentery and leprosy, made an isolated attempt to grow fowl plague virus in an agar medium.[48] He did achieve some proliferation of the virus when he added blood to a glucose-gel in a test-tube. He assumed that a zone had been established in which essential chemical substances from the blood had diffused into the glucose-gel which was then able to support growth of the virus, and believed that he had thus demonstrated the ability of fowl plague virus to grow on an artificial medium. However, his results were soon questioned by Landsteiner and Berliner,[49] who pointed out that in all probability the determining factor was the presence of whole intact blood cells, and that if these rather than a hypothetical substance released from them were necessary for the multiplication of the virus, the claim of successful culture on lifeless material was no longer valid.

On the whole, the decade from 1905 to 1915 saw a growing realization of the inability of viruses to grow outside living cells. There was no one dramatic piece of solid evidence, no one presentation of irrefutable fact; just a slow accumulation of suggestive data, confounding those who continued to attempt to grow viruses *in vitro*. In 1908, when Marchoux' paper was published in Paris, M'Fadyean had declared in London: "Another character common to all the ultravisible organisms is that they appear to be obligatory parasites".[50]

Landsteiner and Berliner published their paper in 1912. In the same year, and in fact in the same volume of *Centralblatt für Bakteriologie . . .*, a staff veterinarian in the German armed forces by the name of Mrowka wrote: "Our knowledge of the nature of the virus strongly favours the view that the filterable virus flourishes only in the body of the host and is very probably tied to a specific protein of the living body, being able to multiply only within the living organism. The periodic, frequently seasonal, outbreaks of disease originate from reservoirs among immune animals without frank clinical manifestations . . .".[51] The statement is contained in an article called, simply, "Das Virus der Hühnerpest ein Globulin". It is an interesting paper,

in which an attempt is made to apply the recently formed concepts of the young science of colloid chemistry to the even younger notion of filterable viruses. Mrowka based his experiments on separation by centrifugation, and explained: "The following experiments were planned on the assumption that in the case of the filterable viruses there can be no question of organised elements, whether bacteria or protozoa. The fact that it is possible to subject infective suspensions to hour-long centrifugation . . . without any change in infectivity in the different layers, has led me to the thought, absurd though it may seem at first, that the virus is in some way dissolved in the fluid".[52] And later: ". . . the indisputable ability of the filterable virus to pass through capillary walls repeatedly suggested to me the colloid nature of the virus. Corroborative evidence comes from studies of bacterial antigens and antitoxins which are generally regarded as colloidal in nature".[53] In Mrowka's experiments the globulins were precipitated out of infective serum by means of tannin, then washed, resuspended, and the suspension subjected to renewed centrifugation. In all cases the globulin fraction remained virulent, whereas the supernatant both before and after washing and resuspension contained no active virus.

Mrowka came to the conclusion that fowl plague virus behaved in all respects as a colloid globulin, and must be regarded as such. Much of his theorizing appears hazy or even ambiguous; in some passages he seems to suggest that the virus globulin is native protein which through external influence has become virulent, but he does not bring this line of thought to any conclusion. However, the concluding paragraph of his paper[54] is provocative in its very vagueness and ambiguity: "The protein nature of the filterable virus also makes us recognise that the search for microscopically visible agents, as well as the attempts to obtain visible growth of the virus, will probably remain unsuccessful. As far as experiments made until now allow us to conclude, artificial cultivation of the virus appears to be possible under certain circumstances. The culture method consists of a transfer of native hen's egg albumen to the virus. More of this later."[55] This passage leaves us anxious for the sequel, eager to learn whether Mrowka intended perhaps to enlarge on the theme introduced with Centanni's egg experiments; especially as he had included infectivity tests on developing eggs from ovaries of infected hens, and been puzzled to find differences in infectivity between eggs from one and the same ovary. Perhaps the complexity of the proposed method of culture defeated Mrowka; perhaps the Great War which broke out the following year put a stop to experiments with fowl plague, and there is no evidence that Mrowka ever returned to them. What is surprising in retrospect is that while various types of tissue culture were being tentatively attempted in the intervening years, it does not seem to have occurred to anyone to try to grow fowl plague virus in eggs until after Woodruff and Goodpasture in 1931 had demonstrated the suitability of the chorio-allantoic membrane for growing fowl-pox virus.[56]

