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A CONCEPT OF THE ULTRAMICROSCOPIC VIRUS DISEASES AND A CLASSIFICATION[1]

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WE[2] have recently attempted to define a filterable virus as a particulate agent, probably endowed with life, of a size and carrying an electric charge which permits it to pass through the pores of ordinary filter candles, as a rule ultramicroscopic (though there may be exceptions), related in many instances to the formation of intracellular inclusion bodies (intracytoplasmic, intranuclear or both). Since disease phenomena have focused our attention upon them they appear to be capable of producing in many instances specific pathologic processes in several different forms of life, including man, lower animals, fowls, fishes, insects and plants.

It is exceedingly difficult to formulate a definition for this large group of agents, which we now, perhaps unfortunately, speak of as the filterable viruses. One may appear foolhardy in attempting such a definition until the exact nature of these agents is known. However, certain developments in the fields of pathology and bacteriology make it of paramount importance at the present time that even with our limited knowledge of the nature of viruses, we attempt to define our problems in order that in the future there may be less confusion than exists at the present moment concerning this group of disease-producing agents. For this reason we have attempted a tentative definition of the filterable viruses and will later present a classification which we suggest as a working basis in thinking of this group of agents.

The history of the virus group is well known and needs no repetition here. Suffice it to say that most

[1] Address at the annual meeting of the American Society for Experimental Pathology, Philadelphia, Pennsylvania, on April 28, 1932.

[2] E. B. McKinley, *The Sci. Monthly*, 32: 398, 1931.

of the discovery which has been made with the virus diseases has taken place during the past four decades since the demonstration by Iwanowski[3] forty years ago that the infecting agent of tobacco mosaic is filterable. Since 1892 the list of virus diseases has grown prodigiously and they now constitute one of the most important and complex group of diseases which challenges the ingenuity and imagination of those in the fields of plant, animal and human pathology. Since 1903 no less than fifteen reviews have been published regarding the filterable virus diseases in attempts to define the field and evaluate the progress which has been made. In recent books by Rivers[4] and his colleagues and our own,[5] published in 1929, the subject has been treated exhaustively, though not conclusively, upon the basis of existing knowledge. In spite of these attempts to keep the virus field defined there has developed a greater confusion and one sees evidence of this not only in the Sunday supplements of the lay press, but also in the minds of some of our colleagues in the biological sciences. It is time therefore that we endeavor to define what we should all have in mind when we think of a filterable or ultramicroscopic virus. Let us therefore consider what is now known concerning the nature of these agents.

There has been a vast amount of discussion regarding the nature of the filterable viruses. While it is true that we know considerable regarding the properties of some of the viruses, such as approximate size, resistance of these agents to physical and chemical agents, cells attacked by them, etc., it is also true that we do not know just what a filterable virus really is. One of the most frequent discussions centers around the question of the living nature of viruses. Are viruses animate or inanimate? Are they minute organisms related to bacteria? Are they a separate species in themselves? Or do they represent inanimate chemical principles or enzymes, etc.? None of these questions have been answered with certainty. Boycott[6] states that he believes that this question of "live or dead" is a stupid one because such a question does not exhaust the possibilities. He would infer that there are varying degrees of "deadness" or "aliveness" and degrees of transition between the two. He would place the filterable viruses in this intermediate group. Be that as it may, there are certain attributes which we associate, in the light of our knowledge and experience, with living things and with dead things. Philosophers have for ages attempted to explain life and death and to determine where one leaves off and the other begins, but up to the present time no one has been able entirely to dissociate such things as reproduction, assimilation and adaptation from living things or the lack of these attributes from dead things. Our difficulty with the filterable viruses lies in the fact that we can not demonstrate that they may reproduce themselves in the test-tube under artificial conditions as do bacteria. Still, there is ample evidence that they do reproduce themselves *in vivo* and with many of them this can be easily demonstrated by introducing a very minute quantity of virus into a susceptible host and when such a host succumbs to the specific infection the virus may be detected in large quantities in different parts of the host's tissues. Furthermore, with a relatively small number of filterable viruses multiplication can be demonstrated in living tissue culture and even specific pathology, if we regard inclusion bodies as such, may be produced in some cases within the living cells of such culture media. There is also evidence that some viruses may be preserved "alive" for a considerable length of time, though not multiplying, outside the living body. For example, if one passes a strain of vaccine virus through a calf one obtains a large quantity of virus in return for a very small original inoculum. Such a virus may be stored in glycerin at a low temperature for a considerable length of time and is again capable of reproducing itself in the body of a susceptible host. But if we store it long enough the time comes when it will not infect a susceptible host . . . it becomes "inactive" and we are justified in considering it "dead" in so far as our purpose is concerned. Certainly then we have abundant evidence which indicates that viruses are probably living things and there is little to suggest the contrary. If we could provisionally agree upon this concept of the living nature of viruses it would serve as a fundamental upon which to base a further analysis.

