HANDBOOK OF FILTERABLE VIRUSES

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TO
P. L. F.

PREFACE

FILTERABLE Viruses or Viruses have been the subject of a considerable amount of investigation during the past decade, and, while our knowledge is still far from complete, there is no doubt that important advances have been made. Voluminous literature has naturally accumulated; much of this is, however, scattered in somewhat obscure journals, and is consequently relatively inaccessible to the general reader. The writer, in preparing this small book, has attempted to present a general survey of the viruses, particularly those causing disease in man, in the hope that the student or general reader may thus be able to acquaint himself with the work that has been carried out in this sphere. A knowledge of viruses is obviously important, as many of the common infectious diseases are caused by these disease-agents. It is hoped that the research worker may also find the book useful as a review of the recent work done on subjects outside his own.

Detailed accounts of special technical methods have been purposely omitted, as they are beyond the scope of all but research workers, who usually prefer to consult the original articles, reference to which is given in most instances. It has been assumed that the reader is conversant with the ordinary bacteriological procedures, many of which have been em-

ployed in the study of viruses, and details of these have also been omitted.

In case readers may wish to study special points more extensively, an endeavour has been made to include the latest and most important references to the various subjects. On many questions divergent views are held by equally competent workers; these controversial points have been given in some detail with possibly a certain amount of personal bias. In some instances work has been encountered, which has either not stood the test of time or which the author considers to be based on unsatisfactory experimental evidence; in such cases this has either been omitted or received only brief mention, as the field is sufficiently extensive without perpetuating obsolete ideas.

In the opening chapters several general questions have been considered, and in these reference has been frequently made to work carried out with viruses causing disease in animals. In the remaining chapters the various diseases of man, in which a virus is the possible cause, have been discussed.

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CHAPTER I

HISTORICAL OUTLINE

It is an outstanding fact that two of the earliest and most important advances in immunology were made before even the knowledge of bacteria was on a satisfactory basis; they were moreover advances connected with virus diseases, and not with diseases of bacterial origin. The first of these we owe to Jenner (1800), who investigated the Gloucestershire dairy workers' contention, that an attack of cow-pox rendered them immune to small-pox, and subsequently introduced the process now universally known as "Vaccination." In an attempt to explain this phenomenon, he stated: "May it not then be reasonably conjectured that the source of the small-pox is morbid matter of a peculiar kind, generated by a disease in the horse, and that accidental circumstances may have again and again arisen, still working new changes upon it, until it has acquired the contagious and malignant form under which we now commonly see it making its devastations amongst us." The practice of vaccina-

tion was first officially adopted in England during 1853, since when the incidence and severity of the disease have diminished in a striking manner.

Many years afterwards Galtier (1879), Pasteur, Chamberland and Roux (1881) transmitted rabies to rabbits ; this step was soon followed by the successful immunization of man against rabies by the inoculation of the rabbit-fixed virus in various stages of attenuation (Pasteur, 1885). As the immunity response appeared analogous to that encountered in bacterial diseases, Pasteur considered he was dealing with an organism which he was unable to see with the microscope then available. Two very important steps were thus made without any definite indication of the nature of the causative agent being available and before the question of filtration was even considered.

Further progress was slow and uneventful until the demonstration of the filterability of viruses in 1892, when a new and extensive field of research was opened. Guarnieri (1894), however, stated that in 1892 he had observed some bodies in the protoplasm of the epithelial cells of variolar and vaccinial lesions, and considering they were sporozoa, he gave them the name " Cytoryctes vaccinæ et variolæ." Previous workers had also found peculiar bodies in the vicinity of vaccinial lesions, which they had claimed were of a similar nature (Renault, 1881). The presence or absence of the Guarnieri bodies is still considered an important feature in the diagnosis of a vaccinial lesion.

The discovery of the filterable nature of viruses was due to the work of a botanist, Iwanowski (1892), who found that even after filtration, the juice of a tobacco plant infected with mosaic disease was infective for healthy plants. This observation was confirmed some time later by Beijerinck (1899), who considered the causative agent to be a "Contagium Vivum Fluidum." In the meantime, Loeffler and Frosch (1898) had found that lymph from vesicles of a case of foot and mouth disease reproduced the disease even after filtration through a Kieselguhr filter; the filtrate was bacteriologically sterile. This was the first human or animal disease which was shown to be caused by a filterable virus. Shortly afterwards, Reed, Carroll, Agramonte and Lazear (1901) demonstrated the filterable nature of the causal agent of a human disease when they found that blood, from a case of yellow fever, filtered through a Chamberland "F" bougie impermeable to ordinary bacteria, was still infective. Following this, Remlinger (1903) advanced evidence supporting the view, expressed previously by Pasteur, that the incitant of rabies was related to the bacteria. He showed that, after finely emulsifying the nervous tissue containing the rabies virus, the virus would traverse a Berkefeld "V," but not the "N" or "W" filters, and reproduce the disease in rabbits after a lengthened incubation period on intracerebral inoculation. Remlinger also pointed out that while previous attempts at filtration had generally failed, in 1899 Blasi and Russo-Travali passed the disease through one generation only after filtration and

consequently considered the reaction involved was of a toxic nature.

Many human and animal diseases have since been shown to be caused by filterable viruses; some of these are mentioned below: Vallée and Carré (1904, etc.) transmitted anæmia of horses to healthy horses by the inoculation of blood collected from infected animals; they also found that the virus would pass through a Berkefeld " V " filter. Negri (1905) passed the vaccinia virus through a Berkefeld " V " filter after maceration of the lymph in water and inoculated the filtered virus with success by scarification of the skin of the cow and the cornea of the rabbit; Juliusberg (1905) claimed that he had filtered the virus of molluscum contagiosum through a Chamberland candle and reproduced the disease after a slight prolongation of the incubation period; Dorset, Bolton and McBryde (1905) showed that swine fever was due to a filterable virus, and that *Bact. suipestifer* or other organisms played only a secondary *rôle*; Landsteiner and Popper (1909) transmitted poliomyelitis to the monkey by intraperitoneal inoculation of an emulsion of the cord of a child dying of this disease; Landsteiner and Levaditi (1909) and Flexner and Lewis (1909) then found that the causative agent was equally infective after passage through Berkefeld and Chamberland filters. Grüter in 1924 stated that during the years 1910–11, in the course of cross-immunity experiments with the vaccinia virus, he had transmitted the virus of herpes simplex to the cornea of rabbits and had obtained a definite specific reaction. Levaditi and

Harvier (1920) confirmed this, and also demonstrated the filterability of the virus by infecting rabbits with a strain obtained from a case of epidemic encephalitis after passing it through Chamberland L1 and L3 candles; the filtrate, bacteriologically sterile, on intracerebral inoculation into rabbits produced a fatal encephalo-myelitis.

In many other conditions the evidence while suggestive of the presence of a filterable virus has not been so definite. In some cases a satisfactory experimental animal has not been discovered, while in other instances the complete exclusion of the participation in the disease-process of bacterial or spirochætal organisms has not as yet been possible. Although the nature of the causative agents in these cases is still unsettled they must, however, receive consideration in any discussion on diseases caused by the filterable viruses.

In recent years further additions have been made to the list of virus diseases. Dunkin and Laidlaw (1926) proved conclusively that dog distemper was caused by a filterable virus; Bedson, Western and Simpson (1930) showed the incitant of psittacosis was filterable and had no relationship to Bact. ærtrycke or any other known organism; Marchal (1930) described an epizootic disease of mice (ectromelia), the causal agent of which was found to be a virus. The latest development concerns influenza; Smith, Andrewes and Laidlaw (1933), by the use of ferrets, obtained results suggesting that the ætiological agent of influenza might be a filterable virus. Other animal diseases, as louping ill of sheep, Rift

Valley fever and swine influenza, have also been found to have a virus as the causal agent, while the common cold of man is considered by many workers to be primarily a virus disease.

It is probable that, in the near future, filterable viruses may be definitely shown to be ætiologically related to other diseases which have previously been considered of bacterial origin. Rich (1932) recently made some interesting observations which suggested that the causative agent of whooping cough was a filterable virus and that the Bordet-Gengou bacillus was merely responsible for the secondary phenomena. On the other hand, it is also possible that certain diseases, believed to be due to filterable viruses, may yet be shown to be of definite bacterial origin. An example of this is found in bovine pleuro-pneumonia, the causal agent of which was at one time considered to be a virus. As a result of detailed cultural and morphological examinations, Ledingham (1933) has recently suggested that the organism should be placed, provisionally, in the family "Actinomycetaceæ."

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CHAPTER II

THE NATURE OF FILTERABLE VIRUSES

Introduction. The nature of filterable viruses is a problem which has excited much speculation. Many theories have been suggested and much work has been performed in attempts to provide a solution to this question.

Beijerinck (1899) postulated that the infective agent of the tobacco mosaic disease was a "contagium fluidum vivum"; it has, however, been proved conclusively by the application of various physical and chemical tests, that most, if not all, viruses are definitely particulate bodies.

Other workers have suggested that viruses represent a filterable phase occurring in the life-cycle of certain bacteria. The present opinion held, however, by the majority of workers, is that the viruses are independent minute living organisms closely allied to the bacteria, and the bulk of evidence at present available appears to support this conception.

The study of bacteriology has been very closely associated with the development of the microscope. Before the construction of instruments capable of high magnification the nature of the bacteria was the subject of as much speculation as the nature of the

viruses is at the present moment. Thus the view, expressed by Fracastorius in the sixteenth century that infection was due to a contagium vivum, was accepted for a long time. The development of the microscope, however, led to the recognition of the bacteria, and, as a result of the work of Pasteur, Lister and Koch in the latter half of the nineteenth century, the relationship of these organisms to disease was established. When, however, diseases of an infectious nature were encountered and the ætiological agent could neither be seen nor cultivated, the disease-incitant was once again considered by some as a contagium vivum. Pasteur, however, during his research on rabies, considered that he was dealing with a micro-organism which was not visible with the microscope then available. This explanation unfortunately did not suffice and other theories were postulated. There is, however, no evidence to indicate that the viability of an organism is in any way connected with the limit of resolution of the ordinary microscope. In fact, it is well recognized that many bacteria possess filterable forms, which are not invariably visible (Hadley, 1927).

The failure of cultivation on lifeless media also seemed to dissociate the viruses from the bacteria. This failure is a problem which is still difficult to explain, although if the researches of Eagles and McClean on the growth of the vaccinia virus in a cell-free medium are confirmed much light will doubtless be thrown on this question. It has been found that, with the exception of the so-called

viruses of bovine pleuro-pneumonia and agalactia, all attempts at cultivation on the usual media employed in bacteriological work have proved futile. Tissue cultures have admittedly been satisfactory in many instances, but until a cell-free medium is discovered the results obtained by cultivation will give rise to controversy.

The two factors, invisibility and failure to obtain cultures on ordinary media, serve to distinguish the viruses from the bacteria. It is important, however, to remember that, in the case of certain parasitic bacteria, artificial cultivation is only accomplished by the application of special technical methods, and in the case of *B. leprœ* the results have not as yet been certain and reliable. Other criteria, as epidemiology, immunity, transmissibility to animals, tissue-reactions and resistance to outside agents, cannot be employed in any way to differentiate these two groups of disease-incitants. They rather tend to unite the two, as, in spite of the marked individual variation found in each, there exists much general similarity between the two groups.

Boycott (1928) pointed out that there is no sharp line of demarcation between the living and the dead ; one merges imperceptibly into the other. This fact should be afforded much prominence in considering the viruses, especially in forming analogies to the bacteria. Bacteria differ widely in their morphology ; some forms are relatively large, as *B. megatherium*, while others are extremely small, as *Bact. pneumosintes*. The growth requirements of the different bacteria also differ considerably ; in some

instances, as *B. leprœ* and *H. influenzœ*, these requirements are very highly specialized. Thus, turning to the viruses, is it not probable that the same marked differences exist? In the case of some, as the vaccinia virus and the psittacosis virus, the indications are that these are of a comparatively large size and are probably visible under the highest magnifications of the ordinary microscope. Such viruses as these appear to be closely allied to the parasitic bacteria, *i.e.*, to the living. At the other end of the scale, as the virus of foot and mouth disease, the disease-agents appear to be much more minute, the particles are simpler, and approach nearer to the enzymes, *i.e.*, to what one terms dead. Within these limits of size it is recognized that a wide range of viruses exists. While marked individual peculiarities are found, it has been shown that the larger viruses exhibit many of the properties associated with the parasitic bacteria, but others are less differentiated, and their classification naturally becomes more difficult.

In considering viruses the ultimate limit of living matter is approached and the difficulties encountered by the earlier investigators in dealing with the bacteria are accentuated. The knowledge of the bacteria has increased considerably since the nineteenth century, but it is still far from complete. The bacteria have been divided into numerous sub-groups for which even now an accepted classification of general application does not exist. There is every indication that the viruses as arranged at present also form a very heterogeneous group; sub-group-

ing, however, is impossible. Each member should therefore be considered quite distinct from the others until the knowledge of the subject is enlarged and clarified.

Many methods have been employed in the investigation of the nature of filterable viruses, but only those yielding useful information will be considered.

Filtration. The term "filterable virus" is itself sufficient to indicate that filtration has played an important *rôle* in the study of viruses. The process of filtration has, since the work of Loeffler and Frosch (1898), served as a rough means of separating this group of infective agents from the bacteria. As many of these infective agents passed through filters retaining the bacteria they were naturally considered to be of a smaller order of size than the bacteria. It must also be recognized that certain minute bacteria, as *Bact. prodigiosum* and *Bact. pneumosintes*, readily traverse the coarser bacterial filters. It is consequently impossible by the results of filtration alone to separate essentially the viruses from the bacteria.

The use of bacterial filters is beset with difficulties, and it is therefore necessary to consider this question in some detail.

Varieties of Filters. Three main types of bacterial filter are in use :—

(1) Diatomaceous earth or Kieselguhr filters.
(2) Chamberland filters.
(3) Seitz filters.

(1) The two best known types of diatomaceous earth filters are the Berkefeld and the Mandler. The Berkefeld is the original German filter and is

made from sifted kieselguhr mixed with asbestos and organic matter. This filter is arranged in the form of a small candle and is prepared in three grades :—

(i.) " V," the coarsest grade, will not hold back the smaller bacteria.

(ii.) " N," the intermediate grade, is the common type in this country and does not allow bacteria to pass through.

(iii.) " W " is the finest grade.

As the individual filters of the various grades vary considerably in their pore size, any comparison of the results obtained by different workers using filters of the same type is not practicable. The filters are good, but, as they are extremely fragile, they require careful handling. The Mandler is an American modification of the Berkefeld.

(2) Chamberland filters or bougies are unglazed porcelain candles used extensively in France. They are made in nine grades of porosity : L1, L1 bis, L2, L3, L5, L7, L9, L11, L13—the L1 candle is the coarsest grade and the coarseness decreases through the intermediate grades, with the L13 candle the finest.

(3) Seitz filters are asbestos discs of varying diameters (3 cm., 6 cm. and 14 cm.) and are made in two grades of porosity : " K " for clarifying, and " EK " for removing bacteria. These filters are used on a large scale at the present time—they are both efficient and easy to put up ; as a new disc is employed for each filtration, the trouble of cleaning does not arise.

The arrangement and manipulation of these filters

are described in most text-books on bacteriology, and consequently will not be discussed here. There are, however, certain points which must be remembered :—

(1) The various components should be carefully set up and sterilized, usually by means of the autoclave, before use.

(2) The filter should be tested for efficiency to hold back bacteria ; this is usually carried out by the addition of a small organism, as *Bact. prodigiosum* to the material being filtered, and the subsequent cultural examination of the filtrate for its presence.

(3) The filters must be thoroughly cleaned after use.

In the case of viruses, before commencing filtration, it is advisable to centrifuge the virus-suspension, which is usually prepared by grinding virus-containing tissue in saline or broth with the addition of sand or broken glass to assist the process, in order to remove the gross particles. The presence of such extraneous material tends to clog the filter and so renders filtration tedious and difficult.

Filtration is an exceedingly complicated process involving a consideration of many factors. It is by no means a simple mechanical procedure governed only by the relative sizes of the pores of the filter and the particles to be filtered. The most important factors implicated are : the composition and electrical charge of the filter, the *p*H and electric charge of the material undergoing filtration ; the amount of protein or other extraneous material in the virus-suspension ; the duration of filtration ; the tem-

perature at which the process is carried out; the pressure employed. Adsorption plays an important *rôle* in filtration; a virus may be adsorbed either on to the filter or on to albuminous material present in the suspension, and so fail to pass through a filter. In recording results of filtration it is therefore necessary to give complete details of the technique employed.

The importance of these factors in the study of viruses is illustrated by the results of different workers: Ward (1929) found that the vaccinia virus readily traversed a Berkefeld "V" filter after emulsification of the virus-containing tissue in hormone broth instead of saline. Sawyer and Frobisher (1929) showed that the yellow fever virus as existing in infected mosquitoes would pass through a Berkefeld "N" filter if suspended in normal monkey serum, while a similar suspension in normal saline would not. Green and Eagles (1931) found that the vaccinia virus, within a *p*H range of 6·4–8·4, would pass Berkefeld "V" and "N" and Chamberland L3 filters; they considered that emulsification in hormone broth instead of saline and the lining of the filters with egg-albumen, as suggested by some Japanese workers, were probably advantageous when small quantities were being filtered, but were not necessary if large quantities were used.

The value of bacterial filters in the study of viruses has obviously been limited. They, however, serve several useful functions, particularly for the removal of contaminating bacteria and extraneous material from virus suspensions and as a means of

providing a rough estimate of the relative sizes of the different viruses. Thus the viruses of foot and mouth disease and poliomyelitis traverse readily such fine filters as the Berkefeld " N," whereas the viruses of vaccinia, rabies and dog distemper only pass through the coarse filters, Berkefeld " V," with difficulty.