A critical assessment of, and reply to, Mrowka's paper came in 1914 from Andriewsky, a young Russian working in Bordet's laboratories at the Pasteur Institute in Brussels. Again, this was labelled report no. 1 and gave the impression of being intended as the first of a series of papers; again, no more were forthcoming. Andriewsky confirmed Mrowka's experiments, but pointed out that the virus might simply be brought down with the settling globulin; nevertheless, he was convinced

that it could not possibly exist in the form of any previously known animal or plant cells. Andriewsky continued: "We know that Professor Beijerinck evolved the theory of a 'contagium vivum fluidum'[57] in order to explain certain contagious diseases in plants. In view of the results presently obtained with the ultrafiltration method, it is tempting to adopt his hypothesis in the case of fowl plague virus. In the circumstances it is to be hoped that Bechhold's ultrafiltration method will prove to be useful in the study of the nature of other invisible filterable viruses similar to that of fowl plague. Our investigations continue along these lines".[58] Andriewsky was making a serious attempt to determine the size of the infective agent of fowl plague, and presumably of other filterable pathogens in the continuing experiments of which no record has come down to us. Using Bechhold's ultrafiltration method,[59] and comparing the pathogen with haemoglobin, Andriewsky concluded that it was smaller than the size generally taken to represent the haemoglobin molecule, i.e. 2.3–2.5μμ (1μμ=1mμ=1nm=10^{-6}mm.) But although Bechhold as well as other authors[60] continued to improve the ultrafiltration method, there were still too many unknown factors in what were essentially comparative studies; even the very basic value of comparison, the diameter of the haemoglobin molecule, was a very rough approximation based on an empirical formula.[61]

Hence little real progress was made in this area until the early nineteen thirties, when Bechhold and Schlesinger attempted to determine the particle sizes of viruses by means of centrifugation analysis,[62] while Elford developed a new series of graded collodion membranes[63] to improve beyond measure the ultrafiltration method first developed for colloid studies by Bechhold in 1907.

Elford reviewed the history of ultrafiltration[64] and pointed out that the first attempt to introduce an ultrafiltration technique had been made in 1896 by Martin,[65] who used a Pasteur-Chamberland candle which he impregnated with gelatin or silicic acid. By using pressures of from 40 to 50 atmospheres he was able to separate colloids from crystalloids, and hence to establish the potential of the method. Bechhold then discovered that when he impregnated filter paper with acetic acid collodion of varying concentrations, the permeability decreased with increasing concentrations of the solution used, so that he was able to establish a graded range of permeabilities and pore sizes.

With the improved methods of filtration and centrifugation in the nineteen thirties, and with the aid of formulae painstakingly worked out to try to eliminate various sources of error, it became possible for the first time to present tables of comparative, and even hopefully of absolute sizes of known viruses. Today when electron microscopy has come to our help, we are able to confirm many values obtained in the thirties as reasonably accurate. The cubic symmetry characterizing a large number of viruses meant that the results of calculations which presupposed a spherical or near-spherical morphology in many cases compare well with present-day values. When in 1938 Elford[66] compared values for particle diameters of various viruses obtained by different methods, he quoted for fowl plague virus the following values in millimicrons:

Ultrafiltration	*Microscopy and U.V. light photography*	*Centrifugation*
75	75	88

The comparable value given for influenza A, human and animal strains, in modern textbooks[67] is 80–120 millimicrons (allowing for the pleomorphic appearance of the influenza group of viruses.[68] For the size of fowl plague virus Schäfer quoted the figure of 70–80 millimicrons.[69]

However, at the same time there was a growing realization that a definition based solely on particle size such as was implied by the general acceptance of the term "filterable viruses" in contemporary textbooks and elsewhere, was not viable and in no way justified classifying together an assortment of disparate organisms. There were different views with regard to the attitude one should take concerning the one known biological characteristic common to most, but not all, "filterable viruses", i.e. obligate intracellular parasitism, manifest by their inability to grow on artificial media. In 1933 Burnet and Andrewes wrote: "The history of bacteriology and the existence of the micro-organisms of pleuro-pneumonia and Agalactia which have similarities with viruses and yet can be grown on artificial media suggest the possibility that additional information from the areas of physiology and cell metabolism might make it possible to compose artificial media which would sustain the growth and reproduction of at least some viruses".[70] Ledingham on the other hand interpreted the same facts differently, concluding that these agents had no claim to the name virus "in view of their frankly bacterial nature when cultivated on artificial media, so simple as serum agar or serum broth".[71] Doerr's remark when commenting on the latter statement in 1938 illustrates the confusion which persisted in this area of definition: "Of course this does not mean that a virus cannot be a bacterium, or, in more precise terms, that it is here necessarily a case of two biologically or even only systematically incompatible concepts, but only that a virus whose bacterial nature has been confirmed may be included with the bacteria".[72] In the same volume Doerr had written of ". . . the unflagging tenacity of the attempts to identify with the word 'virus' a concept which considered rationally and according to its history of origin it could not be, i.e. a specific category of infectious agents characterised by common criteria".[73]