The susceptibility of various viruses to such physical agents as chemicals, high temperatures, ultra-violet light; the demonstrable fact, now adequately confirmed, that certain viruses may be propagated in tissue culture medium; the gradual deterioration, or destruction, of viruses stored in glycerol at low temperatures; the invasive properties and indeed special affinities of viruses for certain types of cells, etc. . . . all, in our opinion, suggest the living nature of these agents, and such observations offer no indications that we are dealing with inanimate matter. We would insist then at this point that the filterable viruses are minute particulate agents which are most probably endowed with life. Whether they exist as cells, in forms much smaller than those now visible with our present optical instruments, is yet to be determined, but it is conceivable that these extremely minute bodies may exist as miniature granules endowed with

[3] D. Iwanowski, *Beiheft Bot. Centralblat.*, 3: 266, 1893; *Zeit. Pflanzenkrankheit.*, 13: 1, 1903–04.

[4] T. M. Rivers, "Filterable Viruses." Williams and Wilkins, Baltimore, 1928.

[5] E. B. McKinley, "Filterable Virus and Rickettsia Diseases." Bureau of Science, Manila, 1929.

[6] A. E. Boycott, Smithsonian Report for 1929.

properties which constitute positive living attributes and they may possibly be below the structure level of organized cells which we have always considered the unit basis of living matter in the scheme of living things. It is interesting to speculate on these questions and attempt to visualize these minute agents, but in the final analysis it must be admitted that, at the present time, we do not know the precise nature of the filterable viruses nor do we know in what form they exist in nature.

Much has been written regarding bacterial filters and the mechanical technique and physics of filtration. While research on filtration has brought to light many interesting facts and aided greatly in our study of the viruses it has not contributed a great deal to the actual nature of these agents. As Zinsser has so well stated, filtration is only a relative matter. What is known to-day as a result of studies on cataphoresis to determine electric charge can not be regarded as conclusive for the reason that, up to the present time, no virus has been obtained in a pure state. Even the size of viruses is at present only an approximation. However, the study of filters and filtration from the standpoint of the influence of hydrogen-ion concentration, viscosity, surface tension, size of filter pores, density and character of virus suspensions has been exceedingly worth while and has contributed general knowledge which is certainly helpful. Detailed treatments of this subject have appeared in special articles and in monographs and will not be considered here. Suffice it to say that in most of these studies the basic thought in the background has been one related to the determination of the actual size of the filterable viruses. Unfortunately, even if we knew the actual size of a given virus it would not aid us materially in the solution of the more fundamental problems concerning its nature and mode of action as compared with other better-known agents of disease, such as bacteria, protozoa, etc.

Rivers and others have emphasized the fact that viruses differ markedly in their effects upon tissue cells. Not only in the type of influence, whether stimulating or destructive, but also in the degree of effect they produce. Viruses possess certain tendencies to attack definite cells. The virus may be essentially neurotropic or epitheliotropic. Some are characterized by their stimulating influence on cells, such as in molluscum contagiosum and warts, while others may quickly bring about destructive processes, such as smallpox, herpes zoster and foot-and-mouth disease virus. Cells attacked by certain viruses may increase markedly in size and a certain group of viruses are well known to be associated regularly with the formation of inclusion bodies. Woodruff and Goodpasture[7] have presented evidence in the case of fowl-pox that the inclusion bodies contain the actual virus of the disease. A salient point to be remembered is the marked tendency for viruses to attack tissue cells from within . . . that is, they are *intracellular* parasites or disease-producing agents. They differ decidedly in this respect from most bacteria, the greater part of which attack tissue cells from without and are therefore *intercellular,* although the *Mycobacterium leprae* of leprosy is an outstanding exception to this rule. Recent work on the cultivation of the leprosy bacillus, it being an intracelluar parasite, as are most viruses, has suggested the possibility of cultivating viruses on artificial mediums under the influence of various gaseous tensions of carbon dioxide and oxygen. Work along this line is now in progress and other gases, such as helium and neon, are also being employed in the study of certain viruses as well as certain known anaerobes. A report on this work will be presented later. The actual cultivation of a virus on artificial medium is perhaps the most fundamental problem dealing with this subject.