Ultrafiltration. More precise studies of the size of different viruses have recently been made by the use of ultrafiltration, which was first employed for the study of colloidal suspensions by Bechhold (1907). This process involves the use of collodion membranes which are less influenced by outside factors than the ordinary bacterial filters. It is important to note that in any work of a comparative nature definite standards should be fixed for the preparation of the filters. The need for this is apparent after consideration of the divergent results obtained by workers in the study of the herpes virus : Levaditi and Nicolau (1926) found that the herpes virus passed through membranes which retained hæmolysin and certain toxins and diastases ; Zinsser and Tang (1927) obtained similar results and considered the virus to be within the limits 20–100$\mu\mu$; Bedson (1927) obtained quite opposite results—he found that membranes permeable to certain serum proteins failed to allow the passage of the herpes virus. It is thus obvious that very variable results from the use of collodion membranes have been reported ; this state of affairs is probably the result of the use of faulty membranes in many experiments.

The application of ultrafiltration to the study of

viruses has recently been investigated by Elford (1929), who considered that " a graded series of filter membranes of progressively finer permeability, such as may be made under appropriate conditions from collodion, enables the separation of more and more finely dispersed fractions and furthermore permits the order of size of the suspended particles to be ascertained." The essential points involved in the technique are : the grading of acetic acid membranes in terms of their average pore diameters, calculated according to Poiseuille's law by measuring the rate of flow of water through the membrane under standard conditions ; constant area of the filtering surface (1·05 sq. cm.) ; the use of constant pressure and membranes supported on a perforated plate.

This technique has been applied by Elford and his colleagues to the study of the size of several viruses with interesting results, which are given below. The

Virus.	Size in microns (μ).	
Vaccinia	0·125–0·175	(Elford and Andrewes).
Herpes	0·1 –0·15	(Elford, Perdrau and Smith).
Ectromelia	0·1 –0·15	(Barnard and Elford).
Borna disease	0·085–0·125	(Elford, Barnard and Galloway).
Fowl plague	0·06 –0·09	(Elford and Todd).
Rift Valley fever	0·023–0·035	(Brown and Findlay).
Bact. coli bacteriophage	0·02 –0·03	(Galloway and Elford).
Yellow fever	0·017–0·028	(Findlay and Brown).
Louping ill	0·015–0·02	(Elford and Galloway).
Foot and mouth	0·008–0·012	(Galloway and Elford).
(Oxyhæmoglobin)	0·003–0·005	(Galloway and Elford).

Staphylococcus = 1μ.
1 Micron or μ = 1/1,000 mm. or 1/25,000 inch.
1 Millimicron or $\mu\mu$ = 1/1,000μ.

figures correspond very well with many of those obtained by other workers using less reliable methods.

An interesting point is the minute size of the virus of foot and mouth disease, all strains of which appeared to be of similar dimensions ; this virus was found to be only three or four times the size of the oxyhæmoglobin molecule.

Centrifugalization. The preparation of virus suspensions for filtration frequently involves the use of the centrifuge in order to remove the coarse particles. For this purpose the emulsion is spun for a short time after which the supernatant fluid is collected.

The centrifuge has also been employed to obtain a concentration of a virus. Bland (1928) centrifuged a suspension of a guinea-pig strain of the vaccinia virus at about 6,000 r.p.m. for two to three hours and found, by inoculation of guinea-pigs, that the virus was present in a greater amount in the deposit than in the supernatant fluid. Tang (1930) by a similar method obtained some concentration of the vaccinia virus from a cell-free virus filtrate ; Bedson and Western (1930) produced similar results with suspensions containing the psittacosis virus. It was claimed that the above findings showed that the vaccinia and psittacosis viruses were particulate, and that they were of a comparatively large size. The main objection to this claim is that prolonged spinning would also deposit the protein particles contained in the suspension and the concentration of the virus in the deposit might have been the result of adsorption on to the protein. The use of

filtrates tended to overcome this objection as by this means all coarse particles were eliminated. Eagles and Ledingham (1932), using a new model of high-speed centrifuge capable of speeds up to 14,000 revolutions per minute, were able to deposit almost entirely the virus content of Berkefeld filtrates of vaccinia virus suspensions. The deposit, containing the virus, was found to consist in the main of Paschen bodies. Bechhold and Schlesinger (1931), as a result of physical calculations based on high-speed centrifugalization, estimated the size of the vaccinia virus to be 0·21–0·23μ, and the fowl-plague virus 0·12–0·13μ. These figures are greater than those obtained by ultrafiltration, which method appears to have a much higher degree of accuracy.

The Electrical Charge of Viruses. It has been pointed out that the electric charge of the filter and the material undergoing filtration may influence the results of filtration. Thus in order to obtain the best results it is necessary that the charge of the virus under certain conditions should be known, and consequently the electric charge carried by many viruses has been examined. Several methods have been devised, the most important are those involving the use of cataphoresis (Todd, 1927; Olitsky, Rhoads and Long, 1929), and the simple filter-paper test (Bedson and Bland, 1929).

In cataphoresis an electric current is passed through the virus suspension, which is arranged in suitable tubes, and after a definite period of time samples, collected from around the kathode and anode, are examined for the presence of the virus.

The virus usually travels to the anode within certain limits of *p*H, whereas the tissue proteins travel to the cathode. Cataphoresis may thus provide a useful means of freeing a virus from tissue proteins. Most viruses carry a negative charge if the virus-suspension has a *p*H in the region of 7·0 : vaccinia virus (*p*H 5·5–8·4) ; poliomyelitis virus (*p*H 6·0–6·8) ; yellow fever virus (*p*H 5·2–7·0) ; rabies virus (*p*H 6·0–9·3) ; fowl-pox virus (*p*H 6·6–8·0). Cataphoresis has also been employed in the case of poliomyelitis virus to recover the active virus from immune-serum-virus mixtures, which were themselves inactive (Olitsky and his colleagues, 1929).

The filter-paper test is very simple ; one end of a strip of sterilized filter-paper is dipped into the virus suspension, when, if the virus has the same charge as the paper, *i.e.*, negative, it will tend to travel up the strip of paper. Portions from different levels of the paper are examined for the presence of virus. Bedson and Bland, by this method, found that the vaccinia virus carried a negative charge over a *p*H 6·8–8·0, while the herpes virus had a negative charge at *p*H 7·6–8·0.

Resistance to Physical and Chemical Agents. It must be recognized in speaking of the resistance of a virus that, under the conditions of examination, the virus has probably never been entirely free from such extraneous material as tissue proteins. The resistance in certain instances may consequently have been influenced by these outside factors. The information obtained by examination of the resist-

ance of viruses to various agents is thus of uncertain value. It is however possible to eliminate much of the extraneous tissue either by cataphoresis or by adsorption of the virus on to kaolin. It may be stated briefly that viruses, as a whole, tend to resemble vegetative bacteria rather than the spore-bearing organisms in their resistance to physical and chemical agents.

The viruses exhibit a varied degree of resistance to physical and chemical agents. The majority are destroyed by moderately high temperatures, 50–55° C., but remain unaffected by low temperatures ; individual differences are naturally found. Many are also very resistant to drying and may be preserved in a dried state for some time.

Most viruses are resistant to glycerine, and consequently 50 per cent. glycerine is frequently employed for the preservation of virus-containing tissues at low temperatures ; under such conditions the poliomyelitis virus has been shown to be little altered in virulence after a period of eight years. The glycerine, in all probability acts by dehydration, so retarding autolysis of the tissues.

Ether, alcohol and chloroform do not appear to have much influence on many viruses, which however appear to be frequently very susceptible to oxidizing agents. There is, however, very marked individual variation in the reaction of viruses to chemical agents.

Weak concentrations of phenol and formalin are frequently used in making vaccines for the purpose of inactivating the virus.

The Photodynamic Action of Methylene Blue. Clifton (1931) found that the inactivation of the staphylococcus bacteriophage when mixed with methylene blue did not take place in the absence of light. He also showed that the presence of oxygen was essential for the process and concluded that the inactivation of the bacteriophage was due to an oxidation of the phage by photosensitized methylene blue in the presence of oxygen. Perdrau and Todd (1933) confirmed these observations and found that the optimal concentration of the dye was 1 part in 100,000, and that, while free bacteriophage was highly sensitive, it became comparatively resistant after union with the corresponding living bacterium. These workers also showed that viruses did not react in a uniform manner to the photodynamic action of the methylene blue. Some, the viruses of vaccinia, herpes, distemper, were found to be highly sensitive while others, the viruses of foot and mouth disease and ectromelia, proved very resistant. They later found that after inactivation by methylene blue the distemper virus was highly antigenic. This point will be considered at a later stage.

Summary. The evidence, available at the present time, indicates that viruses are particulate bodies varying considerably in size. Their reaction to physical and chemical agents does not differ materially from that exhibited by the vegetative bacteria.

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CHAPTER III

CULTIVATION AND MICROSCOPY

THE cultivation *in vitro* of filterable viruses has been the subject of experimental work for many years. Many claims of success have been made from time to time but it is only recently that true multiplication of a virus *in vitro* has been conclusively demonstrated. These successful results have been obtained by the use of media containing growing or living cells ; the application of media employed for the cultivation of bacteria has, with the exception of the so-called viruses of pleuro-pneumonia and agalactia, been unsatisfactory. This is interesting, as earlier workers claimed that such ordinary media had proved successful in many instances and concluded that the particular viruses under examination were related to definite bacteria. Indeed the view that certain filterable viruses merely represent a filterable phase in the life-cycle of certain micro-organisms is upheld by a few workers at the present time. A brief consideration of the results obtained by the application of lifeless media is thus necessary.

The Application of Lifeless Media. The earlier workers on virus diseases frequently obtained cultures of bacteria from the diseased tissues, but in most instances these micro-organisms have been

definitely excluded as primary incitants of the disease-process. The readiness with which many bacteria grow on comparatively simple media and the difficulty of demonstrating the presence of viruses have probably been responsible for many of the fallacies connected with their growth. In many instances the possibility of contamination occurring during the collection and examination of the virus-containing material must not be overlooked. It is also well known that under certain conditions of growth some bacteria pass through the coarser grade filters. In certain virus diseases the bacterium, considered responsible by some workers, is one which multiplies readily on the ordinary bacterial media.

A so-called streptococcus has been held responsible for a number of conditions, especially those in which the central nervous system is involved in the disease-process. Rosenow and his colleagues (1924) have repeatedly claimed that a peculiar streptococcus-like organism, which is said to have a filterable phase, is responsible for poliomyelitis, epidemic encephalitis, epidemic hiccup, and more recently epizootic encephalitis of foxes. This view is not generally accepted ; Long, Olitsky and Stewart (1928) and Fairbrother (1929) found, in the case of poliomyelitis, that this organism was not ætiologically related to the disease but was merely an air-borne contaminant, probably obtained during the collection for examination of the brain or spinal cord.

Other organisms have also been claimed as the causal agents of various virus diseases. Kuczynski

and Hohenadel (1930) claimed that the causative agent of yellow fever was a peculiar diphtheroid, which they termed *B. hepatodystrophicans*. This finding has not been confirmed by other workers.

Tunnicliff (1917, etc.) and others, isolated green-producing cocci from the blood and nasopharyngeal swabs of cases of measles and considered these cocci to be the primary cause of the disease-process; Kermogant (1925) obtained results which indicated to him that the causative agent of mumps was a spirochæte. These results, while difficult to disprove entirely owing to the absence of suitable experimental animals, have not been substantiated.

The presence of a secondary infection has presented difficulties in other instances, *e.g.*, the hog-cholera bacillus in swine fever, *Past. bronchisepticus* in dog distemper, and *Bact. aertrycke* in psittacosis. It is also suggested with some justification that *H. influenzæ* in epidemic influenza, various bacteria in acute coryza and *H. pertussis* in whooping-cough play a similar *rôle;* further developments on this question may be expected in the near future.

In the case of bovine pleuro-pneumonia and agalactia, definite growth of the virus has been obtained on serum-agar and in serum-broth. The growth cycle of pleuro-pneumonia has been studied in detail by several workers. The latest line of investigation has been carried out by means of colonial impressions. In the early stages of growth actinomyces-like branching foci were found, later " nucleated " masses were formed from the mycelia, followed about the fifth day by the formation of

spore-like bodies. These findings suggested affinities to the fungi, although mycologists consider that there is at present insufficient evidence to warrant classifying the ætiological agent of pleuro-pneumonia as a true fungus (Ledingham, 1933). Whether it is justifiable to consider this condition as a true virus disease in view of the morphological findings is an open question. As a result of further observations Ledingham (1933) suggests that the organisms of pleuro-pneumonia and agalactia should be placed in the family " actinomycetaceæ."

" Globoid Bodies." The question of the " globoid bodies " deserves attention, as the statement is still seen in some text-books that these bodies represent the virus of poliomyelitis. Flexner and Noguchi (1913) obtained, by cultivation of poliomyelitic nervous tissue at 37° C., peculiar minute " micro-organisms," which they termed " globoid bodies." The medium employed was human ascitic fluid containing a small piece of rabbit kidney, covered with sterile vaseline or paraffin, and arranged in long narrow tubes. The medium was also rendered semi-solid by the addition of a suitable quantity of 2 per cent. agar. Growth occurred in the form of a faint granular deposit after five to seven days ; while primary cultures were only obtained with difficulty secondary growth took place readily. These workers considered that these bodies had no relationship to ordinary saprophytic bacteria but possibly were associated with the virus. These observations were confirmed and elaborated by Amoss (1914), and Flexner, Noguchi and Amoss (1915). Rosenow,

Towne and Wheeler (1916), in a study of a streptococcus-like organism isolated from poliomyelitic material, found that under anaerobic conditions very small forms, comparable with the " globoid bodies," were obtained. Smillie (1918) later prepared cultures of " globoid bodies," but found them to be quite distinct from the streptococcus-like organism of Rosenow, which he considered a contaminant. Loewe and Strauss (1920), using a similar technique, cultivated globoid bodies from the nervous tissue of cases of encephalitis and considered that they were associated with the virus. Harris and Duval (1924) also by the same methods obtained these bodies from the blood removed from cases of dengue fever. Long, Olitsky and Rhoads (1930), reconsidering their earlier views, could obtain no convincing evidence to indicate that these bodies represented the virus of poliomyelitis ; the presence of the virus was frequently associated with the appearance of the bodies in culture tubes, but this was considered to be probably an adsorption phenomenon.

The nature of these bodies is by no means settled ; some consider them to be minute micro-organisms, while others state that they are probably non-specific protein precipitates. It is however highly improbable that they represent the actual virus of such distinct conditions as the diseases named above.

Media Containing Growing or Living Cells. There is little doubt at the present moment that several viruses can be successfully cultivated in the presence of living cells. In the successful cultures naked-eye evidence of growth, such as shown by bacteria, is not

found; it is necessary in all cases to carry out titration tests on susceptible animals in order to demonstrate the multiplication of the virus.

The first successful results were obtained by the use of cultures containing actively growing cells; Steinhardt, Israeli and Lambert (1913) claimed to have obtained multiplication of the vaccinia virus in tissue-cultures. These were prepared on cover glasses by the addition of a small quantity of the virus emulsion to small pieces of the cornea and some plasma of normal guinea-pigs or rabbits; the cover-glasses were then inverted over hollow ground slides, sealed and incubated at 37° C. for fourteen to eighteen days, after which the virus-content was examined by skin inoculation tests in the rabbit. These results were confirmed later (Steinhardt and Lambert, 1914). Parker (1923–24) prepared tissue-cultures containing rabbit's testis and plasma. By the use of this technique he carried the vaccinia virus through several generations and considered that this was the direct result of multiplication of the virus. Carrel and Rivers (1927) obtained multiplication by the use of seven- to ten-day chick-embryo. This, after mixing with suitable amounts of Tyrode's solution * and seed virus, was allowed to stand in

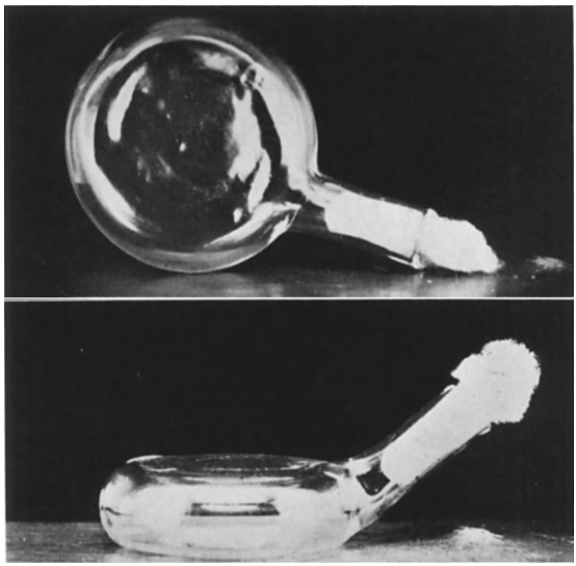
* The composition of Tyrode's solution used by different workers has not been constant; the following formula is that given by Eagles and his colleagues :—

NaCl	6·0 gm.	KH_2PO_4	0·18 gm.
KCl	0·4 ,,	$CaCl_2$	0·275 ,,
$MgSO_4$	0·25 ,,	Glucose	1·0 ,,

Distilled water to 1 litre. Adjusted to *p*H 7·3 with standard NaOH solution and sterilized by filtration through a Seitz filter.

the cold room for a short period in order to fix the virus to the tissue. The virus-pulp-suspension was then further diluted by the addition of Tyrode and hen plasma, after which the mixture was rapidly distributed, before clotting occurred, into special flasks, Carrel " D " tissue-culture flasks, in 2 c.c. amounts ; the tissue plasma clot was subsequently fed with an extract of seven- to ten-day chick embryo minced in Tyrode's solution every two to three days. After incubation at 37° C. for at least eight days, the contents of the flasks were removed, ground up, and the virus-content determined by the inoculation of rabbits. In this manner multiplication of the vaccinia virus was readily demonstrated ; it was also found that this was accompanied by a definite proliferation of the tissue cells. These observations were soon confirmed (Haagen, 1928 ; Eagles and McClean, 1929) ; the results of the latter workers were, however, distinctly irregular. As it was found that the growth of virus was invariably associated with growth of the tissue-cells, it was generally assumed that the presence of actively proliferating cells was essential for the multiplication of a virus by *in vitro* cultivation.