More than twenty years earlier Doerr had himself entered upon a study of fowl plague virus which with different collaborators he pursued on and off for nearly two decades. The first paper in the series[74] was published in 1915, when work was interrupted by Doerr's departure for the Army Medical Corps.[75] The title of the paper is "Studies on the fowl plague virus", but in the introduction the authors make it clear that the agent of fowl plague is once again serving as a convenient model; the immediate problem under scrutiny in this case was the overwintering of seasonal disease agents, specifically the pathogens of the so-called phlebotomus-fevers.[76] In this connexion they refer to the findings of Landsteiner and Russ[77] of the uneven distribution of fowl plague virus in the blood of infected hens, i.e. that after defibrination of virulent blood the serum proved to be less infectious than washed erythrocytes. From these beginnings, Doerr's investigation of fowl plague virus soon became centred on the behaviour of erythrocytes in virulent blood, the phenomenon of adsorption of the virus on to red blood cells, and the possible protective effect of red blood cells on the virus.

The total of eight papers in the series[74,78,79] reflects the very considerable volume

of work on many viruses of both plants and animals in the years between the wars, in many centres in all parts of Europe and the United States. Like most of this work, it was competent and determined, and yet few decisive facts emerged. Much of it was concerned with determining whether viruses were more closely related to bacteria (i.e. plant cells) than to protozoa (i.e. animal cells), by measuring their sensitivity to, e.g. tannin, saponin, bile, etc. When fowl plague virus was used as model the verdict was usually in favour of a closer relationship with the protozoa since the infectivity was destroyed by agents affecting trypanosomes but not bacteria.

Nevertheless, a change in attitude took place over this period. In 1921 in the second paper in the series, Schweizer wrote: "With certain obvious exceptions it is permissible to use this agent [fowl plague virus] as a characteristic representative of the whole group of microscopically unseen or perhaps even intrinsically invisible pathogens. It shares with other viruses in this category many properties such as resistance to glycerol, and the ability to induce in various types of tissue the formation of characteristic reaction products, etc., and in one respect it is even superior to the rest, that is, with respect to filterability".[78] By 1936, three years after the appearance of the last paper in the series, Doerr himself no longer believed in the justification of using *any* "invisible" virus as a representative model for all the rest of the ones included at that time in the "filterable virus" group, and wrote: "In order to get an idea of the unreality of this type of reasoning it is necessary only to imagine that the resolution of the microscope were considerably lower than is in fact the case, and to picture the results of a research discipline which aimed at integrating the world of 'inframicrobes' defined in this way into a natural entity".[80] This was written the year after Stanley had succeeded in preparing what he cautiously called "a crystalline protein possessing the properties of tobacco mosaic virus".[81] Stanley's results, and the subsequent demonstration by Bawden and Pirie[82] of the true character of tobacco mosaic virus, i.e. its nucleoprotein nature, stimulated the more biochemical approach to research into the nature of viruses. In the first year of the second world war, Weineck in Leipzig published three papers,[83] which, while pointing both back to Doerr and ahead to things to come, were at the same time a classic example of experiments cleverly conceived and competently executed but alas wrongly interpreted only because the time was not yet ripe for a thorough understanding.

Weineck was familiar with the original observation made by Landsteiner and Russ[84] of the uneven distribution of virus between the serum and erythrocyte fractions of fowl plague infected blood, and with the subsequent work done by Doerr and his co-workers. He was especially interested in the so-called "Potenzierungseffekt",[85] and used developing chicken embryos, in which the red blood cells begin to form after a lag period of thirty hours, in an attempt to show that the "potentiating effect" was due to an activation of the virus infection by the erythrocytes.[86] He then began to investigate the nature of the fowl plague virus in greater detail, and by the use of hydrolysing enzymes came to the conclusion that although the virus was closely associated with the globulins, it could not be a protein of any known configuration as it resisted decomposition by the known corresponding enzyme systems. In a series of experiments Weineck showed[87] that while the virulence was unaffected by proteolytic enzymes, it was destroyed by organic solvents; taking into account also the

"potentiating effect" induced by erythrocytes as well as by certain inorganic adsorption agents, he proposed the structure shown in his own diagram in Fig. 2.[88] Weineck concluded that fowl plague virus consisted of two components, one a protein, the other a lipid. The lipid represented the active, infective principle, and he believed that the protein might be playing a part in the processes of immunization.