If we may review at this point let us return for a moment to our definition of a virus in which we state, as a working hypothesis, that a virus is a particulate, animate agent, of a size and presumably carrying an electric charge which permits its passage through ordinary filter candles, that it is as a rule ultramicroscopic and is related in many instances to the formation of intracellular inclusion bodies. Some viruses, to be sure, have as yet not been found associated with intracellular bodies, but the list of inclusions is growing rather than decreasing. If we provisionally accept these general characteristics of viruses as a working hypothesis, then we should ask a question concerning which there is a growing source of confusion in this field of study, *i.e.,* What relation, if any, have the filterable viruses to bacteria and more especially to the reputed "filterable forms" of bacteria?

The existence or non-existence of filterable forms of bacteria we do not care to challenge. We regard this question as distinctly outside the realm of the filterable virus problem. Unfortunately, however, the literature has recently associated the terms "filterable forms of bacteria" and "filterable viruses" in a very loose fashion. In our opinion this has been exceedingly unfortunate, for it lacks discrimination and an appreciation of certain fundamental considerations which are inherent in the virus problem and have nothing whatever, except perhaps the superficial question of filtration, to do with the true filterable viruses. As we have pointed out elsewhere[8] there are several fundamental characteristics which set the filterable

[7] E. C. Woodruff and E. W. Goodpasture, *Am. J. Path.,* 5: 1, 1929.

[8] E. B. McKinley, "The Etiology of Epidemic Encephalitis." In press.

viruses apart from bacterial forms. For example, there is a distinct tendency for virus diseases to produce a lasting immunity to subsequent infections, while this tendency is not so marked in bacterial diseases. Furthermore, artificial immunity can only be produced with living viruses, while killed bacteria commonly induce antibody formation when injected into the animal body. Virus infections, as a rule, attack tissue cells from within, while, in most instances, bacteria attack tissue cells from without. A large number of virus diseases are associated with the formation of intracytoplasmic or intranuclear (or both) inclusion bodies. Bacteria do not produce inclusion bodies in tissue cells. We can omit the difference in size between viruses and bacteria as a relative matter. Bacteria are easily cultivated as a rule on artificial culture medium, while no virus as yet has been cultivated, except in the presence of living cells. In addition there are certain epidemiological features of virus diseases which are distinctly different from most bacterial diseases. These are a few of the most important differences between viruses and bacteria, but sufficient, we believe, to permit the statement that with the filterable virus group we are dealing with something quite distinct from bacteria. This is a point which we should like to emphasize in an attempt to define the virus problem.

It may assist some in further elucidating the virus problem to attempt a classification of the diseases now thought to be associated with the filterable viruses. Here again one treads on difficult and debatable terrain in attempting a classification of over eighty diseases, the virus nature of which surely no group of individuals will entirely agree upon at the present time. A few years ago we attempted a classification of the virus diseases along most general lines based upon host susceptibility. This classification was helpful but, of course, too general in scope. During the past three or four years new information concerning many of the virus diseases has come to hand, and we feel it is possible to attempt a classification based upon finer characteristics. Imperfections will exist in any classification of viruses and only the future will give us an ideal grouping of these very interesting diseases. The following classification is based upon the presence or absence of inclusion bodies, upon transmissibility and upon filtrability of the virus in question. Subheadings dividing the diseases according to host susceptibility are also included.

Classification OF *Virus Diseases*

I. VIRUS DISEASES WITH CELL INCLUSIONS WHICH ARE DEFINITELY PROVED TRANSMISSIBLE AND FILTRABILITY OF THE CAUSATIVE AGENT IS ESTABLISHED

A. *Diseases of Man:*
- 1. Smallpox
  - Varioloid
  - Vaccinia
  - Paravaccinia
  - Alastrim
- 2. Verruca (Common warts)
- 3. Molluscum contagiosum
- 4. Rabies
- 5. Herpes febrillis
- 6. Papilloma of the larynx
- 7. Yellow fever

B. *Diseases of Lower Animals:*
- 1. Pox diseases
  - Cow pox
  - Sheep pox
- 2. Virus III infection of rabbits
- 3. Borna disease
- 4. Distemper
- 5. Foot-and-mouth disease
- 6. Myxomatosis of rabbits
- 7. Rabies
- 8. Hog cholera
- 9. Infectious pustular stomatitis of horses
- 10. Salivary gland disease of guinea-pigs
- 11. Rift Valley fever (Enzootic hepatitis)
- 12. Louping-ill