Maitland and Maitland (1928) made further progress when they found that growth of the vaccinia virus was possible in cultures in which tissue-proliferation was not detected. The following method was employed : into a flask were put approximately 0·66 c.c. of minced hen kidney and 1·3 c.c. of the inoculum, which was a testicular strain of the vaccinia virus, diluted 1 in 6·6 with Tyrode's



Carrel Tissue Culture Flasks, Type D (5 cm. diameter).

[*To face p.* 30.

solution. The mixture was allowed to stand in the cold room for four hours. Then 1 c.c. of Tyrode and 6 c.c. of hen's serum were added. The mixture was distributed in amounts of 2 c.c. into Carrel's tissue-culture flasks, type "D," incubated at 37° C., and the virus content determined by the intradermal inoculation of rabbits after various intervals of time.

This technique was an important advance owing to its simplicity and the method, with the substitution of the serum and kidney or testis of the rabbit for those of the hen, has since been repeatedly employed with success (Maitland and Laing, 1930; Eagles and McClean, 1930). This step was revolutionary, as it demonstrated that the presence of multiplying tissue-cells was not essential for the multiplication of viruses. Not only was tissue growth not detected but it was found that the tissue showed extensive disintegration. This suggested that the growth of the virus had taken place in the absence of viable cells. This question was investigated by Rivers, Haagen and Muckenfuss (1929), who found that, under the required conditions of cultivation, the tissue-cells survived at least five days, while in certain instances multiplication of the cells was observed; they also showed that growth of the virus did not occur in the presence of cells, which had been killed by alternate freezing and thawing. These experiments indicated that living cells, but not necessarily multiplying cells, were essential for growth of the vaccinia virus; this suggested that the virus either multiplied within the

living cells of the tissue or depended for its growth upon some substance obtained from living cells. Muckenfuss and Rivers (1930) found that the vaccinia virus placed in a mixture of rabbit serum and Tyrode's solution survived longer when kept separated from living cells by a collodion membrane than if placed in serum and Tyrode alone. This indicated that some diffusible substance, favourable for the survival, and possibly the growth of the virus, was obtained from the cells. There is thus no doubt that living cells play an important *rôle* in the cultivation of viruses.

The Maitland medium, or some slight modification, has since been employed extensively and definite multiplication of various viruses has been reported : vaccinia, virus III. disease of rabbits, herpes, salivary gland disease, psittacosis, common cold and foot and mouth disease. In the case of the virus of foot and mouth disease growth occurs less readily than with the other viruses.

The use of tissue-cultures has not been limited to the propagation of viruses, this technique has also been applied to the study of the cellular changes and the immunity reaction involved in certain virus diseases. These aspects will be discussed later in their respective chapters.

Cell-free Media. The cultivation of viruses has passed through various processes of simplification, of which one of the most important steps was the substitution of living for actively growing cells. The elimination of cells from the medium is the next step desired and recent experiments suggest that

this may perhaps be possible. Eagles and McClean (1930) claimed that they had obtained multiplication of the vaccinia virus by cultivating it in a medium of minced rabbit kidney extract and Tyrode's solution, from which the cells had been almost entirely removed by centrifugalization. These workers (1931) later published the results of confirmatory experiments, and also found that even after filtration through a Chamberland L2 candle the kidney extract gave satisfactory results; by this means the presence of single cells or fragments of cells was definitely excluded. The cultures, however, were by no means invariably successful. Similar results were obtained later by Eagles and Kordi (1932), who prepared a richer medium by extracting the minced kidney with 9 per cent. saline and centrifugalizing for two periods of one hour, after which the supernatant fluid, rendered isotonic by the addition of distilled water, was added to equal parts of fresh rabbit serum and Tyrode's solution. In this medium the vaccinia virus was generated through ten subcultures and showed an increase of 10^{20}. Eagles (1932), considering this question in a review of virus-cultivation, stated "that an ideal method of releasing essential substances from cells has not been achieved is evidenced by the difficulties encountered in securing a culture at all. There appears, however, no doubt that substantial increases in virus were realised in a medium containing no demonstrable cells."

Unfortunately this work has not been confirmed by other workers; Maitland, Laing and Lyth (1932),

Rivers and Ward (1933), in attempts to repeat the experiments of Eagles and his colleagues, have obtained completely negative results. The explanation of this is not obvious, and, until these divergent results have been reconciled, the findings of Eagles and his co-workers can only be accepted with some reservation. The question, however, is one of great fundamental importance; if viruses can be grown in a cell-free medium the presence of extraneous cells will be eliminated, and it may be possible to study a virus in a "free" condition. The problem of cultivation would be considerably simplified and the technique brought in closer relationship with that employed in the cultivation of bacteria.

There is no doubt that a marked advance has been made recently in the technique for virus-cultivation. It must, however, be recognized that little is known of the exact requirements necessary for virus-growth. Many variable factors are involved, and much work is needed before they can be understood and adjusted. Some slight alteration of any one factor may exercise a considerable influence on the results of an experiment; this point is well illustrated in the conflicting results of different workers. The individual requirements of the various viruses apparently differ considerably; in only a comparatively few instances has *in vitro* cultivation been successful. In the case of certain viruses the only available method of obtaining multiplication is by the inoculation of a susceptible animal. There is still much work to be accomplished in this field.

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MICROSCOPY

One of the criteria of viruses has been their invisibility under the microscope; while this statement has been accepted up to the present time, it may, however, require some modification when the nature of the elementary bodies is definitely decided. On account of the failure of the microscope to identify viruses, they have been termed at various periods in their study ultramicroscopic, infravisible and invisible viruses. These terms have never been considered satisfactory, although the failure of the microscope to detect filterable viruses is doubtless due to their small dimensions.

Filtration experiments have indicated that viruses are smaller than $0{\cdot}2\mu$ in their largest diameter; this figure is also approximately the limit of resolution by the ordinary microscopic methods. The limit of resolution is the term used to indicate that condition when no further separation of the elements of structure is obtainable. The resolving power of an objective is governed largely by the wave-length of light employed and the numerical aperture of the

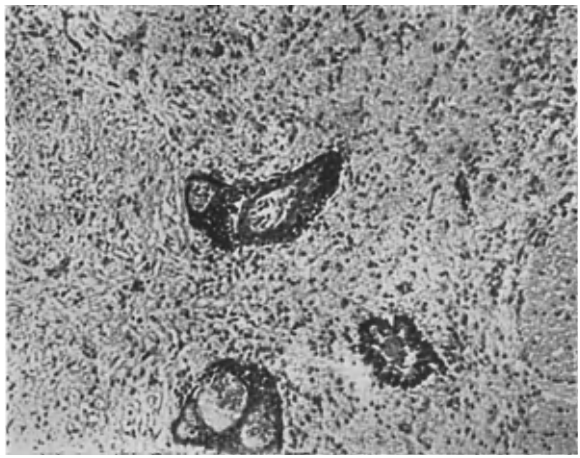
objective used, and is determined by the formula $\frac{\frac{1}{2}\lambda}{\text{N.A.}}$ (λ=wave-length of light employed, and N.A. = the numerical aperture of the objective). It has thus been found that the smallest particle that can be resolved by dark-ground illumination using visible rays, under ideal conditions, is approximately $0{\cdot}2\mu$; under the usual laboratory conditions, however, the figure is usually about $0{\cdot}25\mu$. Barnard (1925) found that this limit could be extended to $0{\cdot}075\mu$ by using photography with ultra-violet light and a special optical system of quartz lenses. By the application of this technique it has been possible to examine the growth changes of the ætiological agent of pleuro-pneumonia.

While the viruses themselves cannot be identified by the microscope, microscopy has proved of great importance in the examination of lesions produced by them. Viruses in many instances exhibit an intimate relationship with certain of the tissue-cells of the host; this affinity or tropism for special types of cells is frequently very well defined, as evidenced by the marked affinity of the viruses of rabies and poliomyelitis for the nerve cells of certain animals. It is, however, uncertain whether viruses as a whole multiply within the cells or whether growth occurs extracellularly as a result of some substance or substances produced by the cells. Nevertheless, during the reaction between the virus and the cells characteristic changes are frequently produced. The type of lesion varies in different diseases. In some, molluscum contagiosum, warts and fowl-pox, there

is a distinct tendency to cell-proliferation and the appearances suggest that there might be some connection between these conditions and the filterable fowl-tumours. In other diseases, variola, poliomyelitis, etc., there is a definite inflammatory reaction with changes of a degenerative nature. This is illustrated by the vacuolation, degeneration and ultimate destruction of the nerve-cells of the cord found in poliomyelitis.

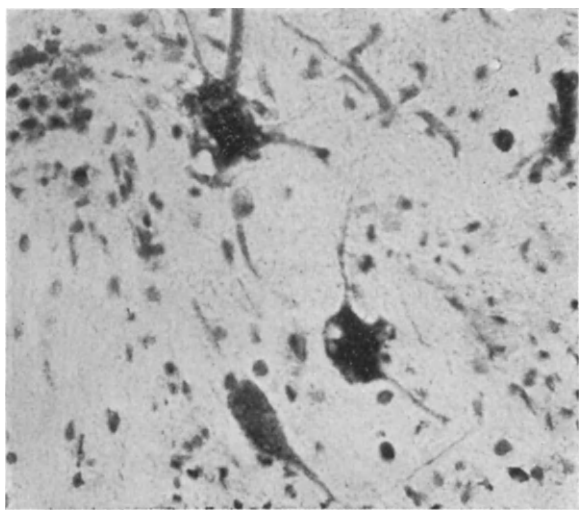
Inclusion Bodies. One of the most important features of the histological picture is the presence in the affected cells of definite abnormal bodies, which may be situated either in the cytoplasm or within the nucleus; in some instances they are found in both situations. These bodies have been termed "inclusion bodies," when present in the cytoplasm they are called "cytoplasmic inclusions," and when in the nucleus "intranuclear inclusions." Inclusion bodies which vary greatly in shape and size, 0·25–20μ, were first given prominence by Guarnieri, who found them in the epithelial cells of variolar and vaccinal lesions. They can frequently be demonstrated in sections stained by hæmatoxylin and eosin or by Mann's method, and have been found in association with many virus diseases; these are given on p. 40.

While the presence of inclusion bodies is of diagnostic importance in many instances, their nature and composition are by no means understood. Much work has been carried out in attempts to solve these problems, but only the major points will be mentioned here. More detailed accounts can be studied elsewhere (Ludford, 1930; Cowdry, 1928).



EXPERIMENTAL POLIOMYELITIS

Spinal Cord. Perivascular and diffuse infiltration.



EXPERIMENTAL POLIOMYELITIS

Spinal Cord. Vacuolar degeneration of nerve cells. Above and to left, a focus of neuronophages at the site of a vanished nerve-cell.

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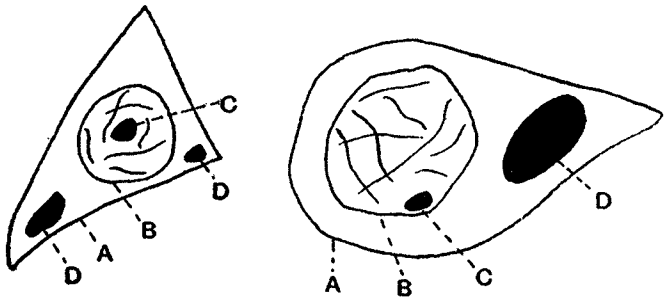
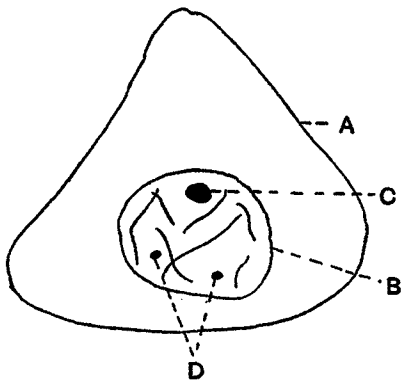


CYTOPLASMIC INCLUSION BODIES (RABIES)

A = Nerve Cell.
B = Nucleus.
C = Nucleolus.
D = Inclusion (Negri) Body.



INTRANUCLEAR INCLUSION BODIES (EXPERIMENTAL POLIOMYELITIS)

A = Nerve Cell.
B = Nucleus.
C = Nucleolus.
D = Intranuclear Inclusion Body.

	Human Diseases.	Animal Diseases.
Intranuclear inclusions.	Herpes simplex. Varicella. Poliomyelitis. Yellow fever.	Virus III. Salivary gland disease of guinea-pigs. Borna disease. Rift Valley fever.
Cytoplasmic inclusions.	Rabies. Vaccinia. Molluscum contagiosum.	Sheep-pox. Fowl-pox. Infectious myxomatosis of rabbits. Ectromelia.
Combined intranuclear and cytoplasmic inclusions.	Variola.	—
Bodies of an *Indefinite nature.*	Measles. Epidemic encephalitis. Warts. Trachoma.	Distemper. Swine fever. Fowl plague.

The bodies frequently contain both basophilic and acidophilic material, but, while our knowledge of them is increasing, the precise chemical composition of any one inclusion body has not yet been determined.

The bodies are found in those cells actively involved in the disease process; in rabies and poliomyelitis they are found in the nerve cells, while in molluscum contagiosum, varicella and vaccinia the epithelial cells are involved. The presence of inclusion bodies in the epithelial cells of vaccinial lesions is interesting, as Ledingham (1924) found that, after inoculation of the virus into the skin, the local

reticulo-endothelial tissue was primarily and dominantly involved.

Many views have been expressed regarding the nature of these bodies; the main theories are briefly :—

(1) These bodies, when first discovered, were considered to represent the causative agents of the disease, which were placed among the protozoa. Guarnieri (1894) termed the bodies he found in vaccinia and variola lesions "*Cytorcytes vaccinæ et variolæ*"; Negri (1903) also considered that the bodies found in the nerve cells of cases of rabies were of a similar nature. Evidence supporting this theory was not easy to obtain, and as it was difficult to reconcile this contention with the filterability of many viruses and the irregular appearance of some of the bodies in virus-containing tissues, this view was gradually abandoned. In recent years the question has again been raised; Manouelian and Viala (1924) considered that the Negri bodies represented degenerating intracellular agglomerations of the parasite "*Encephalitozoon rabiei*," which was of a similar nature to the causative agent of spontaneous encephalitis of rabbits, the *Encephalitozoon cuniculi*; Covell and Danks (1932) were not able to obtain any evidence to support this hypothesis, and suggested that the Negri body was in the main an alteration in the basophilic Nissl substance. Levaditi and his colleagues (1926) added to the confusion by suggesting that the Negri bodies represented a stage in the life cycle of a protozoon, *Glugea lyssæ*, which in another phase was filterable. This parasite was

considered to be quite distinct from the *encephalitozoon cuniculi*.

(2) As the protozoon theory did not prove satisfactory it was postulated that inclusion bodies were simply reaction products produced by the cell as a result of infection with the virus. Traces of the material probably giving rise to intranuclear inclusions have been found in several instances, herpes and poliomyelitis, but there is no proof that this material comprised the whole of the final inclusion. There is, however, no question that a cell is profoundly modified by the activity of a virus. Findlay (1933) considered that the intranuclear inclusions found in Rift Valley fever and in yellow fever were probably degeneration products due to a loss of water from the nucleus.

(3) A compromise hypothesis was next put forward in which it was contended that the inclusions represented the virus together with the products of cellular reaction against the infection. These bodies were accordingly termed "*Chlamydozoa* = mantle animals" by v. Prowazek (1907), and "*Strongyloplasmata*" by Lipschutz (1907). The recent researches of Woodruff and Goodpasture (1929, 1930) on fowl-pox have an important bearing on this subject; these workers found that the inclusion or Bollinger body was composed of hundreds of minute bodies enclosed in a fatty capsule. It was shown that aside from the lipoid the Borrel bodies were the major constituents of the inclusion, and they were judged to represent the actual virus of fowl-pox. These workers later (1931) demonstrated that

molluscum bodies could be broken up by tryptic digestion to form myriads of minute Lipschutz granules, which were almost identical morphologically with the Borrel bodies of fowl-pox. Carrying their investigations further (1932), Guarnieri bodies were shown in mesodermal cells by infecting chorio-allantoid membranes of chick-embryos with vaccinia virus. It was considered that these bodies were composed in part of Paschen bodies. Thus in three conditions minute elements, which are said to represent the virus in question, have been definitely found to be major constituents of the inclusion bodies. The relation of these minute bodies, which have been termed elementary bodies, to the actual virus, will be considered later.

In view of the great variation in the type of virus bodies found, it is improbable that any one theory would suffice for them all.