In Weineck's work we find again the use of embryonated eggs in fowl plague experiments. It was a logical choice for Weineck, since he was concerned with the relations between virus and red blood cells during the period of formation of the latter. At the same time, the cells of the developing egg (with its embryo at the half-way stage, i.e. after 10–12 days' incubation) had been found to support the growth of a number of viruses since the technique was first introduced for the cultivation of fowl-pox virus by Woodruff and Goodpasture in 1931.[89] In 1936 Burnet succeeded in growing influenza virus in the developing egg[90] where others had failed;[91] two years earlier, in 1934, Burnet and Ferry had shown that they could titrate fowl plague virus by a quantitative assessment of its capacity to kill developing chick embryos, more than thirty years after Centanni first recorded a deformity in a chick embryo after inoculation with fowl plague virus.[92] Burnet and Ferry were using this technique in a comparative study of fowl plague virus and Newcastle disease virus. The two diseases are similar in their clinical manifestations, and after Newcastle disease was first recognized in 1927[93] it was suggested that they might prove similar in other respects.

However, as World War II dragged on for longer than anyone could have foreseen, research and resources were concentrated in the areas of most immediate concern, above all in the development of penicillin, and in the attempts to develop vaccines against that notorious killer which swept through continents at the close of World War I, influenza. Even in its milder forms, influenza is a perennial nuisance to the human race. The memory of the sudden increase of its virulence and its tragic consequences in 1918–1919 was an inducement which spurred on work on vaccines,[94] and many promising young microbiologists were recruited into the field. Perhaps fresh eyes are more sensitive to the unexpected. Burnet wrote later: "Then came a discovery which I should have made but did not".[95] It was made simultaneously and independently by George Hirst at the Rockefeller Institute,[96] and by McClelland and Hare at Toronto.[97] George Hirst had recently moved into influenza research from work on rheumatic fever and streptococci; Ronald Hare had arrived in Toronto in 1936 having considered himself always "an inhabitant of the curative camp", to find himself in Canada catapulted into the position of a "permanent inhabitant of the opposite camp [preventive medicine] for the next ten years".[98]

The discovery was haemagglutination,[99] and it has since become an eminently valuable tool in the case of the myxoviruses. The sentiments expressed by Burnet could well have been shared by a number of other veteran workers in the field of influenza viruses. More especially, Robert Doerr who worked for so long on the affinity between erythrocytes and fowl plague virus, had reason to feel as Burnet did. But Doerr died in 1952, three years before Schäfer delivered the final and elegant proof of the true identity of fowl plague virus as an avian influenza A,[100] and thus neither he nor Landsteiner, who had died in 1943, lived to see the work which had begun with the observation of the preferential adsorption of fowl plague virus on to

erythrocytes in 1906[101] brought to its final and very useful conclusion. There was to be one more extension of the use of the phenomenon when Vogel and Shelokov in 1957 showed that erythrocytes may be adsorbed to the surface of animal cells in tissue culture if they have been previously infected with a haemagglutinating virus.[102]

Even since Landsteiner and Berliner suggested that the presence of living blood cells was the determining factor for the replication of the virus in Marchoux' experiments, tissue culture had been another area of research confounding many able and enthusiastic workers. Rhoda Erdman in 1916 was able to keep fowl plague virus alive for six days in a tissue culture of red bone marrow; after six days it became gradually "attentuated".[103] Real progress was made in the twenties with vaccinia virus in tissue culture,[104] and in 1931 Hallauer[105] applied the principles which Carrel and Rivers[106] had used to grow large amounts of vaccinia and Rous sarcoma virus, in a tissue culture study of fowl plague virus. Hallauer ascribed previous failures to the use of tissues which were unsuitable to sustain virus multiplication; instead he obtained good results with a mixture of all the tissues of eight-day-old chick embryos ground into a pulp. He found that living tissues were necessary to sustain growth of the virus, and that rate of multiplication depended on the degree of proliferation of the tissue. Histologically he found characteristic cytopathic effects, and mentioned the possibility of developing a method of virus titration in tissue culture to replace animal experiments.