C. *Diseases of Fowls:*
- 1. Fowl pox (Avian diphtheria)
- 2. Fowl plague
- 3. Virus disease of parrots and parrakeets

D. *Diseases of Insects:*
- 1. Polyhedral diseases
  - Gipsy-moth caterpillar
  - European moth caterpillar
  - Tent caterpillar
  - Jaundice of silkworms

E. *Diseases of Plants:*
- 1. Mosaic diseases
  - Common examples
    - Tobacco
    - Tomato
    - Potato

II. VIRUS DISEASES WITH CELL INCLUSIONS WHICH ARE TRANSMISSIBLE BUT FILTRABILITY OF THE CAUSATIVE AGENT HAS NOT BEEN ESTABLISHED

A. *Diseases of Man:*
- 1. Varicella
- 2. Inclusion Blennorrhoea

B. *Diseases of Lower Animals:*
- 1. Horse pox

C. *Diseases of Insects:*
- 1. Polyhedral disease of the black arches moth caterpillar

D. *Diseases of Fish:*
- 1. Lymphocystic disease

III. VIRUS DISEASES WITH CELL INCLUSIONS WHICH ARE NOT TRANSMISSIBLE AND FILTRABILITY OF THE CAUSATIVE AGENT HAS NOT BEEN ESTABLISHED

A. Diseases of Man:
1. Herpes zoster

B. Diseases of Fish:
1. Carp pox
2. Epithelioma of Barbus

IV. VIRUS DISEASES WITH NO CELL INCLUSIONS WHICH ARE TRANSMISSIBLE AND THE CAUSATIVE AGENT HAS BEEN DEFINITELY ESTABLISHED

A. Diseases of Man:
1. Epidemic parotitis
2. Foot-and-mouth disease
3. Pappataci fever
4. Poliomyelitis
5. Dengue fever
6. Psittacosis
7. Common colds

B. Diseases of Lower Animals:
1. Nairobi disease of sheep
2. Catarrhal fever of sheep
3. Equine influenza
4. Vesicular stomatitis in horses
5. Rinderpest (Cattle plague)
6. Ephemeral fever in horses and cattle
7. Epizootic in guinea-pigs
8. Guinea-pig paralysis
9. Novy's rat disease
10. Cattle warts
11. Fox encephalitis
12. Noguchi's (*Dermacentor andersoni*) virus infection in guinea-pigs and monkeys
13. Infectious bulbar paralysis
14. Pleuropneumonia in cattle
15. Agalactia of sheep
16. Anemia of rats
17. Equine infectious anemia
18. African horse sickness
19. Mad itch

C. Diseases of Fowls:
1. Rous' chicken sarcomata
2. Philippine fowl disease (Newcastle or Ranikhet disease)
3. Leukemia of chickens

D. Diseases of Insects:
1. Sacbrood disease of bees

V. A GROUP OF DISEASES IN SOME CASES FOR WHICH CLAIMS HAVE BEEN MADE FOR SPECIFIC (BACTERIAL, PROTOZOAL, RICKETTSIAL, ETC.) ETIOLOGICAL AGENTS; IN MANY OF WHICH THE FILTRABLE NATURE OF THE CAUSATIVE AGENT REMAINS QUESTIONABLE BUT SUGGESTIVE; AND IN OTHERS INCLUSION BODIES HAVE BEEN REPORTED AND THEIR IDENTIFICATION IS EITHER ESTABLISHED OR REMAINS DOUBTFUL

A. Diseases of Man:
1. Scarlet fever
2. Trachoma
3. Encephalitis
   - Epidemic encephalitis
   - Vaccinial encephalitis
   - Australian X disease
   - Encephalitis following measles
   - Encephalitis following mumps
   - Encephalitis following varicella
4. Epidemic influenza
5. Measles
6. German measles
7. Multiple sclerosis
8. Tsutsugamushi disease
9. Psoriasis
10. Condyloma acuminatum
11. Visceral disease

B. Diseases of Lower Animals:
1. Puppy disease
2. Swine pox
3. Goat pox
4. Kurloff bodies in guinea-pigs
5. Swine influenza (Hog flu)

C. Diseases of Fowls:
1. Fowl paralysis (Neurolymphomatosus)
2. Macfie's disease of fowls
3. Fowl laryngotracheitis

D. Diseases of Insects:
1. Grasserie of the caterpillar of the large white cabbage butterfly
2. Nuclear disease of the caterpillar of the large white cabbage butterfly

E. Diseases of Amphibia:
1. Todd bodies

In offering this classification we are well aware of the difficulties involved. We expect and invite constructive criticism. Objection will come no doubt that certain diseases, such, for example, as scarlet fever, should have no place whatever in the consideration of this field, and yet some investigators insist that it is not fully established that a virus may not be associated with the *Streptococcus scarlatinae* in this infection. Others will question the basis or selection of experimental work upon which a place in the classification is given to a specific virus, but this, again, is a matter of opinion. The classification is not conclusive. It is based upon the literature in the light of our evaluation and interpretation of it. We would expect in such a field of study as the virus field to find disagreement, but on the whole we feel that this grouping, in the form presented, represents a measure of progress in an attempt to bring about some order out of chaos and to assist in a statement of the virus problem which sorely needs definition at this time.