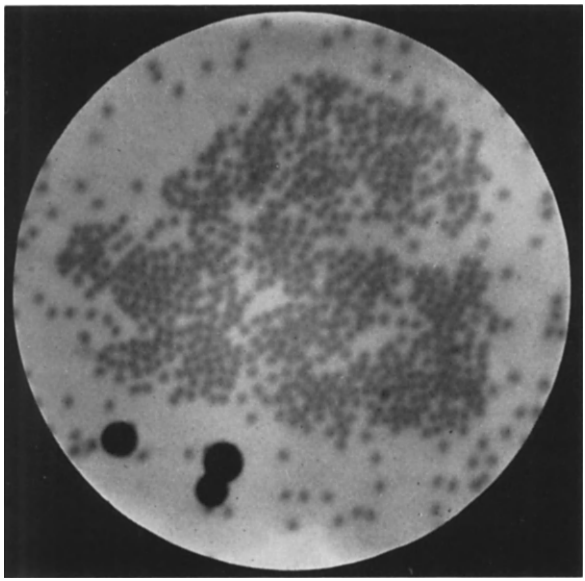
The formation of inclusion bodies in tissue-culture under varying conditions has been the subject of recent investigations. Andrewes (1929), working with virus III. disease of rabbits, found that inclusion bodies were formed with marked regularity in the presence of normal testis and plasma, and were definitely associated with survival or growth of the virus ; the bodies were not formed in the presence of immune testis in immune serum or plasma. This method thus provides a means of studying *in vitro* some of the immunity reactions of certain viruses.

Elementary Bodies. The presence of minute coccal bodies in the exudates found in lesions formed by some of the larger viruses has suggested that

these elementary bodies might be associated with the actual virus. Borrel (1903) demonstrated minute bodies in fowl-pox lesions, while Paschen in 1906 discovered similar bodies in material from vaccinial lesions, and claimed that they were related to the ætiological agent of the disease. These claims received little attention until recent years, when they have been re-examined, and much evidence has been obtained to indicate that the elementary bodies do actually represent the virus in certain diseases.

Ledingham (1931) prepared from dermal vaccinial lesions a homogeneous suspension of the bodies in formolized saline, which he claimed was free from other elements. The elementary bodies were stained either by the prolonged Giemsa method or by a special technique. While unstained the bodies were probably less than $0{\cdot}2\mu$ in diameter, but when stained they were found to be $0{\cdot}2$–$0{\cdot}25\mu$; the increase in size was doubtless influenced by the deposition of stain. The suspension was agglutinated by the sera of animals either recovered from vaccinia or hyper-immunized with the virus. This reaction was demonstrated by the hanging-drop technique using a $\frac{2}{3}$-inch objective. Further evidence that the ætiological agent of vaccinia is the Paschen body was obtained by Nauck and Paschen (1932), who found that the elementary bodies increased in numbers during *in vitro* cultivation of the virus.

Similar bodies have also been demonstrated in the lesions of ectromelia, while Paschen (1933) has found some in the vesicular fluid obtained from cases of varicella and herpes zoster.



Elementary Bodies. The Borrel bodies of fowl-pox with three staphylococci; note that the diameter of the staphylococci, approximately 1μ, is about 5 $\times$ that of the Borrel bodies. (Giemsa.) $\times$ 4,000.

[*To face p.* 44.

The relationship of elementary bodies to the inclusions found in lesions of vaccinia, fowl-pox and molluscum contagiosum has already been discussed (p. 42).

Elementary bodies have also been demonstrated in the lesions of psittacosis. Bedson and Bland (1932), using dark-ground illumination and also stained preparations, found, both by the intra-peritoneal inoculation of mice and the subsequent examination of their spleens and by culture of the virus in mouse-spleen cultures, that the psittacosis virus had a definite growth-cycle, in which the formation of elementary bodies was the last phase. The development-cycle suggested to these workers that the virus was not a bacterium but possessed affinities with the mycetozoa or myxomycetes, or possibly with the microsporidia. This complex growth-cycle has, however, only been demonstrated in the case of psittacosis. It will be interesting to observe whether such a complicated growth-cycle can be demonstrated in the case of other viruses.

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CHAPTER IV

EPIDEMIOLOGY AND IMMUNITY

THE incidence of infectious disease depends largely on two factors : the activity of the infecting agent and the susceptibility of the community as a whole to the infection. These factors are consequently of paramount importance in studying the epidemiology of virus diseases. Cases have been recorded where the introduction of a virus, such as the virus of variola, poliomyelitis or measles, into virgin soil, as illustrated by the population of an isolated island, has given rise to extensive epidemics of marked severity. Paul and Freese (1933), during an investigation of the incidence of the common cold in an isolated Arctic community at Spitzbergen, found that the arrival of the first boat of the shipping season was almost invariably followed by a sudden epidemic, which involved the whole community in a short period of time. An admirable survey of the human adaptation to infection has been given by Dudley (1929), in which particular reference is made to the importance of infection-pressure and herd immunity in measles, influenza, common cold and poliomyelitis.

The epidemiology of virus diseases presents, perhaps, as much variation as does the epidemiology

of bacterial diseases. Many virus diseases, as smallpox, measles, distemper and foot and mouth disease, appear to be readily communicated by direct or indirect contact and are extremely contagious, while others, as poliomyelitis, molluscum contagiosum and encephalitis, exhibit only a mild degree of infectivity. In the case of poliomyelitis at least this lack of infectivity appears to be more apparent than real. In view of the demonstration of immune bodies to the poliomyelitis virus in the serum of apparently normal individuals and as a result of a statistical survey of the incidence of the disease, it has been considered probable that many people undergo subclinical infection with this virus without displaying any obvious manifestations of the activity of the virus (Aycock and Kramer, 1930). The occurrence of subclinical infection probably plays an important *rôle* in the epidemiology of many other virus diseases, as alastrim, variola, measles and mumps. In the case of distemper, the presence of immunity in dogs resulting from subclinical infection interfered considerably with experimental work ; Laidlaw and Dunkin (1926), in order to overcome this difficulty, used a special stock of dogs bred under the strictest isolation.

The presence of herd immunity naturally constitutes an important factor in deciding the extent of an epidemic or epizootic. This is illustrated by the appearance of waves of severe epidemics of certain diseases at intervals of several years. The disease, during the intervening periods, is held in check by the lack of a suitable soil, due probably to the fact

that many members of the population possess some degree of immunity after the subsidence of a severe epidemic. An excellent example of epidemic waves is seen in the introduction of measles into Fiji. In 1874 measles occurred for the first time, and the estimated morbidity rate was 100 per cent. of 100,000 population with a fatality rate of 25 per cent. A high degree of general immunity followed and the disease became infrequent. As years passed another susceptible community developed, and in 1907 another outbreak of measles took place in which 40 per cent. of the population acquired the disease and the fatality rate was about 40 per cent. The importance of latent immunization in determining the spread of infection has recently been emphasized by Stocks (1930, 1932) in a statistical analysis of the epidemiology of measles, German measles, chicken-pox and poliomyelitis.

The portal of entry of the virus also plays an important *rôle* in the incidence of infection. The necessity of introducing viruses by selected routes in order to reproduce experimentally some diseases, is a common experience; thus while fowl-pox readily spreads in an infected poultry farm, it is extremely difficult to obtain infection under laboratory conditions by placing a healthy bird in contact with an infected bird. In order to ensure the reproduction of poliomyelitis in monkeys it is necessary to inoculate the virus directly into the central nervous system; spontaneous cage infection of this disease has not been reported. In man the virus is frequently transmitted by droplet infection,

and in these cases the site of entry is probably the nasopharynx; in some diseases, as measles and small-pox, the virus appears to be carried by the blood-stream to the various tissues, which manifest the signs of infection. In the case of poliomyelitis the virus does not reach the central nervous system by the blood stream. Recent experimental evidence indicates that the virus passes to the nerve-cells of the brain-stem and spinal cord by way of the axons of the olfactory nerves (Fairbrother and Hurst, 1930; Hurst, 1930). This may account for the comparatively low incidence of the disease, as, in order to produce infection, the virus must reach the axons of the nerves; the presence of the virus in the naso-pharynx does not necessarily lead to the production of the disease in its recognizable form. Such a mode of infection, which has also been shown to occur in rabies, may exist in other neurotropic diseases (Goodpasture, 1925).

It is probable that CARRIERS play as important a part in the dissemination of virus diseases as in bacterial diseases. It is known that many virus diseases are endemic in different regions, and during the quiescent period a virus-reservoir must exist somewhere. In view of the apparently parasitic nature of viruses it is probable that, during this period, they are harboured to a large extent in the human and animal body. The difficulty of eradicating animal virus diseases, as foot and mouth disease and rinderpest, from infected areas is well known, and is probably largely due to the presence of carriers. Owing to the difficulty attached to the

identification of viruses, experimental investigations of the carrier rate in virus diseases have been limited. Statistical evidence in many instances, however, indicates the prominent part played by carriers in the spread of the disease.

Direct communication by contact of an animal virus disease to man is extremely rare. One important instance has recently been demonstrated; there is no question that the recent epidemic of psittacosis was due to the importation of infected parrots from South America. In the majority of cases there was a definite history of contact, frequently close contact, with infected birds.

In diseases transmitted by human contact, age is frequently an important factor in the incidence and severity. The young are frequently the most susceptible, while the mortality rate is often greatest at the extremes of life.

The epidemiology of certain other diseases, herpes and warts, is very uncertain. These infections are not usually transmitted to other individuals; they, however, tend to render the host more liable to further infections with the same virus. There appears to be a marked individual susceptibility; in these cases, the virus may persist for an indefinite period, giving rise to either a continuous or an intermittent infection.

Another important method of infection is the direct implantation of virus into the human tissues. Two such modes of infection are seen. (1) The virus may gain entry and give rise to infection as a result of the handling of infected material when abrasions

or open wounds of the skin are present. Examples in man of this type are the various animal-pox diseases and foot and mouth disease. This type of infection has also been considered probable in certain cases of yellow fever (Lowe and Fairley, 1931). (2) The introduction of the virus through the skin may be performed by some outside agent—bites from infected animals in rabies, insect bites in yellow fever, dengue and pappataci fever; the recent suggestion that rabies-encephalitis in Trinidad was communicated by vampires also deserves mention (Hurst and Pawan, 1931). The incidence of these diseases is obviously limited by the distribution of the intermediary hosts of the virus, and it is natural that suppression of these agents has led to a decreased incidence of the diseases. This is illustrated by the rarity of rabies in Great Britain as a result of the enforcement of the muzzling laws, while organized campaigns against mosquitoes have been mainly responsible for the control of yellow fever. Transmission of these diseases directly from man to man does not occur.

Seasonal Incidence. A definite seasonal incidence is a special feature of only a few virus diseases. Thus the incidence of poliomyelitis is greatest during the late summer and autumn, while that of epidemic encephalitis is maximal in the earlier months of the year; measles is most frequent in spring and autumn. The question of a virus-reservoir during the interepidemic periods has already been mentioned. The explanation of the seasonal incidence of these diseases is not known. It is probable that

the virulence of the virus and the resistance of the individual may both be affected by changes of the environmental conditions occurring at the different seasons. The alteration in the resistance of the individual may be either a local or a general phenomenon. The local changes probably take place at the site of infection. The general or constitutional changes are even more problematical. It has recently been suggested that in the case of poliomyelitis at least the comparative resistance of adults to infection is non-specific, and is the result of a physiological maturation of the tissues (Jungblut and Engle, 1932).

Changes in the virulence of viruses are not easy to determine owing to the difficulty experienced in demonstrating their presence. Experimental work indicates that it is highly probable that changes of virulence do occur. In many instances the viruses are extremely active ; in the case of yellow fever it has been found experimentally that 0·0001 c.c. of blood removed from an infected animal will reproduce the infection on subcutaneous inoculation. After keeping for some time the virus deteriorates and much larger doses are required to produce infection.

The Modification of Viruses. In view of the uncertainty surrounding the ætiology of a number of the virus diseases, one point arises which appears of paramount importance. Is it possible that a virus may become modified under different environmental conditions and give rise to an altered form of reaction ? This question is of particular interest in

the study of certain neurotropic diseases, especially encephalomyelitis, the origin of which is still obscure. It is well known that by passage through the lower animals the variolar virus becomes decidedly modified—so also does the rabies virus; the *virus de la rue* is distinct in many respects from the *virus fixe*. Many workers consider that the identity of the ætiological agents of zoster and varicella is established, although the clinical manifestations of these conditions are quite distinct (Netter and Urbain, 1931). The experimental evidence indicates that there was a definite relationship between the rabies virus and the Japanese encephalitis epidemic in 1923. The prevalent type of infection with the variolar virus, termed alastrim, is much less severe than that found in earlier outbreaks. This modification is considered to be the result of infection of a partially immunized community. It has recently been found that the addition of testicular extract to the vaccinia virus considerably modified the nature of the reaction after intradermal inoculation, due probably to increased permeability of the tissues (Duran-Reynals, 1929; and McClean, 1930). There is thus no question that a virus or the reaction evoked by a virus may, in certain instances, be profoundly modified. The postulated connection of the herpes virus with epidemic encephalitis deserves mention in any consideration of the question of the mutability of viruses.

The modification of the infectivity for man of viruses by passage through lower animals is of great importance in the study of immunity. The vaccinia

virus, the rabies fixed virus, and more recently the yellow fever virus adapted to the brain of the mouse have been, and are still being, used for the production of immunity in man. It has also been suggested that the poliomyelitis virus, after frequent passage through monkeys, probably becomes similarly modified, and might be used for prophylactic inoculations. It is, however, important to realize the danger of generalizing in so far as viruses are concerned.

Plurality of Strains. Mention must be made of the possibility of the existence of several strains of one virus. This plurality of types of virus is an important point in the epidemiology of foot and mouth disease in cattle; three strains ("A," "O" and "C") have been described, each of which gives rise to a distinct immunological response (Vallée and Carré, 1928); infection with one strain does not lead to immunity against either of the other two strains. This factor has not as yet been found to play an important *rôle* in the propagation of human virus diseases. In the case of rabies, variations in the characters of various viruses have been reported; Remlinger and Bailly (1930), however, investigated this question, and concluded that, while there may be differences in "aggressiveness" in viruses isolated in different parts of the world, the viruses are essentially identical.

Symbiosis. The modification of the activity of a virus when combined with a bacterium, merits attention. Shope (1913), in a study of swine influenza, found that the intranasal instillation of

Berkefeld filtrates of infectious material in susceptible swine resulted in a mild illness of short duration. He mixed the filtrates with pure cultures of *H. influenzæ suis*, an organism which was cultured regularly from the respiratory tract of infected swine but which completely failed to reproduce the disease on intranasal inoculation. On inoculating swine with this mixture a disease identical with swine fever resulted. Thus the combination of two infecting agents, both of which were incapable separately of inducing the disease, produced positive results. The mild disease produced by the virus was probably accentuated by the activity of the bacillus. The recent work of Smith, Andrewes and Laidlaw (1933) indicates the possibility of a similar trend of events in epidemic influenza. These workers suggest that a virus is responsible primarily for the disease and this infection facilitates the invasion of the body by bacteria, particularly *H. influenzæ*.

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IMMUNITY

It is important to recognize that the terms immunity and susceptibility are only relative. An individual although resistant to a low grade of infection with a virus or bacterium, may equally develop the disease in the presence of an overwhelming infection. A person may thus be either susceptible or immune according to the virulence and dosage of the infecting organism ; the terms essentially overlap.

Diseases due to filterable viruses were, until quite recently, generally considered to be followed by a state of "*permanent or solid*" immunity against the particular virus causing the disease. The opinion was consequently prevalent that the immunity-response in diseases of this character was probably of an entirely different nature from that following infection with bacteria. However, it is now almost universally recognized that the condition of im-

munity resulting from attacks of virus diseases has numerous resemblances to anti-bacterial immunity and varies considerably in different diseases. Some diseases, herpes and the common cold in man, and foot and mouth disease in animals, are succeeded by a very transient state of immunity; second attacks may follow after a short interval. Nevertheless, after the majority of virus diseases a high degree of immunity ensues, although in certain isolated instances second attacks of particular diseases have been recorded. Thus it is obvious that no general statement can be propounded with regard to the degree and duration of immunity succeeding diseases due to filterable viruses. The use of such terms as "solid immunity" and "permanent immunity" is consequently misleading, and should be avoided.

The nature of the immunity response evoked by viruses is still uncertain. It has been recognized for some time that immunity to the filterable viruses is frequently associated with the presence of immune bodies in the serum, and it is now accepted that these bodies play an important *rôle* in the body defence. There does not thus appear to be any justification for the view taken by some workers, that immunity in virus diseases is fundamentally distinct from that occurring in bacterial diseases. Evidence is now accumulating which indicates that the reactions concerned are of a similar nature to those involved in bacterial infections. The procedures employed in the study of bacterial immunity have consequently been adopted, and the subject will be considered first under the headings of natural

and acquired immunity, followed by a review of the immunological reactions invoked.

Natural Immunity. Natural immunity with regard to viruses is frequently considered in a loose manner. It has long been recognized that natives of tropical regions are relatively insusceptible to yellow fever and that adults appear to be immune to poliomyelitis. These, until quite recently, were considered examples of natural immunity. It has now been shown that this is not correct—the immunity in these instances is acquired. This question will therefore be considered later. As a general rule it may be stated that natural racial immunity, in so far as filterable viruses are concerned, does not exist. All persons, if not in some way rendered immune, are liable to infection with any of the viruses.

Species immunity is of great importance. The spontaneous occurrence of human diseases in animals is extremely rare; in some cases the experimental production of the disease is also very difficult, and this constitutes one of the chief factors interfering with investigations on the ætiology of certain diseases. In epidemic encephalitis, varicella, zoster and others, no satisfactory experimental animal has been found. In poliomyelitis only the monkey has been shown to be susceptible to the disease; in yellow fever the asiatic monkey and the mouse are the only animals not markedly refractory to inoculation of the virus. The reason for this insusceptibility is not obvious. Immune bodies to the different viruses cannot be demonstrated in the

serum of the refractory animals. It is thus highly probable that the refractory state is evidence of an unsuitability of the tissue for the growth of the virus referable to a species peculiarity. The tissues of lower animals, unlike the tissue of human beings, do not permit the virus to multiply and produce infection. The virus in certain cases does not appear to be destroyed immediately after inoculation into insusceptible animals, but may persist around the site of inoculation for some time ; Amoss (1918) found that, after intracerebral inoculation of the poliomyelitis virus into rabbits, which are refractory to this virus, he could demonstrate, by re-inoculating the brain around the site of injection into monkeys, the presence of the virus after four, but not after seven, days.