The introduction of the chick embryo and its sheets of uniform cells as a rather special kind of experimental animal, also in 1931,[107] probably deflected attention from these other types of tissue culture for a while. In 1951 it at last proved possible to emulate for viruses the plaque counting method used for titration of bacteriophages, when Gey and Bang[108] showed that eastern equine encephalitis virus produced discrete lesions on sheets of rat sarcoma cells. It was developed into a quantitative plaque titration method by Dulbecco,[109] and modified and applied to fowl plague virus by among others Hotchin[110] and Waterson.[111] Perfect though fowl plague virus may be in many ways as a model for tracing the development of the concept of the virus, there lie between the discovery of haemagglutination in 1941 and the realization, in 1955, that fowl plague is an avian variant of the virus of influenza A, two discoveries of major importance for the concept of the general nature of viruses which did not at all involve study of the agent of fowl plague. One was made by O. T. Avery *et al.* at the Rockefeller Institute in 1944.[112] If its importance was not immediately recognized as widely as it deserved,[113] it was probably because it was published at the height of war, and in a journal which was perhaps not read by a maximum number of the people who were then beginning to think in terms of molecular biology. Avery identified the "transforming principle" which was able to turn avirulent pneumococci into a virulent form, as DNA, and the transformation was shown to consist in a permanent change in the hereditary characteristics.

A few years later, when radioactive isotopes were becoming available for biological research, Hershey and Chase[114] working with bacteriophage, were able to demonstrate the manner of entry of the infective principle into the cell invaded, i.e. that the nucleic acid of the bacteriophage particle is released from its protein component to infect the cell, leaving its protein coat at the cell surface. If the importance of the

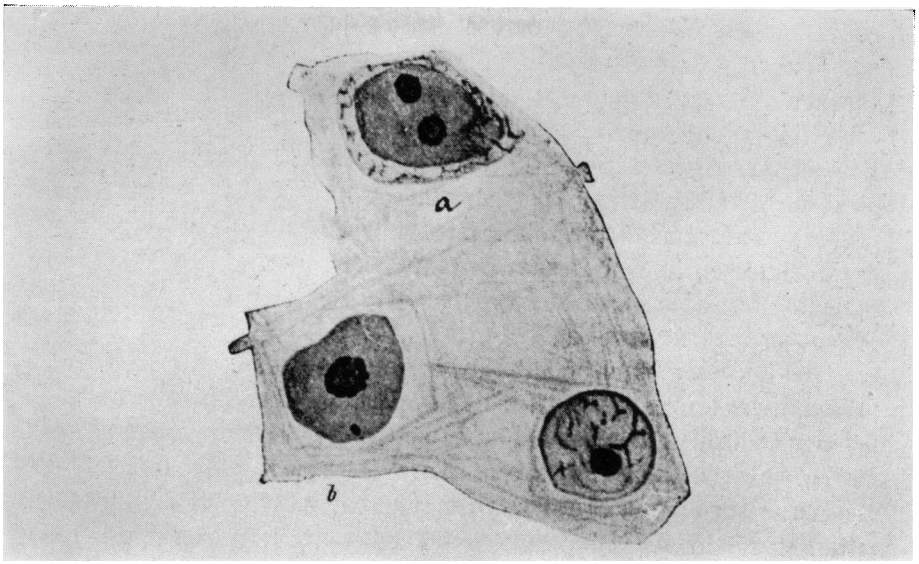
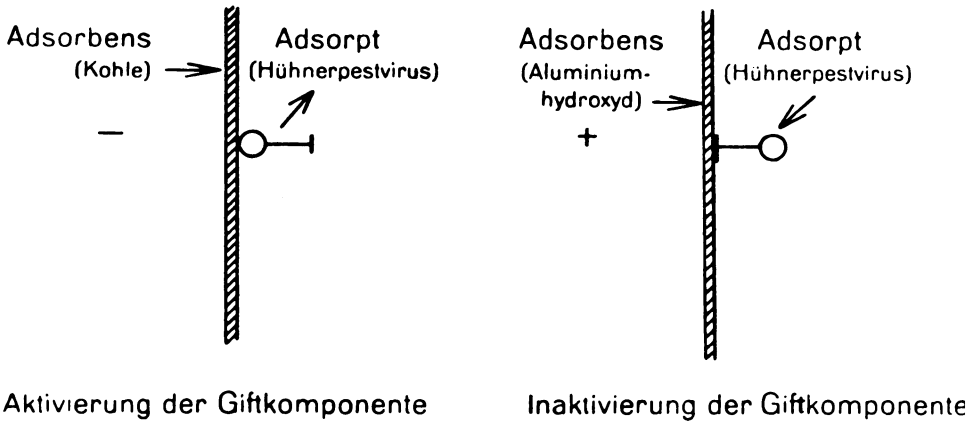


Figure 1
Prowazek's presentation of "Fowl plague bodies" (*Archiv für Protistenkunde*, 1907).