SUMMARY

To summarize, we have attempted a tentative definition of a filterable virus and have reviewed briefly some of the known facts concerning the nature of these agents. Fundamental differences between the true filterable viruses and bacteria, either filterable or non-filterable, have been discussed in an effort to

define the virus problem in contradistinction to the latter. The point of view that a true virus represents a distinct type of disease-producing agent has been presented and emphasized. To further assist in defining the virus field a classification of virus diseases based upon the presence or absence of inclusion bodies, transmissibility of the virus and filtrability of the infecting agent is presented for consideration.

THE ESTABLISHMENT OF PRIORITY IN SCIENTIFIC DISCOVERIES

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A STUDY of the individual claims for precedence in making discoveries constitutes an interesting chapter in the history of science. The history of the discovery of oxygen and of other chemical elements is filled with controversial claims of this character and the opinions of the critics, who have weighed the evidence in these and other similar cases, have unfortunately been often influenced by feelings of national bias.

An interesting case of recent significance relates to the discovery of the missing element, Number 61, which was named Illinium by Harris and Hopkins in 1926, and Florentium by Rolla and Fernandes, who claim priority for the discovery upon the fact that they deposited a sealed note relating to their work with the Royal National Academy of the Lincei of Florence in 1924. The method of assuring priority by the deposition of sealed notes has been practiced for many years in various European countries. The *Chemiker-Zeitung* in Germany, for example, has conducted a bureau for this purpose since 1895, which gives their subscribers the right to deposit in its keeping sealed communications regarding work which is not yet ripe for publication. The date of the receipt of the deposition is registered and at any time upon request of the sender the sealed envelope may be opened and the dated communication published. The editorial office of the *Chemiker-Zeitung* is careful to inform its clientele, however, that, while the deposition of a sealed note may establish the intellectual ownership of a discovery (das geistige Eigentumsrecht), it does *not* provide for the vindication of patent claims.

Notwithstanding certain conveniences of the sealed deposition procedure, this method of establishing priority has been very generally and correctly frowned upon in English-speaking countries. If an individual, A, for example, deposits such a note, it simply indicates that at the time of the deposition A was not perfectly sure in his own mind of the validity of his discovery. If further work on A's part contradicts the statement in the sealed note, he orders it to be destroyed and is thus saved from the embarrassment of a refutation. It should be remarked, however, that if a rival investigator, B, makes a published announcement of the same discovery before A has completed the confirmation of his work, then B is entitled to the credit, as he anticipated A *in having confidence in the validity of his work.*

The official date of the publication of an article does not usually determine the time when a discovery was made. Months may elapse between the time when an article is received in an editorial office and the official date of publication. On the other hand, cases are known with journals, that were behind with their monthly issues, when the official date of publication of an issue preceded by many months the actual time when a discovery reported therein was made.

The date of receipt, which is usually attached to an article, is now usually accepted as the decisive index of priority. Yet cases are known where authors have inserted later discoveries in the proof sheets of an article that was previously submitted. In all such cases there should be a footnote regarding the date of such insertions. Charges of unfair preference in permitting the dating back of articles for the purpose of securing priority for favored contributions have been made against certain journals, but such unethical procedures, so far as the writer is aware, have never been confirmed.

Authors who are working in closely competitive fields are often anxious to indicate that their findings antedate the appearance of articles by rival investigators—a trait of human nature which Emerson once referred to as a "habit of saliency"—and hence we note the occasional attachment to articles of footnotes regarding the time of making a discovery, the date of a verbal statement or other matters irrelevant to the main subject of the paper. Personal remarks of this character can, of course, have no weight in the establishment of priority.

Some investigators are exceedingly cautious about drawing conclusions from their work, even when these conclusions may seem to be almost self-evident. Cases are known where a clever interpreter, after reviewing the publications of an over-cautious investigator, has foreseen the probable final outcome of an incompleted