In the case of other viruses the limitation of susceptible species is less strict ; for example, the viruses of vaccinia and herpes can produce infection in a number of animals, while the viruses of rabies, psittacosis, Rift Valley fever and foot and mouth disease, may all produce natural infections in man.

Acquired Immunity. Acquired immunity may be either active, in the sense that the immunity response is the result of the reaction of the person's own tissues, or passive, in the sense that the increased resistance is due to the presence of immune bodies produced in some other person or animal and introduced from without.

Active Acquired Immunity. Active immunity may arise naturally or may be artificially induced. In each of these the reaction is primarily the same,

and consequently there is a close connection between the two. In artificially inducing immunity, the attempt is made to produce a definite degree of immunity with the minimum of risk and discomfort.

Natural Immunity is exemplified by those cases in which a single attack of the disease confers an immunity against subsequent infection with the same virus. The majority of the virus diseases fall into this category, and in many instances the immunity so acquired is long standing and sufficient to afford protection against a second attack. Immune bodies can frequently be demonstrated in the serum of these cases many years after the infection has subsided. The principal exceptions to this statement are herpes simplex, influenza and the common cold in man, and foot and mouth disease in animals, in which cases the immunity response is very uncertain, and, when present, it is only transient.

There is also another manner in which immunity may be naturally acquired, and that is by the occurrence of infections so slight and transient that they escape observation. It has been pointed out previously that subclinical infection plays a very important *rôle* in the epidemiology of a number of virus diseases. The occurrence of unrecognized infections is offered as an explanation of the comparative immunity of natives of infected regions to yellow fever. The intraperitoneal protection test in mice, which is now being widely applied, has shown the presence of immune bodies in the serum of many inhabitants of endemic zones, who have been

apparently free from the disease. Infants are very refractory to this disease, and during this period it is probable that they become immunized without presenting any signs of the disease (Marchoux and Simond, 1906).

Artificially Acquired Immunity. The theory that immunity to a disease may be induced artificially by the deliberate production of the particular disease in a modified form is very old. The first universal adoption of this procedure was that of Jennerian vaccination with cow-pox material in order to prevent the ravages of small-pox. Further advance was not made until the epoch-making researches of Pasteur on anthrax and rabies in the latter half of the last century. Since then the spread of prophylactic inoculations has been enormous, but unfortunately its practical application to the virus diseases did not meet with further success until recent years.

In the case of prophylactic vaccination against small-pox the living virus, modified by passage through calves, was employed. In performing the inoculations against rabies the method introduced by Pasteur, which was the one generally adopted for a long time, was the injection of the spinal cord of rabbits, infected with fixed virus, attenuated by drying over KOH at 22° C. for varying periods. Thus it is seen that in both cases virus, which had been modified in infectivity for man but which was still living, was employed for carrying out the immunization process. This was in marked contrast with bacterial immunization, in which bacteria

killed by various methods had been successfully employed. This suggested to many workers that the reactions involved in immunization with bacteria and viruses were of a totally different nature. In the light of recent researches it will be seen that this differentiation is not so sharp. Prophylactic immunization is now carried out in a large number of conditions and several methods of preparing the vaccines have been adopted.

(1) *Living Virus.* The use of living virus is far from being an ideal method owing to the difficulty of controlling the virus after its inoculation, and on a few occasions serious accidents have resulted. In some human diseases, as rabies and small-pox, living virus, modified and attenuated by animal passage, is still used. Living virus is also used in certain animal virus diseases, as distemper, to supplement preliminary injections of the killed virus.

(2) *Living Virus with Immune Serum.* The combined use of living virus and immune serum has been successful in several diseases. Different methods of combining the virus and the immune serum have been adopted. In some instances simultaneous inoculations into different parts of the body is carried out. This method has been applied largely to animal diseases, *e.g.*, rinderpest, distemper, and swine fever. When inoculated into an animal an active virus is sometimes difficult to control, and instead of an attack modified by the simultaneous inoculation of the immune serum a severe form of the disease may develop which may end fatally. This method is thus not entirely free from risk, and

consequently cannot be employed to give protection against human diseases. In giving convalescent serum six to nine days after exposure to infection with the measles virus, an attempt is made to modify the disease so that an active immunity follows a mild attack.

In other cases the virus and immune serum are mixed before inoculation. Rhoads (1931) claimed that by the use of immune-serum-virus mixtures, themselves inactive, he had produced immunity in the rabbit with the vaccinia-virus, and in the monkey with the poliomyelitis virus. Fairbrother (1933) found, however, that in the case of vaccinia virus, the virus in such mixtures was not entirely inactivated, but that its activity was merely reduced. This method therefore involves the inoculation of living virus, and is consequently altogether not free from risk. The use of immune-serum-virus mixtures has been found to give excellent results in the case of yellow fever: mixtures of human immune serum and the neurotropic mouse virus are inoculated, followed after a short interval by separate injections of human immune serum to prevent the appearance of the virus in the blood.

(3) *So-called Killed Virus.* A third method of immunization is by the use of inactivated or so-called killed virus. This is obviously the method of the greatest practical importance as the risk of accidents is considerably reduced. The so-called killed viruses have been employed with success in many diseases, and the most satisfactory methods of inactivating viruses have been by the addition of formalin or

phenol. Viruses inactivated by heat have proved very unsatisfactory. Rabies virus inactivated by phenol or ether is now being used on a large scale, and it is claimed that the phenolized vaccine is safer and more reliable than the living attenuated virus (Stuart and Krikorian, 1929). In other human diseases the application of this type of vaccine is still in the experimental stages. Hindle (1929) showed that the inoculation of the yellow fever virus, inactivated by formalin or phenol, produced a high degree of immunity in monkeys. The application of this vaccine to prophylaxis in man has not, however, been a striking success, and it has been replaced by the injection of immune serum-virus mixtures. Inactivated vaccines are now being used on a more extensive scale for the immunization of animals against several diseases ; distemper, African horse sickness, rinderpest, fowl-pox and foot and mouth disease. In the case of distemper the virus inactivated by photosensitized methylene blue is said to be highly antigenic.

One important question arises. Is the virus in the inactivated vaccines really killed ? The inoculation of large doses of the inactivated virus into a susceptible animal has invariably failed to produce infection. This is possibly the severest test possible, and suggests that the virus has been killed. Kligler (1931), however, has demonstrated by cataphoresis living virus in a phenolized fowl-pox vaccine that the active virus was not entirely destroyed but was only considerably reduced. It may be possible that by such means living virus may be demonstrated in

other vaccines, but the indications are that in many inactivated vaccines the virus has been actually killed.

Passive Immunity. Passive immunity, as in the case of active immunity, may also arise naturally or be induced artificially. The natural form is dependent on the passage of immune bodies from the mother to the child during intrauterine life or later by means of the milk. This form of increased resistance is not usually of very long duration but may persist for several months. By means of the neutralization test with the poliomyelitis virus and the serum of twelve mothers and their newly-born infants, Aycock and Kramer (1930) found that immunity was present in ten out of the twelve cases with a complete correspondence between mother and infant.

Artificially induced passive immunity is carried out by the introduction from an outside source of serum containing immune bodies against the particular virus causing the disease concerned. Serum containing abundant immune bodies can usually be obtained from individuals, who have recently recovered from the particular diseases. This convalescent serum has been applied with success in several human virus diseases both as a prophylactic and a therapeutic measure. The therapeutic application of convalescent serum in poliomyelitis has been used for some time on a comparatively large scale, but the results have been rather disappointing. In view of the demonstration of immune bodies in the serum of many normal individuals, such serum

has been also employed in a few instances. As a prophylactic measure, Flexner and Stewart (1928) recommended that, in the event of an epidemic of poliomyelitis, convalescent serum should be injected intramuscularly into all contacts. Immune horse serum is now available for this purpose. The immunity so obtained is only of short duration, and consequently the inoculation should, if necessary, be repeated at the end of four or five weeks. In measles the use of convalescent serum to immunize contact children was first tried by Nicolle and Conseil (1918). This serum, as well as the serum of healthy adults, who have had a definite attack of measles, is now used extensively to produce both a passive immunity and a modified attack, and is said to yield very good results (L.C.C. report, 1933). The ætiology of acute encephalomyelitis following the routine process of vaccination is not known. However, the treatment of these cases with the serum of recently vaccinated individuals has proved very successful (Hekman, 1930). While the use of this serum is advocated, it is uncertain whether it acts in a specific or non-specific manner.

Before the collection of blood from convalescents or apparently normal adults, it is necessary, in all cases, to carry out rigorous tests to exclude the presence of syphilis, tuberculosis, malaria or any acute infectious disease.

Convalescent serum has thus been applied rather widely in the treatment or prevention of human virus diseases. The procedure has many disadvantages; the serum is frequently difficult to obtain,

and when this is overcome, the quantity that can be procured is often very small. In order to obviate these disadvantages many attempts have been made to produce antisera in animals, but not altogether with success. The serum of large monkeys hyperimmunized with the poliomyelitis virus has proved useful, but here again the quantity available is very limited (Pettit, 1929). It has recently been proved conclusively that it is possible to produce an antiserum in animals which are naturally insusceptible to infection with the virus. Fairbrother and Morgan (1930), by the hyperimmunization of the horse with poliomyelitis virus, obtained a serum comparable in specific antiviral activity with human convalescent serum. It was also found that horses differed considerably in their response to the virus inoculations. This step is of particular interest in that, if this procedure can be carried out with other viruses, the application of serum therapy in virus diseases will be enormously increased.

In the administration of serum for therapeutic purposes, it should be remembered that when viruses become established in the body cells they are very difficult to reach. Consequently serum therapy commenced late in the course of certain diseases is of limited value. For example, the preparalytic stage, when nerve cell involvement is probably not extensive, is without doubt the best time to administer serum in poliomyelitis. The vaccinia virus has been demonstrated in the leucocytes of the blood at the same time that immune bodies are present in the serum, for a period of several days. This

suggests that the virus is protected from the action of the immune bodies by its intracellular position (Smith, 1929). Andrewes (1929) also found that, if the vaccinia virus were inoculated intradermally in rabbits a short time before the immune serum, complete neutralization of the virus did not take place. This indicated a rapid penetration of the cells by the virus with subsequent protection from the action of the serum.

The persistence of certain viruses in the body tissues after recovery from the disease, when antiviral bodies are present in the serum, has been observed. Osgood and Lucas (1911) demonstrated the poliomyelitis virus in the nasopharynx of a monkey five months after recovery from the experimental condition ; Olitsky, Rhoads and Long (1929), by cataphoresis, obtained the poliomyelitis virus from the central nervous system of a monkey twenty-three days after infection. The simultaneous presence of antibodies and the vaccinia virus in the tissues of rabbits after recovery from infection has been repeatedly observed (Douglas, Smith and Price, 1929). Such an occurrence has also been noted in several virus diseases of animals. Consequently it has suggested that immunity in a virus disease may be linked with the persistence in the body of living virus (Olitsky and Long, 1929). These conclusions have, however, not been confirmed by many independent workers, and this must at present remain an open question.

Immunological Reactions. It has been seen that the immunity responses in virus diseases bear a close

relationship with those associated with bacterial diseases, in that recovery from many virus diseases or immunization with certain virus suspensions gives rise to a condition of increased resistance. Moreover, this state of immunity is frequently associated with the presence of specific immune bodies in the serum.

The presence of immunity may be tested either actively by noting the resistance to inoculation with an infective dose of virus or passively by examination of the serum for antiviral bodies The former method is the severer test, but, with a few exceptions, it is unfortunately limited in its general application to laboratory animals. Such an examination in man with either the poliomyelitis virus or the virus of yellow fever is obviously impossible. Consequently the method almost universally adopted in the study of immunity in virus diseases is the examination of the serum for immune bodies. Many investigations have been performed in this direction, and the most reliable results have been obtained by the use of the neutralization test. Flocculation, agglutination and complement fixation tests have been applied with a varying degree of success. The individual tests merit special attention.

The Neutralization Test. This test has been applied in many virus diseases, and in all instances the same principle appears to be involved. The serum, either undiluted or diluted in a graduated scale, is mixed *in vitro* with the active virus, which may be used either as a filtrate or suspension ; after remaining in contact for varying lengths of time, the

mixture is inoculated in the necessary manner into a susceptible animal. If infection does not take place or occurs after a marked increase in the incubation period, the serum is said to contain immune, viricidal or antiviral bodies. A control test, in which saline is substituted for the serum, should always be made simultaneously.

The *modus operandi* of this reaction is uncertain. In all instances a marked degree of specificity is exhibited. There is much evidence to indicate that the virus is not destroyed by the serum during contact *in vitro*. Andrewes (1928) found that, even after contact for some hours, the vaccinia virus could be recovered in an active state from an otherwise neutral mixture either by simple dilution or by absorption with kaolin or euglobulin. Olitsky, Rhoads and Long (1929) were able to recover, by cataphoresis, active poliomyelitis virus from a serum-virus mixture, which was itself non-infective on intracerebral inoculation of monkeys. There is, however, evidence indicating that an immune serum does form some kind of union with its corresponding virus. Leiner and v. Weisner (1910), working with the poliomyelitis virus, found that contact *in vitro* for several hours was necessary to ensure complete neutralization of the virus by the immune serum. Bedson (1928) showed that with the herpes virus contact *in vitro* was an important factor in obtaining complete neutralization of the virus. In the case of the vaccinia virus the need for contact *in vitro* is not essential ; immediate intradermal inoculation of the immune serum-virus mixture has proved as effective

as inoculation of the mixture after prolonged contact in the test-tube (Andrewes, 1928). Evidence that the vaccinia virus and its immune serum do unite has been obtained by means of cross-absorption tests with the herpes virus (Smith, 1930). Andrewes (1930) also found that, after prolonged contact *in vitro*, it became increasingly difficult to obtain active virus from the immune-serum-virus mixture. It would thus appear that the union between a virus and its corresponding immune serum varies in degree with the different viruses. In some instances the union is unstable and easily broken down. There seems little doubt, however, that some form of union does take place. The exact nature of this union is obscure. It has been seen that in certain cases the virus is not destroyed by contact *in vitro* with the serum, and it is probable that the serum may sensitize the virus, with the result that it is more easily attacked by the body cells. The phenomenon would thus be analogous to the phagocytosis encountered in bacterial reactions. Evidence, suggesting that this mode of reaction takes place in the neutralization tests with the vaccinia virus, has been put forward by Douglas and Smith (1930). These workers found that the leucocytes attacked the vaccinia virus with increased avidity in the presence of immune serum. Fairbrother (1933) also found that the action on the vaccinia virus of immune serum was more marked in the presence of leucocytes and that neutralization of the virus was more easily demonstrated by intradermal than by intracerebral inoculation. A similar state of affairs is

probable so far as the poliomyelitis virus is concerned. Fairbrother and Morgan (1930) found that, all other things being equal, the condition of the monkey employed for the inoculation had an important bearing on the results of the neutralization tests. Thus, as in bacterial immunity, both " humoral " and " cellular " factors are involved in the resistance of the body to infection.

Many workers have stated that complement is not concerned with this reaction. Serum inactivated at 56° C. has been shown to be as potent as the unheated serum in a number of instances: vaccinia, rabies, poliomyelitis, herpes. The possibility of the complement of the inoculated animal taking part in the reaction must not be ignored. Gordon (1925), in a small experiment with the vaccinia virus obtained evidence in one instance that the addition of complement to inactivated immune rabbit serum increased its antiviral activity. This point has not received very close attention and the participation of a thermolabile factor, in a minor *rôle*, may easily have escaped notice.

Further indications that the immunity reactions produced by viruses are closely allied to those occasioned by bacteria are given by the distribution of the immune body in the serum. By means of serum fractionation experiments it has been found that the various antibacterial bodies are largely associated with the euglobulin fraction. Work of this nature with the viruses has been very limited. The results available indicate that the antiviral bodies are also present mainly in the euglobulin

fraction of the serum ; in rinderpest (Hartley, 1914), in poliomyelitis (Morgan and Fairbrother, 1930), in vaccinia (Findlay, 1931), and in distemper (Laidlaw and Dunkin, 1931).

Flocculation and Complement-fixation Tests. The application of the neutralization test is unfortunately very limited. Animals are essential for this test, and in poliomyelitis the only suitable animal is the monkey ; in such a case it is natural that investigations can only be performed on a very restricted scale. The need for a purely *in vitro* test is obvious, and, while not universally accepted, the application of the flocculation and complement-fixation reactions has in certain instances proved very useful.

The Flocculation Test. This test has been applied chiefly to the studies of the viruses of variola and vaccinia. Nothing of a definite nature was accomplished until the work of Gordon (1925). Gordon, in the course of a detailed investigation of the viruses of vaccinia and variola, employed the flocculation test with success. He prepared emulsions from vaccinial lesions in rabbits, from scabs of variola and alastrim cases, using material obtained from other sources to serve as controls. By the addition of the serum of rabbits hyperimmunized with the vaccinia virus, he obtained positive results, *i.e.*, flocculation, only with the emulsions of vaccinial and variolar lesions. These experiments were only carried out on a small scale. Later Burgess, Craigie and Tulloch (1929) employed the same technique as part of an investigation into an outbreak of small-pox

in Dundee. Altogether ninety-three cases were examined, of which fifty-three were cases of small-pox; the other cases included chicken-pox and other skin conditions. In all these emulsions were prepared from the various skin lesions and tested against the serum of rabbits hyperimmunized with the vaccinia virus. Of the fifty-three cases of small-pox there were only three results in which the interpretation was difficult. The control experiments with the exception of the vaccinial lesions, which were strongly positive, gave completely negative results. These observations were later confirmed and amplified (Craigie and Tulloch, 1932).