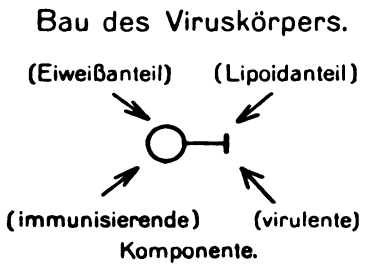


Figure 2
Weineck's diagram of the supposed components of fowl plague virus
(*Zeitschrift für Immunforschung etc.*, 1940).

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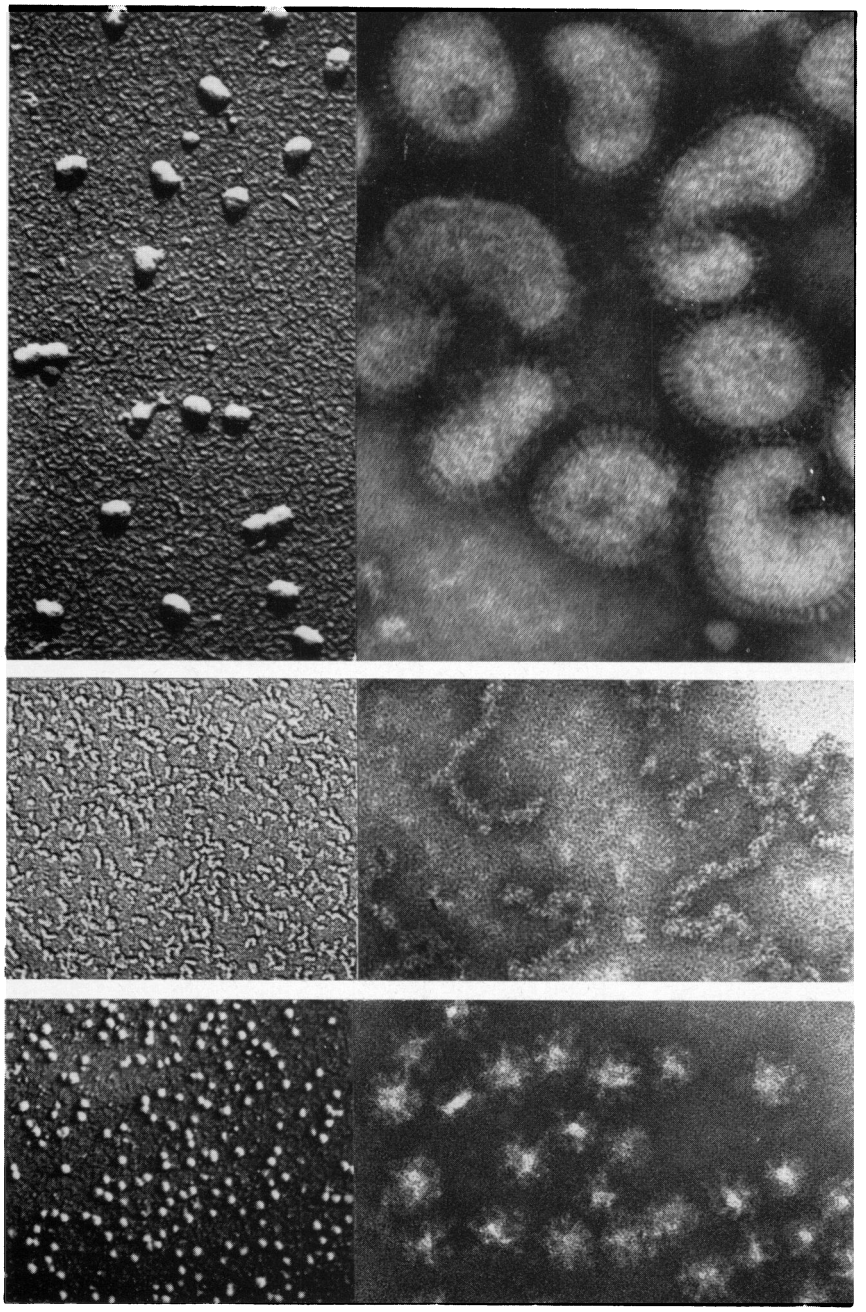

Figure 3

Appearance of fowl plague virus (Avian influenza A) in the electron microscope.