The nature of this reaction is uncertain. Schultz and his co-workers (1928) made an elaborate investigation of the antigenic activity of viruses. In the case of vaccinia, rabies and herpes, they prepared antigens from neurotropic strains, as by this means it was possible to obtain the antigens in a sterile condition. The results of the flocculation tests performed with these viruses were entirely negative; any positive results were shown to be due to non-specific reactions. In all instances the virus emulsions contained an abundance of the virus.

Examining the flocculation reaction given by vaccinia virus suspensions in the presence of immune serum, Craigie (1932) found that two distinct reactions occurred :—

(1) A precipitin reaction, in which a filterable portion containing a specific product of the elementary bodies participated.

(2) An agglutination reaction, in which the elementary bodies were specifically clumped.

Smith (1932), investigating this filterable substance, found it bore a close similarity to bacterial haptenes ; it had the capacity to give specific precipitation *in vitro*, but was unable to stimulate antibody production *in vivo*.

Complement-fixation Test. A similar controversial state of affairs is found in this case. Many workers have obtained positive results and, while there appears to be no doubt of the specificity of the reaction, conclusive proof that the virus is itself solely responsible for the reaction has not yet been obtained. Netter and Urbain (1924) used the scabs of zoster and varicella as antigens and obtained positive results in both cases with the serum of zoster patients ; impetigo and vaccinial scabs as antigens yielded negative results. Gordon (1925), employing diluted calf-lymph and vaccinial rabbit pulp as antigens, obtained fixation of complement with the serum of rabbits hyperimmunized with the vaccinia virus. Bedson and Bland (1929) considered that, as the interaction between the herpes virus and its corresponding immune serum in the neutralisation test took place slowly, the fixation of complement might require a longer time than was usually allowed in these tests. Consequently, using immune guinea-pig serum, they allowed prolonged fixation, either eighteen to twenty hours at 8°–10° C. or two hours at room temperature, and obtained positive results with guinea-pig herpes lesions as antigens ; normal and vaccinial pads, as well as cultures of

contaminating organisms, as antigens gave negative results. Ciuca (1929), working with the virus of foot and mouth disease, prepared a watery antigen from the septic maceration of infected guinea-pig pads. By the use of this he obtained positive results only with the serum prepared from the homologous strain of the virus. Gilmore (1931), using the serum of rabbits hyperimmunized with the vaccinia virus obtained from a culture-medium, against such antigens as calf-lymph and rabbit vaccinial pulp, also obtained positive results.

It is thus seen that positive results have been obtained in many instances, and that in all cases a definite disease-specificity has been noticed.

In the case of the neurotropic viruses, where the virus can be obtained free from bacterial contaminants and the products of bacterial activity, the results have been very disappointing. Kraus and Takaki (1926) immunized rabbits with the rabies virus and with the resultant serum obtained positive results against an antigen from the brains of cases of rabies, but not encephalitis, herpes or vaccinia. The antigens in these experiments were brain emulsions cooked in a water-bath at 100° C. and allowed to stand overnight in the cold room ; after this they were centrifugalized and the supernatant fluid collected. This type of antigen was termed a " Koctoantigen." Schultz and his collaborators, as in the case of the flocculation test, tried complement-fixation tests with neurotropic strains of the herpes, vaccinia and rabies viruses. In no instance were they able to elicit specific fixation of complement ;

their positive results were shown to be due to the presence of non-specific nervous tissue, which could be removed by extraction with ether without interference with the activity of the virus. Experiments with the "Koctoantigen" of Kraus and Takaki also yielded entirely negative results. Tang and Castaneda (1929) were unable to obtain any evidence that specific complement-fixing antibodies existed in the serum of rabbits immunized against the viruses of herpes and rabies. Stuart and Krikorian (1929) found no specific fixation of complement using a fixed rabies virus brain as antigen with the homologous immune serum. Bedson and Bland (*loc. cit.*), using prolonged fixation methods, could not obtain satisfactory results with rabbit strains of neurovaccine against the corresponding immune serum. Tests carried out by the author with the poliomyelitis virus also proved to be unsatisfactory.

Thus it is seen that in the case of the neurotropic viruses the results have been very unsatisfactory. Schultz and his colleagues considered these negative results indicated that the results, obtained by the use of dermal lesions as antigens, were non-specific and probably due to bacterial contamination. The results of Bedson and Bland, and Gilmore (cited above), however, contra-indicate this, as also do the controlled flocculation tests performed by Burgess, Craigie and Tulloch (*loc. cit.*). Also this does not supply an explanation for the marked disease-specificity exhibited by the various dermal viruses, especially the results obtained by Ciuca with the various types of the foot and mouth virus. Here

the specificity was so marked that it was possible to differentiate between the three different types of the virus.

A possible explanation of these divergent results may be that while the virus participates in the reaction it is not, however, the sole responsible antigenic factor. If this were so there seems no explanation to account for the negative results obtained with the neurotropic viruses. These antigens contain an abundant supply of the virus, and, moreover, the virus can be obtained with less extraneous material than in the case of the dermal viruses. Thus it is probable that an antigen containing the virus plus some other factor is necessary and that this factor is present in dermal but not in neurotropic lesions. The nature of this other factor and the *rôle* it plays are obscure—it is possible that it may be an adsorption phenomenon. Whether this hypothesis is correct or not can only be decided by further research.

There is another point worthy of mention. The amount of complement specifically absorbed in the different reactions varies considerably with the individual viruses, but in many cases this amount is small. Thus, in some cases, the negative results might be due to the use of too large a dose of complement, in which case the occurrence of a weak fixation would be obscured.

Much remains to be accomplished in this field of research before these tests can be generally applied, but there is no doubt that a decided advance has been made.

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CHAPTER V

CLASSIFICATION

As our knowledge of filterable viruses is at present incomplete in many respects, attempts at serious classification are inadvisable; a new fundamental observation might entirely revolutionize modern conceptions of the subject. The term "Filterable Virus" itself is far from satisfactory; an alternative, except perhaps "Virus" alone, does not, however, readily suggest itself.

It is well recognized that filterable viruses exhibit at least as much variation as bacteria in their pathogenicity, and in the present state of knowledge classification on such a basis cannot be attempted. Although the process of filtration has been employed as a criterion for the general terminology of this group, it is totally inadequate for classification. The process of filtration is itself very complicated, being subject to the influence of many variable factors, and while all viruses are not filterable, certain definite bacteria, spirochætes and protozoa possess this property. Thus, if this group of diseases were to be classified by the filterability of the causative agents, many definite bacterial or protozoal diseases would justify inclusion, while certain accepted virus diseases would not.

Levaditi (1922), as a result of observations of the main site of virus activity, concluded that viruses had an affinity for particular tissues. Certain diseases, herpes zoster, vaccinia, rabies, poliomyelitis, encephalitis, were consequently classified as "Ectodermoses Neurotropes." He considered that the incitant of these diseases had a special selective affinity for tissue of ectodermal origin—the central nervous system and the skin. This view has not, however, been generally accepted; Ledingham (1924) found that the reticulo-endothelial system, tissue of mesodermal origin, was primarily involved in dermal vaccinial lesions; Hurst and Fairbrother (1930) demonstrated that in vaccinial encephalitis of the monkey and rabbit the lesions were present in tissue of mesodermal origin, and that the nerve cells were practically uninvolved. The viruses of poliomyelitis and encephalitis, moreover, have never been shown to exert the slightest action on the skin tissue; their activity is almost entirely confined to the central nervous system. There does not therefore seem much justification for such a method of classification. The terms, "neurotropic, dermotropic and viscerotropic viruses," are, however, frequently employed in order to indicate the tissue mainly attacked by the virus under discussion.

Wolbach (1912), reviewing the subject, grouped the diseases according to the host involved, and Rivers (1928) arranged a table on similar lines but stressed the fact that serious classification was not attempted; the arrangement was only included for

convenience of discussion. McKinley (1929) also considered that the most useful method of classification was on the basis of host susceptibility. As a working basis he adopted four groups, arranged according to the evidence upon which a disease was said to be caused by a filterable virus. The only accepted method of classification thus appears to be on the basis of host susceptibility. The subgrouping presents more difficulties, but rather than describe each individual disease as an isolated entity, it seems preferable to present some arbitrary form of subdivision. As certain human diseases, as poliomyelitis, yellow fever, etc., have been proved conclusively to be caused by a filterable virus, while the nature of the ætiological agents of others, as influenza, encephalitis and trachoma, is still "sub judice," a general consideration of these together would be merely confusing and of little material value. The most rational procedure therefore appears to be one similar to that adopted by McKinley, viz., grouping according to the evidence upon which a filterable virus is said to cause the disease. Accordingly three groups have been arranged :—

(1) Those diseases considered to be caused by a filterable virus.

(2) Those diseases probably caused by a filterable virus.

(3) Those diseases possibly caused by a filterable virus.

The diseases of man included in the different groups are given in Table I.

TABLE I

Group 1.	Group 2.	Group 3.
Poliomyelitis.	Measles.	Trachoma.
Rabies.	German measles.	Acute Encephalomyelitis.
Japanese Encephalitis.	Mumps.	(Whooping Cough.)
Rabies Encephalitis (Trinidad).	Dengue fever.	
Yellow Fever.	Pappataci fever.	
Variola.	Epidemic Encephalitis.	
Alastrim.	Australian X disease.	
Vaccinia.	Herpes Zoster.	
Other animal pox diseases.	Varicella.	
Herpes.	Influenza.	
Psittacosis.	Acute coryza.	
Warts.		
Molluscum contagiosum.		
Foot and mouth disease.		
Rift Valley fever.		

While it is proposed to discuss these three groups separately, it must be emphasized that there is no hard and fast line of demarcation between them. The various diseases have been arranged in the different categories as a result of a review of the evidence, offered up to the present time, of the nature and transmissibility of their causative agents. In certain instances very discordant views have been formulated, and therefore a definite grouping cannot readily be given. Thus the difficulties of classification must be fully realized and, while only tentative and open to criticism, the above scheme of sub-

division is offered in order to render the description of the various diseases less confusing and disjointed.

Proof of the *rôle* played by filterable viruses in the causation of disease is not easy to obtain. This fact can readily be understood when it is recognized that the presence of characteristic lesions in the various tissues is the only visible manifestation of their activity; the viruses themselves cannot be seen except probably in a few instances, when special technical methods are required for their demonstration. In some instances the failure to detect bacteria in the disease process has afforded *primâ facie* evidence in favour of its causation by a virus. The sole method available of establishing the part played by these agents is the passage of the particular disease to susceptible animals in series. In some instances, as in zoster, varicella, measles, suitable laboratory animals are not available and transmission experiments have been performed on humans. Such a practice has obvious limitations, and the evidence obtained in these cases is rather scanty. In view of difficulty of demonstrating the presence of pathogenic viruses, the impossibility of determining the existence of saprophytic viruses, if such are present, is obvious.

The passage of the disease in series is important, as passage through one generation only might equally be the result of "toxin" activity; subsequent successful passage tends to rule out this objection, as, if a toxin were implicated, the excessive dilution of the original material after several passages would tend to render it ineffective. Thus,

in order to demonstrate the presence of a virus, it is necessary to possess an animal susceptible to the activity of the virus, and in this respect the different viruses exhibit marked variation. The difficulties so encountered in the study of the pathogenic bacteria are accentuated in dealing with viruses. In a number of instances many susceptible animals are available, but in certain other diseases of suspected virus origin laboratory animals are of little value.

The selection of the experimental animals is a procedure demanding much precaution. Rabbits are very prone to spontaneous encephalitis, which is caused by infection with the *encephalitozoon cuniculi;* the use of these animals in the study of the neurotropic viruses may consequently be unreliable and the most rigorous control of all such experiments is essential. The possibility of the presence of virus III. disease must also be considered whenever rabbits are employed. In mice a spontaneous filterable virus disease, ectromelia, is not infrequently present. Monkeys are very susceptible to tuberculosis, and in these animals dysentery is not infrequently activated by the presence of another infection. Secondary infections of this nature are liable to give rise to irregular results. The use of healthy animals for transmission experiments thus constitutes an important factor in the study of virus disease.

Goodpasture (1933) has suggested that filterable viruses should be divided into two sub-groups: (1) filterable viruses, and (2) cytotropic viruses. The

second group differs from the first in that it contains viruses which exhibit a close relationship to single types of cells. He considers that these viruses reproduce exclusively in the interior of these cells. This group is probably closely associated with the Rickettsiæ and the intracellular bacteria, as *B. leprœ*, and also with the hypothetical viral carcinogenic agents. This subdivision, while worthy of consideration, embraces such a wide field that it cannot be recommended for general application.

The Rickettsiæ and the bacteriophage, because of their close relationship with viruses, will be briefly considered at this stage.

The Rickettsiæ. Rickettsia is the name given to minute Gram-negative weakly staining bacterium-like bodies found in the alimentary canal of insects and also in association with certain diseases in man. The name Rickettsia was proposed in honour of Ricketts, who died of typhus fever while investigating that disease. The human diseases caused by Rickettsiæ are typhus fever, trench fever, Rocky Mountain spotted fever, and the Japanese disease, Tsutsugamushi fever.

The classification of these bodies is very uncertain, but while they are frequently included in reviews of filterable viruses, there does not appear much justification for this unqualified inclusion. The bodies are small, being $0{\cdot}3$–$0{\cdot}5\mu \times 0{\cdot}3\mu$ approximately ; they are visible microscopically and do not traverse the ordinary bacterial filters. The pathogenic species, with the probable exception of *R. nipponica*, which is found in Tsutsugamushi fever,

have not been cultivated in the absence of living cells ; tissue cultures have, however, proved successful in Rocky Mountain and typhus fevers. This fact may serve to associate the Rickettsiæ with the filterable viruses.

Many discordant views have been expressed on the nature of these bodies : (1) Woodcock (1921) considered that they were inanimate particles resulting from the disintegration of red blood corpuscles by hæmotophagy. (2) In view of the agglutination of the proteus X_{19} by the serum of typhus patients, it has been suggested that the *R. prowazeki* represented a phase of the proteus in the louse (Felix, 1930). (3) The majority of workers consider that the Rickettsiæ are definite self-supporting organisms capable of giving rise to diseases in man and animals (Cowdry, 1923). This last view appears the most satisfactory from the evidence available, but, in order to avoid confusion, it appears advisable to regard the Rickettsiæ in a group distinct from filterable viruses until the information about these two disease agents is more definite. The Rickettsiæ must not, however, be entirely ignored, as it is possible that they may represent some intermediary stage of biological development between the bacteria and the filterable viruses.

Bacteriophage. In 1915 Twort observed peculiar changes in the colonies of organisms grown on solid media from vaccine lymph. These after twenty-four hours became glassy and transparent, and could not be transplanted successfully. On addition of this

glassy substance to a fresh young culture of the same organism similar changes were produced.

D'Herelle in 1917 obtained changes of a somewhat similar nature in cultures grown in a fluid medium. He found that when fæces from Shiga dysentery cases were emulsified in broth, incubated for eighteen hours at 37° C., and filtered, the addition of a few drops of this filtrate to young broth cultures of Shiga caused growth to be arrested and the occurrence of lysis. This phenomenon was carried on indefinitely by filtering the lysed culture and adding the filtrate to further young cultures. D'Herelle considered this reaction to be caused by a filterable virus, which he called "Bacteriophagum Intestinale." These observations have since been repeatedly confirmed, although the virus theory has not been universally accepted.

Bacteriophage, or phage as it is frequently termed, can be obtained from fæces, sewage, water, soil and sometimes pus by filtration, and while its action is not entirely specific, particular strains are usually more active against certain organisms than others. Phages acting to a marked degree, even in a dilution of $1/10^6$, on a number of organisms, especially those of the coli-dysentery-typhoid group, have been isolated.

The characteristic properties of a phage are the power of clearing young living broth cultures, either temporarily or permanently, and the formation of clear areas, plaques, in young cultures on solid media. It has no action on killed bacteria. While the addition of an active phage to a young culture

sometimes gives rise to complete lysis, unless an extremely active phage is employed, the lysis is usually incomplete ; all the bacteria are not lysed, and on subculture a number of resistant strains, termed secondary cultures, is obtained. The exact nature of these resistant organisms is not known, although they tend to resemble in many ways " R," or rough, variants of the original bacterium.

By means of filtration tests through gradocol membranes the particulate nature of phages has been conclusively proved ; moreover, it has been found that the various phages vary considerably in size, from 0·008–0·075μ (Elford and Andrewes, 1932). It has also been demonstrated that viruses are antigenic and a number of antiphage sera have been produced in rabbits. While there is no doubt that considerable progress has been made in the knowledge of the bacteriophage, its precise nature is still unsettled. Many workers agree with D'Herelle and consider a phage to be an autonomous living agent comparable with a filterable virus. However, while D'Herelle considers that there is only one phage, which exhibits an almost infinite capacity for modification and variation, other workers are of the opinion that there are many separate distinct phages. Important evidence supporting the latter contention has recently been obtained by means of serological tests and by examination of the physico-chemical properties. Burnet (1933), as a result of an extensive and prolonged investigation of the behaviour of bacteriophage, states " these investigations point to a multiplicity of phages rather than to the single

plastic micro-organism envisaged by D'Herelle, and both are inexplicable on any theory which makes bacteriophage autolytic ferments or other bacterial products." The other views of the nature of the bacteriophage are (1) the autolytic ferment theory, and (2) the theory that a phage is a product of the bacterium which is set free by autolysis; this is the one strongly advocated by Bordet (1925).

Bacteriophage has been advocated for the treatment of intestinal, urinary, skin and other diseases, but the results have not been very satisfactory. While there is no doubt about the activity of the bacteriophage *in vitro*, this action appears to be considerably influenced by the body cells and secretions.

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CHAPTER VI

DISEASES CONSIDERED TO BE CAUSED BY FILTERABLE VIRUSES

POLIOMYELITIS

ACUTE anterior poliomyelitis, or infantile paralysis, is an acute infectious disease involving the nerve cells of the spinal cord, medulla, and, to a much less extent, those of the brain. This condition was definitely established as a clinical entity by Heine in 1840, and, as the first careful epidemiological study was made by Medin in 1890, the disease was subsequently known as the Heine-Medin disease. Poliomyelitis was first transmitted experimentally by Landsteiner and Popper in 1908, who reproduced the disease in a monkey by intraperitoneal inoculation of the spinal cord of an infant dying of the disease.

The Virus. There seems no doubt that the causative agent of poliomyelitis is a filterable virus. The claims of the globoid bodies and the streptococcus as ætiological agents have never been substantiated. The virus is ultra-microscopic, passes readily through the ordinary bacterial filters, and has not been cultivated with certainty by the usual technical procedures. Gildemeister (1933) has recently reported the successful cultivation of the virus through eighteen subcultures; the medium

employed contained monkey's serum with chick-embryo brain ; no obvious signs of growth could be detected on visual examination. This work, if confirmed, is an interesting advance.

The study of the virus is obviously a matter of some complexity ; the only means available at present for its demonstration is by animal inoculation experiments. Here again difficulties are encountered, as the only animal susceptible to experimental infection is the monkey ; the usual laboratory animals have proved quite refractory. In order to produce the disease in monkeys it is necessary to introduce the virus as closely as possible to the central nervous system ; intracerebral, intraneural, intrathecal and intranasal inoculations, in order of reliability, are the methods giving the most satisfactory results ; the intracerebral route is the one usually employed for experimental work. The first passage of human cord emulsion into the monkey by no means invariably leads to a reproduction of the disease—only about 50 per cent. of such transfers succeed. After the initial difficulties of adapting the virus to the monkey have been overcome, the virus is usually very virulent for these animals. An active virus produces infection in approximately 100 per cent. of monkeys, after an incubation period of four to seven days, following the intracerebral inoculation of 0·5 c.c. of a 5 per cent. suspension in saline of the virus-containing cord.

The limitations of experimental work are obvious, as the cost and upkeep of monkeys are not inconsiderable. Nevertheless, the following properties of

the virus have been established: it is extremely resistant to freezing, drying, pure glycerine and 0·5 per cent. phenol, but it is readily destroyed by heat over 45° C. and oxidizing agents. It has been found that virus added to milk and left at room temperature for thirty days retained its activity. The virus is conserved by placing the infected cord in 50 per cent. glycerine in saline and keeping at a low temperature; under such conditions the virus has been found to be active after eight years.

Infectivity. The poliomyelitis virus only produces spontaneous disease in man. While the disease can be reproduced in a fatal form in monkeys by intracerebral inoculation, spontaneous cage infection has never been reported. Infectivity in man may be conveniently considered in two phases, dealing (1) with the infection in the community, and (2) infection in the individual.

(1) **Infection in the Community.** The epidemiology of poliomyelitis presents a fascinating but complex problem. In this country the disease is always present to a limited degree, as cases are recorded weekly. The occurrence of extensive outbreaks has not been observed for many years, although small epidemics are frequently reported. In other countries the position is different; in U.S.A. the most extensive and alarming epidemic since 1916 occurred in 1931—in nineteen States there were 13,091 cases. Extensive outbreaks took place in Alsace-Lorraine in 1930 (405 cases), and in Roumania in 1927 (3,133 cases). What explanation can be offered to account for these outbreaks? The view advocated by Aycock

is worthy of close consideration. Aycock (1928), studying the disease side by side with measles, diphtheria and other exanthemata, observed many points of similarity in their epidemiology. He found that in densely populated communities the age distribution in these diseases was very similar, the majority of cases occurring during childhood with a comparative immunity of adults. In isolated districts this age distribution did not hold to the same degree, all individuals proved more or less equally susceptible. It was also observed that large outbreaks in urban communities usually occurred after a long quiescent period. By means of the neutralization test it has been found by many observers that individuals who have never suffered from the disease possess antibodies for the virus in their serum. Antibodies are also found in the serum of convalescent cases and of many infants under six months. In order to correlate the above facts with the epidemiology of the disease the following theory has been propounded. In a populous community the poliomyelitis virus is usually present and infects a large number of people, only a few of whom develop poliomyelitis. The majority undergo "subclinical infection" and obtain a high degree of immunity associated with the presence of antibodies in the serum. These individuals become healthy carriers and assist the dissemination of the disease. Mild unrecognized cases doubtless play a similar *rôle*.

The individuals developing poliomyelitis are usually children between the ages of one to twelve

years. The probable reasons for the comparative immunity of infants under twelve months are that, for a few months after birth, antibodies may be present in the serum due to maternal transplacental passage, and also the risk of exposure to infection is usually limited. Thus here the main factors are increased resistance and decreased chance of infection. In the case of individuals over twelve years the explanation is different. During the first twelve years of life the risk of infection is great, as children tend to make close contact with each other; thus the chance of subclinical infection or the production of the disease itself is great during this period. Consequently, as one advances beyond twelve years, the possibility of the presence of immunity brought about by earlier infection with the virus correspondingly increases. The majority of persons over twelve years are thus relatively insusceptible to infection with the virus. After the outbreak of a large epidemic the majority of individuals possess a marked degree of immunity; the soil is consequently not very suitable for the persistence of the virus. Thus the chance of subclinical infection occurring during the immediately succeeding years is diminished, and after a number of years a susceptible community may again be produced; on the subsequent introduction of a virulent virus another epidemic occurs in which the victims are mainly children. This is the explanation offered for the waves of epidemics occurring in certain countries. It probably also explains the more frequent appearance of the disease in rural populations, as here the

chance of contact is much less than in urban communities and the occurrence of subclinical infections with resulting immunity will be less likely. It is interesting to note that the introduction of a poliomyelitis virus into a virgin population, such as isolated islands, has led to extensive outbreaks involving persons of all ages.

The theory of specific immunization by subclinical infection is not universally accepted. A few workers (Jungeblut and Engle, 1934) consider that the resistance of adults to the disease is due to a non-specific physiological maturation of the tissues. The evidence advanced to support this theory is, however, far from conclusive.

The actual mode of spread of the poliomyelitis virus is difficult to determine. Evidence strongly suggests that the main spread is by contact, the virus being conveyed by droplet infection from the naso-pharyngeal mucosa of one person to another. The criteria on which this has been based are the analogy to many other infectious diseases and by the demonstration of the virus in naso-pharyngeal washings of a few healthy carriers and individuals suffering from the disease. These latter observations are limited, as, owing to the exigencies of experimental work, investigations have only been carried out on a limited scale. Other views of the mode of spread are that the virus may be introduced by contaminated drinking water or milk. Conclusive proof of any of these theories has not yet been obtained.

The seasonal incidence of extensive outbreaks also

remains unexplained. Sporadic cases are reported throughout the year, but epidemics of any proportion are almost invariably encountered during the late summer and autumn months. It has been suggested by Aycock that the individual reaction of the host undergoes some change during this period whereby the resistance offered to the virus is decreased. This change may be of a local nature and consist of an altered permeability of the nasopharyngeal mucosa, or it may be a purely general phenomenon.

(2) **Infection in the Individual.** It has been already pointed out that individuals respond differently to infection with the poliomyelitis virus. In the majority of instances the result of infection with the virus is probably the production of a subclinical infection with resultant immunity. These cases represent the " healthy " carrier. In others there is a manifest reaction to the virus with the appearance of definite symptoms. These cases, found most commonly in children, have been classified according to the nature of the clinical findings, which have been recently discussed in detail by Collier (1927). It is sufficient to mention here the commonest types encountered, viz., (*a*) the spinal form, (*b*) the bulbar form, and (*c*) the abortive form. In the last group are cases showing pyrexia with vague nervous signs, as headache, and presenting typical changes in the cerebrospinal fluid; these recover without any obvious muscular involvement. Further types have been described, but these are comparatively rare and do not

present such well-defined characters as those given above.

The reason for the individual variation in response is not obvious. Two variable factors are concerned : (*a*) the virulence and dosage of the invading organism, *i.e.*, the infection pressure ; and (*b*) the resistance of the individual.

(*a*) It is generally accepted that the greater the dose and activity of an invading organism, the greater is the chance of production of disease. This is doubtless true for the poliomyelitis virus. In many cases the dose may be too small to give rise to the definite disease, whereas a massive dose of an active virus might produce it. There is, however, no means available to determine the strength of the infecting dose of virus during the course of an epidemic, but this is probably an important factor in the production of the disease. The presence of different strains of the virus has been postulated. This question has not been fully investigated ; it is possible that the apparent differences observed in some strains may be simply a question of a different virulence for the monkey.

(*b*) The resistance offered by the body presents a problem of the greatest difficulty. There is no generally accepted opinion of the site of invasion by the virus. The view which received most support, both experimental and epidemiological, is that the virus first invades the tissues of the nasopharynx. The route afterwards taken by the virus on its way to the spinal cord has recently received much attention ; the researches of Fairbrother and Hurst

(1930) indicate that the virus passes to the olfactory bulbs by the axons of the olfactory nerves, and thence to the cord by axonal channels mainly *viâ* the pyramidal tract. The virus probably gains access to the axons of the nerve by the olfactory hairs, which lie exposed in the nasal mucosa. Throughout its progress through the brain, mid-brain and cord, the virus may attack the local nerve cells, particularly the motor nerve cells, but in the majority of cases, human and experimental, the cells most frequently attacked are those in the anterior horn of the lumbar and cervical cord enlargements. The limitation of lesions to the bulb or mid-brain in some cases cannot be satisfactorily explained at present. The primary lesions are found in the nerve cells and not in the interstitial tissue. Evidence supporting this hypothesis of axonal spread has since been produced by Hurst (1932) and Faber and Gebhardt (1933). No evidence has been obtained to support the contention that the virus is spread through the central nervous system by means of either the cerebrospinal fluid or the blood stream, as the virus has never been found in these situations in samples removed from human cases at different stages of the disease.

The clinical picture may be very varied. After an incubation period of two to five days there is frequently a prodromal or preparalytic stage characterized by high temperature, headache, vomiting and malaise with definite changes in the cerebrospinal fluid, viz., a pleocytosis, first polymorphonuclear and then mononuclear, slight increase in

globulin with no alteration in the sugar and chloride content. This stage is followed by recovery in the abortive cases or by involvement of various muscle groups in the other forms. When paralysis follows, the disease usually remains localized, and clears up with a varying degree of permanent damage; the mortality rate varies in the different outbreaks, but it is usually about 10 per cent. and is frequently due to involvement of the respiratory and cardiac centres. In these cases the virus can frequently be demonstrated by animal inoculation in the mid-brain, spinal cord, especially the lumbar and cervical enlargements, and the nasopharyngeal mucosa. The characteristic lesions are found in the grey matter of the central nervous system. These are widespread, and consist mainly of degeneration, vacuolation and neuronophagia of the nerve cells with marked perivascular and meningeal infiltration. Intra-nuclear inclusion bodies have been demonstrated in the nerve cells before degeneration is appreciable. The histological picture of the experimental disease has been studied in detail by Hurst (1929).

The probable course of the disease is, during the incubation period the virus passes from the naso-pharynx, or other site of entry, to the nerve cells of the affected region; the preparalytic or prodromal stage represents the early reactive processes of the body, particularly of the nerve cells, to invasion by the virus. In the experimental disease the virus has been demonstrated, after intracerebral inoculation, in various regions of the cord during the late prodromal phases of the disease; histological

examination also at this stage showed that in these same regions the nerve cells of the anterior horn were already involved. The struggle for supremacy between the virus and the nerve cells may end in victory either for the virus—in which case muscular weakness and (or) paralysis occur—or for the nerve-cells when recovery takes place without muscular involvement, constituting the abortive case.

Immunity. Recovery from the disease is followed by a high degree of immunity, second attacks are extremely rare; this state is associated with the presence of antiviral bodies in the serum. These bodies are also present in the serum of many adults, who have displayed no previous manifestation of the disease. Antibodies have also been produced in animals not susceptible to the disease, horses (Fairbrother and Morgan, 1930), sheep and goats (Howitt, 1932).

The nature of the antibodies is not definitely known, specific agglutination and complement-fixation tests have not been successful; the usual and most satisfactory method of demonstrating these bodies is by means of the neutralization test. In performing this test equal parts of the serum to be examined, diluted or undiluted, and a 5 per cent. suspension or filtrate of an active cord are mixed, left one to two hours at 37° C. and overnight at 0° C., when 0·5 c.c., after mixing, is inoculated intra-cerebrally into a monkey. To serve as a control, saline is substituted for the serum. The monkeys are examined daily to determine the onset of weakness and paralysis in either or both.

Specific prophylaxis has been attempted experimentally, but the results have not been altogether satisfactory. It may consequently be stated that active immunization is at present of no value in the prevention of poliomyelitis. It has been found, by experimental work on monkeys, that immunity can only be produced with any degree of certainty by the inoculation of living active virus ; killed virus gives little, if any, response. The introduction of living virus in man would be beset with danger, as there is no method available at present of assessing the dose necessary to produce immunity without giving rise to symptoms.

Serum Therapy. The use of various antiviral sera in the treatment of the disease has been investigated by various workers with divergent results. Many workers, including Aycock and Luther (1928) and MacNamara and Morgan (1932), have claimed marked success by the inoculation of human convalescent serum in the preparalytic stages ; Park (1932), however, recently obtained results which indicated that serum therapy was of little value. In a group of more than 1,000 cases, diagnosed in the preparalytic stages, of which approximately half were treated with convalescent serum, no indication of the value of the serum, either in reducing case fatality or preventing paralysis, was found. Further well-controlled investigations are obviously required before a final assessment can be made. The main difficulties in evaluating the results of serum therapy are the necessity of diagnosing the cases in the preparalytic stages and also the not infrequent

occurrence of complete spontaneous recovery of cases, in which slight but definite muscular involvement has been observed.

In the administration of convalescent serum the usual procedure has been to give a large dose, 25–50 c.c., either intramuscularly or intravenously, together with a smaller dose, 5–15 c.c., intrathecally. This is repeated daily until the temperature subsides.

While the therapeutic value of immune serum is still undecided, many authorities recommend the intramuscular injection of convalescent human serum or immune horse serum in doses of 5–15 c.c., as a prophylactic measure in children during the presence of an outbreak of the disease.

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A detailed review of Poliomyelitis with an exten-

sive bibliography is given in the "Survey of Poliomyelitis," by the International Committee for the Study of Infantile Paralysis, Williams & Wilkins, Baltimore, 1932.

RABIES

Rabies or hydrophobia is an inflammatory disease of the central nervous system transmitted to man by bites from infected animals, usually dogs. Rabies was one of the first virus diseases to be investigated, and to Pasteur we owe much of our knowledge of the disease.

The Virus. While rabies is usually designated a filterable virus disease, there appears to be some doubt that the ætiological agent of rabies is a filterable virus. The uncertain nature of the cytoplasmic inclusion bodies, Negri bodies, has raised some question as to the accuracy of this statement in the minds of some workers. When Negri described the inclusion bodies he considered them to be protozoa, which he termed "*neurocytes hydrophobiæ.*" As this theory did not fit in with some of the properties of the virus, it was gradually disregarded, but the question has recently been raised again by Manouelian and Viala (1924), who considered that the bodies represented agglomerated masses of a sporozoon, which they termed "*Encephalitozoon rabiei.*" Levaditi and his colleagues (1926) also investigated this question and decided that the bodies represented a stage in the life cycle of a protozoon, *Glugea lyssæ.* In contradistinction to the other French workers they considered that

the protozoon was not in any way related to the *encephalitozoon cuniculi*. The nature of these bodies was discussed in detail at the International Conference on Rabies held in Paris during 1927, but the Commission was unable to make a definite pronouncement. Until the evidence offered to support the protozoal hypotheses is more definite, it is probably advisable to adopt Pasteur's conception of the causal agent and consider it to be a filterable virus.

Certain properties of the infecting agent have been established: it resists glycerine and low temperatures, but its activity is decreased by drying, phenol (1 per cent.), oxidising agents, ether and temperatures over 45° C.; it is not readily filterable, as it only traverses a Berkefeld V filter with difficulty. Cultivation *in vitro* has never been satisfactorily demonstrated. The virus is conserved by placing infected material in 50 per cent. glycerine and keeping at a low temperature.

Infectivity. Rabies is essentially a disease of canine animals, in which it is enzootic. Actually all mammals and birds are susceptible; in these infection is almost invariably obtained from bites of rabid members of the canine tribe, dogs, wolves or jackals. The virus is present in the central nervous system and frequently in the saliva of these animals. Viruses isolated from various regions have exhibited variations in infectivity for experimental animals. As in poliomyelitis, in order to obtain the most satisfactory results it is necessary to implant the virus into the cerebral nervous system, and conse-

quently the intracerebral route is most frequently employed for experimental purposes. Pasteur found that by serial intracerebral inoculation of rabbits with a freshly isolated strain, *virus de la rue*, the incubation period of the disease in these animals gradually decreased until it became constant at six to seven days ; this modified virus, which he termed *virus fixe*, differed in some respects from the original street virus. The fixed virus exhibited a decreased degree of infectivity for the monkey on subcutaneous inoculation and did not lead to the formation of Negri bodies in the brain of infected rabbits.