The plate shows comparable specimens of fowl plague virus prepared by the metal-shadowing technique (left) and by negative staining (right), demonstrating the greater resolution obtainable by the latter technique. Magnification x 30,000 (L) and x 180,000 (R).

Top: intact fowl plague virus (strain Rostock). *Centre:* Ribonucleoprotein (g-antigen) prepared by ether-splitting of virus. *Bottom:* Haemagglutinin prepared by ether-splitting of virus. The negatively-stained pictures of the two ether fractions are preparations of an influenza A virus morphologically identical to fowl plague. The metal-shadowing was done with platinum-rhodium and the electron micrographs are reproduced from Waterson, A. P., Rott, R. and Schäfer, W. (1961), "The structure of fowl plague virus and virus N", *Z. Naturforsch*, **16b:** 153–156. The negatively-stained preparations were prepared with phosphotungstic acid and the electron micrographs of them are by J. D. Almeida in 1969. The intact fowl plague virus was prepared by one of us (A.P.W.). The two ether-split fractions shown in the negative by stained preparations were supplied by L. Hoyle.

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discovery by Avery *et al.* came to be recognized only slowly, the joint impact of their paper with that of Hershey and Chase (who incidentally did not refer to Avery's work), sparked off an impressive volume of work on both plant and animal viruses which within a few years left no doubt of the genetic message transmitted by viral nucleic acid.

When in 1955 Schäfer's paper "Vergleichende sero-immunologische Untersuchungen über die Viren der Influenza und klassischen Geflügelpest"[115] appeared, it was the culmination of a number of methodical studies of the chemical, physical and biological properties and composition of animal viruses using fowl plague virus as a model,[116] undertaken at the Max Planck Institute for Virus Research in Tübingen. In it, Schäfer demonstrated the existence of a close relationship between the viruses of fowl plague and of influenza A, and placed them in the same group because of their similarity with regard to complement-fixing and cross-immunisation as well as in physical and chemical respects. Schäfer concluded: "The connection revealed between the representatives of influenza A viruses and of fowl plague appears to justify the inclusion once and for all of both pathogens in one and the same group. It could be imagined that the members of this group might occasionally change their host specificity, and that in this way a new type of influenza agent could develop from fowl plague virus or vice versa".[117] In 1942 Burnet[118] and Lush[119] had examined the behaviour of Newcastle disease virus and of fowl plague virus, respectively, and found that they both agglutinated chick red cells in a manner similar to that shown by Hirst[120] and by McClelland and Hare[121] for the influenza viruses. Lush also demonstrated that there was no serological relationship between the two fowl diseases, but no one at that time suspected how close the relationship between fowl plague virus and influenza A virus would ultimately prove to be.

The realization that fowl plague virus was no more and no less than a type of avian influenza A, coincided with a period of rapid advances on many fronts in virus research, and in recent years comparative studies have aimed at elucidating the finer details of structure and biological behaviour of the two groups of myxoviruses.[122] In particular, fowl plague virus has been studied alongside strains of influenza A virus from human infections in all its more important aspects, such as the formation of "incomplete" virus, the so-called von Magnus phenomenon, first recorded by von Magnus in 1951 for the PR8 strain of influenza A,[123] and studied with fowl plague virus by the German school in Tübingen.[124]

Another important area of study in recent years has been the structure of viruses, where remarkable progress has been made, both in electron microscope studies, and by means of biochemical methods. Perhaps the single most outstanding contribution in the early post-war years was made by Leslie Hoyle, who worked with strains of human influenza A, but whose results in a sense gave rise to a long series of studies by the Tübingen school dealing with many biochemical and biological aspects of fowl plague virus. Hoyle's definitive paper on the structure of influenza virus[125] was published in 1952, the same year in which Hershey and Chase showed DNA to be the infective principle of bacteriophage entering the cell.

After half a century of speculation which sometimes verged on the metaphysical, with no real facts to build on except for the filtration and centrifugation values which

allowed only tentative estimates of particle size, the concept of the virus at last came into its own and became a tangible entity. Suddenly facts accumulated fast, and it became possible not only to evaluate the nature of the virus in general, but to distinguish between different types of viruses, and to arrive at some form of taxonomy. While Hershey and Chase showed the infective moiety of bacteriophage entering the cell on infection to be desoxyribonucleic acid, Hoyle's work with the egg-adapted D.S.P. strain of influenza virus A suggested that its elementary body consisted of a soluble antigen, part of which was a self-replicating ribonucleo-protein (soluble antigen was first found in extracts of influenza-infected mouse lung by Hoyle and Fairbrother in 1937[126]), responsible in turn for the production of haemagglutinin, the two components then combining to form an aggregate which is finally completed by acquiring a lipid membrane derived from the wall of the host cell.

On this foundation the Tübingen group built up an impressive corpus of studies on the structure and properties of fowl plague virus. They appear initially to have turned their attention to fowl plague virus in order to compare it with Newcastle disease virus. Having established their dissimilarity both with regard to size, morphology and serological tests,[127] they abandoned Newcastle disease virus and concentrated on fowl plague virus[128] until Schäfer in 1955 unequivocally established its identity as avian influenza A.[129] By this time, enough material had accumulated to make it possible to make an attempt at classification[130] of the myxoviruses which reflects the situation before the appearance of Schäfer's paper, while further subsequent progress is illustrated by comparing this paper with two papers on the taxonomy of the same group of viruses published in *Nature* in 1962[131] and in 1966[132] respectively. The two latter papers reflect the very considerable advance made within the field of electron micrography such as metal shadowing[133] and negative staining with phosphotungstic acid,[134] which combined with the information obtained about the structure of protein molecules[135] to make it possible to create for the viruses a taxonomic arrangement within which groups could be described as, e.g. ". . . enveloped RNA viruses with helical symmetry" (including the myxoviruses) and " . . . DNA viruses with cubic symmetry" (including the viruses of herpes and varicella, as well as cytomegalovirus and adenovirus).[136] The sophisticated techniques of recent years have allowed further revelations of the finer details of structure such as the neat icosahedral arrangement of the adenovirus[137] and even attempts to elucidate the structure of the envelope of fowl plague virus[138] (Fig. 3).

Perhaps paradoxically this last paper brings us to the end of the road and forces us to leave fowl plague at last at a point where it can no longer serve as a model. For as knowledge of the finer details of virus structure accumulated, and the concept of the nature of virus finally acquired a foundation in well-documented fact, the need developed for names to be given to the different parts which could now be distinguished in the electron microscope. According to the nomenclature first suggested by Lwoff, Anderson and Jacob in 1959,[139] and presented in its final form at the Cold Spring Harbor Symposium of 1962,[140] the mature virus, the *virion,* consists of a *nucleocapsid* made up of nucleic acid and protein units (*capsid*). The morphological units discernible by electron microscopy are *capsomeres*. But, in the words of a recent textbook "In practice the use of this terminology is more felicitous with

cubic than with helical viruses. For example, the helical ribonucleoprotein of the myxoviruses is not well termed a nucleocapsid, and in fact the protein units of these viruses are, by agreement, now called not capsomeres but structure units, because they appear to be the chemical sub-unit of the virus".[141]

SUMMARY

An attempt has been made to trace the development of the concept of the virus through papers written on fowl plague virus in the twentieth century. In the first year of the century the agent was shown to be small enough to pass through bacteria-proof filters, thus belonging to the "filterable virus" class. Further than that, all was speculation, with two firmly opposing views established from the beginning.

The adherents of one believed the agent to be of a living, bacterial or possibly protozoan nature, but of an extreme tenuity which would account for the ability to pass through bacteria-proof filters; whereas their opponents were prepared to struggle with the concept, difficult to accept at the time, of a non-living agent, of the nature of a chemical molecule, yet able to multiply by some form of autocatalytic process. With few facts to support either theory, and no microscopes with a resolution high enough to see the filterable agents except for inclusion bodies whose nature and function were in any case arguable, little progress was made for the better part of half the century, in spite of much competent work and lively thinking.

Once it had proved possible to prepare another filterable agent (tobacco mosaic virus) in the form of crystalline nucleoprotein and yet retain its infectivity, and with the added technical advances in electron microscopy, radioactive labelling and crystallography, progress accelerated swiftly and it became evident that the endless discussions of the nineteen thirties, with arguments for and against the living nature of viruses[142] had not only been fruitless, but in fact pointless. We now have electron micrographs showing not just the general outline of individual virus particles, but in some cases the nucleoprotein helix; other electron micrographs show viruses invading cells, and yet others their progeny leaving cells after replication. Contemplating them all one returns with renewed admiration to Centanni's evocative phrase of the agent which he thought might belong in a transitional zone between the realms of living organisms and lifeless complex chemical molecules.[143]

ACKNOWLEDGEMENTS

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