The distribution of the disease in man is dependent on the presence of rabid animals. In some countries, as Great Britain and Scandinavia, the disease has been almost entirely eradicated by strict muzzling and quarantine laws. In others, India, China and Russia, where such precautions have not been enforced, the disease is prevalent. In all cases the infection has been introduced from infected animals ; direct contact cases from man to man do not occur.

The incubation period, twenty-seven to sixty-four days, is long and subject to much variation; it depends largely on the proximity of the bite to the brain, *i.e.*, according to the distance the virus has to travel along the axis-cylinders of the nerves supplying the bitten region. The symptoms are similar to those encountered in the lower animals. Fear and anxiety are prominent from the beginning ; extreme thirst, giving rise to pharyngeal spasms is common —in fact, these spasms are induced by many stimuli and create a condition of aerophobia. Consciousness

is rarely lost and death usually occurs in three to four days after the onset of symptoms. In the absence of prophylactic inoculation during the incubation period the disease is invariably fatal.

The lesions are found in the central nervous system. The nerve cells show degeneration and neurophagia, while in some, particularly those of the Hippocampus major, characteristic cytoplasmic inclusions in the nerve cells are found. These were first described by Negri in 1903, since when their presence has been a point of great diagnostic importance; the significance of these bodies has already been discussed. Perivascular infiltration is also present.

The virus is introduced into the subcutaneous tissues in the saliva, and it is probably conveyed from the region of the bite to the central nervous system by the nerve-axons. It has been found that, in the absence of treatment by specific immunization, rabies resulted in approximately 35 per cent. of persons bitten by proved rabid animals (Cornwall, 1923). The explanation of this low figure is not obvious, as once the disease commences it is invariably fatal. It would appear that the mere introduction of the virus into the subcutaneous tissue is not sufficient to produce the disease; damage of the nerves is necessary in order that the virus can reach the axis-cylinders. It is also probable that the virulence of the virus and the amount inoculated with the saliva are factors influencing the production of the disease.

Immunity. Although rabies, once established, is

always fatal, it is possible to prevent the development of the disease by means of prophylactic inoculation during the early stages of the incubation period. This was one of the many important observations made by Pasteur. The principle is that, in view of the long incubation period following infection with the street virus, inoculation with graduated doses of attenuated fixed virus as soon as possible after infection produces a high degree of immunity and aborts the original infection. Pasteur (1890) employed the spinal cord of rabbits, inoculated with fixed virus, dried for varying periods of time over KOH ; emulsions of the cord were prepared and a course of injections given to the person bitten. The first injection contained virus which had been desiccated for the longest period of time (fourteen days), followed by less attenuated virus, until for the final injection cord, which had only been dried for five days, was used. The results were very striking, a marked decrease in the mortality rate followed, and the practice has been extensively employed. Many modifications of the original method of Pasteur have since been tried, the chief of which are :—

(1) Hogye's method, in which active fixed virus was diluted to 1/10,000 for the initial doses, the amount of virus being gradually increased in the subsequent inoculations.

(2) Babes' method, in which heated fixed virus was used ; virus heated to 80° C. is used for first injection, followed by virus heated at 60° C. and 45° C., and finally by unheated virus.

(3) Fermi and Semple employed phenol (1 per cent.) to inactivate the virus.

(4) Remlinger used fixed virus, which had been attenuated by exposure to ether for long periods.

Claims have been made that the results, obtained by these modified methods, have been more satisfactory than by the original method of Pasteur. The advantages were a decreased mortality rate and a decrease in the incidence of " paralytic accidents " following the inoculations. The paralytic accidents, which take the form of an encephalomyelitis, have fortunately been an infrequent occurrence; the records of the Pasteur Institute show only eight such cases during the treatment of more than 41,000 persons. The following table gives the results of three methods of treatment carried out at the Kasauli Institute, India. These figures are quoted by Stuart and Krikorian (1929), who have also found the carbolized vaccine to be extremely efficient.

TABLE II

Period.	Method.	Number treated.	Total mortality rate, per cent.
1900–1907	Pasteur's dried cord .	5,141	1·53
1907–1912	Hogyes' dilution .	8,435	1·17
1912–1926	Killed carbolized virus (0·28–0·7 grm. dosage)	84,844	0·77

These figures indicate the superiority of the carbolized vaccine. The whole question is discussed

in detail in the reports of the International Conference on Rabies held in Paris, 1927.

The nature of the immunity reaction is uncertain. Recent experimental work indicates that immunity is closely associated with the appearance of antiviral bodies in the serum. These immune bodies can be readily demonstrated by means of the neutralization test.

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JAPANESE ENCEPHALITIS

In 1924, an epidemic of encephalitis occurred in Japan in which over 4,000 people died; the mortality rate was about 50 per cent.

Little difference could be detected from the symptoms seen in encephalitis lethargica (Kaneko, 1925).

Kobayashi (1925) performed a large number of experiments on rabbits, and, by repeated passages, obtained a virus capable of producing a typical encephalitis after an incubation period of four to twelve days in 100 per cent. of animals following intracerebral inoculation. The virus reacted very

similarly to the virus of rabies. Takaki (1925) also isolated a similar virus, which traversed many of the usual bacterial filters, from the brains of six cases at autopsies; this virus appeared to be quite different from the herpes virus. As a result of a detailed histological and immunological study of this condition, Cowdry (1927) also concluded that the virus was identical with the virus of rabies.

It has been suggested that the recent severe outbreak of encephalitis in St. Louis, U.S.A., bore some clinical resemblance to the Japanese encephalitis of 1924. The experimental reproduction of the American disease has not yet been established (Leake, 1933), so the relationship of the two conditions cannot be determined.

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ACUTE RABIC MYELITIS (TRINIDAD)

An outbreak of acute ascending myelitis occurred in Trinidad during the wet seasons of the years 1929–30; seventeen cases were described. The symptoms were those of an acute myelitis and the death rate was 100 per cent. The nature of the condition was not known, but poliomyelitis was considered a possibility. Hurst and Pawan (1931, 1932) carried out a detailed investigation and determined

conclusively that the ætiological agent was the rabies virus. The disease was transmitted to monkeys and rabbits, and, on histological examination, typical Negri bodies were demonstrated in the nerve cells of the Cornu ammonis. Successful cross-immunity tests were also carried out with the virus fixe.

The source of infection was uncertain, as rabies had not been notified in the island since 1914. A peculiar disease diagnosed as botulism had existed among the cattle since 1925, and as the rabies virus was obtained from the brain of a diseased cow, it is probable that this disease was a clinical manifestation of infection with the rabies virus. There was, however, no epidemiological evidence to suggest the direct transmission of the disease from the cattle to man. Hurst and Pawan considered that the vector of the disease was a vampire bat, as a definite history of bat-bite was obtained in several of the cases.

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VARIOLA OR SMALL-POX

Variola or small-pox, now as in ancient times, is a widely distributed infectious disease, which may give rise to serious epidemics.

The Virus. Variola is only reproduced experimentally with difficulty; the most suitable animal is the monkey, which responds to cutaneous inocula-

tion by the production of a definite local lesion with moderate constitutional changes. The inoculation of lower animals, as the rabbit, merely results in a slight local inflammatory lesion with little, if any, general reaction. However, the subsequent passage to lower animals of material, collected from variolous lesions of monkeys, is more successful—a marked local lesion with a constitutional reaction is produced without difficulty. The virus thus becomes distinctly modified by animal passage and becomes identical with the " vaccinia " virus. Owing to the readiness with which this condition can be transmitted to the common laboratory animals by scarification or intradermal inoculation, it is only to be expected that the vaccinia virus has been the subject of extensive investigations. Gordon (1925), in a detailed study, determined the following properties of the vaccinia virus : it did not readily traverse Berkefeld filters ; it was comparatively resistant to glycerine, phenol, ether and low temperatures, but was readily destroyed by heat over 55° C. and potassium permanganate. It has also been found that the virus can be cultivated readily in tissue cultures (see Chapter II.). Its dimensions, as tested by filtration through graded collodion membranes, have been found to lie between 0·125–0·175μ. A number of small coccal bodies, which have been termed elementary or Paschen bodies, have been demonstrated in the vesicular fluid of the lesions (Paschen, 1906). Craciun and Oppenheimer (1926) separated these bodies from calf-lymph and also claimed that they had obtained them in tissue-

culture preparations ; the fluid part of the vaccinial lymph which remained after the removal of the " granules " was found to be quite inactive. These elementary or Paschen bodies have recently been the subject of much work, and are now considered to represent the actual virus (see p. 44).

The vaccinia virus can produce lesions in most mammals, and the presence of cytoplasmic inclusions in the epithelial cells involved in the lesions, which were first described by Guarnieri (1894), constitutes an important diagnostic feature. The calf is the animal employed in this country for the production of the vaccine lymph, while the rabbit is largely used for experimental purposes. The guinea-pig is also a useful experimental animal (Gildemeister and Herzberg, 1925). Lesions follow the inoculation of the virus by various routes. Marie (1920) produced an encephalitis in rabbits by intracerebral inoculation of the virus. Levaditi and Nicolau (1923) found that infection of the central nervous system of the rabbit was greatly facilitated by preliminary passage of the virus through the rabbit's testicle. After several passages the virus became very active, and was termed " neurovaccine." By the intracerebral inoculation of this neurotropic strain a meningo-encephalitis can be produced in the monkey (Hurst and Fairbrother, 1930). It was considered by Levaditi and Nicolau (*loc. cit.*) that the vaccinia virus acted solely on ectodermal tissue. Ledingham (1924 and 1927), as a result of an extensive study of the lesions produced by the vaccinia virus in the different tissues, found that the reticulo-endothelial

elements, mesodermal tissue, were primarily involved in the reaction. This observation was confirmed by the results of Hurst and Fairbrother (*loc. cit.*), who found that the mesodermal tissues were primarily concerned in the encephalitis produced by intracerebral inoculation of the vaccinia virus.

The vaccinia virus, after inoculation by any route, exhibits a tendency to generalize and may be demonstrated in many tissues of the body. In some cases a severe generalized disease, in which necrotic areas are found in many tissues, has been produced in rabbits (Douglas, Smith and Price, 1929). The vaccinia virus is frequently found in the blood of children several days after dermal vaccination (Herzberg-Kremmer and Herzberg, 1930).

Infectivity. Prior to the introduction of vaccination by Jenner at the end of the eighteenth century, small-pox was one of the dreaded diseases. The high mortality rate and the extensive ravages and scarrings of the disease are frequently indicated in the literature of the earlier periods. At the present time such outbreaks are rarely experienced in civilized communities; the disease in its severest form is rarely encountered, and extensive outbreaks are also very infrequent.

The infection is considered to occur through the agency of air-borne infected particles, which settle in the upper respiratory tract. The virus is then transported by the blood stream to the skin tissues, where it settles and multiplies in the capillary circulation.

After an incubation period of about nine to fifteen days the onset is characterized by headache, rise of temperature, vomiting, and backache with the appearance of an erythematous rash. On the third day the rash develops in the form of discrete shotty papules on the face, hands, and later on the legs; three days later the papules become vesicular and then pass on to a pustular stage, following which they dry up and after decrustation form scars. Lesions may occur around the mouth and along the mucous membrane of the upper respiratory tract.

The disease has apparently become considerably modified; the most prevalent form encountered in immunized communities at the present time is a mild disease, which has been termed "Alastrim."

Alastrim. This is a mild form of variola occurring throughout the world, although it has only been observed in this country during the last ten years. It is practically never fatal and the symptoms are often very mild, the pustular stage being frequently absent. Blaxall (1923), investigating the relationship to the virulent form of small-pox, reproduced typical papules by dermal scarification of alastrim material in monkeys, which were subsequently immune to vaccinia; also he found that monkeys vaccinated previously, did not respond to inoculations of material from either variola or alastrim patients. Ledingham (1925), as a result of a review of the experimental work performed with alastrim and variolar material, considered that these two viruses were essentially the same.

It is now accepted that the virus of alastrim is a

variant of the variola virus. Consequently, owing to the mild nature of this disease, some consider that the risks involved in the practice of vaccination are not justified, as it is preferable to allow the immunization process against variola to be carried out naturally by an attack of alastrim (Millard, 1929). A serious objection to this is the possibility that, in the presence of an unimmunized community, a change of type from the weak alastrim virus to the virulent variola virus may occur.

Immunity. An attack of small-pox or alastrim is succeeded by a high degree of immunity; second attacks rarely, if ever, occur. Immunity can also be induced by means of infection with the vaccinia virus. This fact was investigated by Jenner and has been the main factor responsible for the diminution in the incidence and severity of the disease. The process known as vaccination has been carried out in most countries under State control; it was first adopted officially in England in 1853. The type of virus employed varies—in France and other Continental countries the potent neurovaccine is employed. In England calf-lymph is used, and recommendations for its preparation were drawn up by the Royal Commission on Vaccination, 1889–96. These recommendations found expression in the Vaccination Act, 1898. The preparation of lymph in this country has been further controlled by the Therapeutic Substances Regulations, 1927, in which provision is made for the condition of the vaccinifers, and the preparation, purity and potency of the lymph.

The Preparation of Vaccine Lymph. The vaccinia virus is maintained virulent by cutaneous passage through rabbits, while the vaccination is carried out on young calves four to six months old. These are shaved and cleaned over one side of the abdomen, and scarification made 3–6 inches long and about ½ inch apart, as aseptically as possible. On the fifth day, 120 hours after vaccination, the infected region is cleaned up and the vesicles removed by a Volkmann spoon—this forms the pulp. This is titurated with 50 per cent. glycerine and distilled water until no particles visible to the naked eye are present. The pulp is then sealed in suitable tubes and placed at — 11° C. Aerobic and anaerobic cultures are then prepared; these are repeated periodically. Lymph containing anaerobic organisms or streptococci is not issued. When aerobic organisms are reduced to 5 or less per mgm., or 5,000 per c.c., the lymph is ready for issue. This is tested for potency and is issued in capillary tubes containing about $\frac{1}{30}$ c.c. of emulsion, which is sufficient for one dose. The following tests for potency were selected at an International Commission (1927) :—

Gins' test consists in inoculating the scarified cornea of a guinea-pig with dilutions of vaccine lymph 1–100 to 1–1,200. A keratitis develops which in three days causes definite opacity of the cornea, which should be apparent to the naked eye.

Sobernheim's test consists in inoculating four patches on the shaved or depilated skin of a rabbit. On each patch three linear incisions are made and the patches are inoculated with different dilutions

(1–1,000 to 1–10,000) of lymph. After three days a specific reaction, showing papules and perhaps pustules, should be produced along the line of the incision.

Groth's method consists in injecting 0·1 c.c. of dilutions, from 1 in 10 to 1 in 100,000, of vaccine lymph intradermally into the depilated or shaved skin of a rabbit. The reaction, which follows in twenty-four hours with the strongest dilutions but with the weakest is delayed until the fourth or fifth day, consists in the appearance of red raised infiltrated areas at the points of inoculation. These can be measured by calipers.

The Calmette-Guerin test consists in inoculating the shaved backs of rabbits with different dilutions—1 in 100 to 1 in 1,000. An eruption of isolated vesicles numbering 3–4 per sq. c.c. of the field inoculated should be produced following the inoculation of 1 c.c. of the 1 in 1,000 dilution.

Vaccination in man is carried out by cutaneous scarification. The results of this process have been beyond dispute. In recent years, however, the occurrence of nervous symptoms following primary vaccination has, in some circles, caused much adverse criticism. While the cases of post-vaccinial encephalomyelitis were undoubtedly associated with the process of vaccination, it is almost universally accepted that the vaccinia virus was not the direct cause. This question is discussed later (p. 180). However, the problem received serious consideration, and as a result of a Ministry of Health investigation (1928), the following recommendations were made:—

(1) In place of the officially advocated four insertions trial be made of vaccination and re-vaccination in one insertion with a minimum of trauma, and that multiple scarification, and (or) cross-hatching be deprecated.

(2) Primary vaccination be performed in infancy, between the ages of two and six months as at present, and that re-vaccination be offered at the time when a child enters school (five to seven years) and again on leaving (fourteen to sixteen years).

(3) Vaccination in multiple insertions be available for such persons as desire to obtain the maximum protection against small-pox obtainable at one operation.

(4) In public vaccination, parents be informed that if in consequence of vaccination a child requires medical attention, it is the duty of the public vaccinator concerned to provide such attention without cost to the parents.

(5) Instead of the one inspection now required in the case of public vaccination, there be two; the first not earlier than the seventh or later than the tenth day, and the second not earlier than the fourteenth or later than the seventeenth day.

In some districts vaccination is not enforced; this is probably one of the chief reasons for the increased incidence of the disease in recent years.

The use of living virus is essential for the production of a high degree of immunity; virus, killed in various ways, has been tried experimentally, but the results have been unreliable and inconsistent.

The state of immunity is definitely associated with

the presence of antibodies in the serum. These can be readily demonstrated by means of the complement-fixation test, agglutination and flocculation reactions and the neutralization test. The various immunity reactions have been employed extensively in establishing the identity of the viruses of variola, alastrim and vaccinia. The flocculation test has also been used for the diagnosis of variola with good results (Burgess, Craigie and Tulloch, 1930).

ANIMAL POX DISEASES

The attendants of cows, sheep, goats, swine and horses sometimes become infected with the various animal poxes as a result of direct inoculation through an abrasion of the skin with infected material. The lesions are local and occur usually on the hands and arms; generalization is rare. There seems no doubt that the vaccinia virus, or some modification of it, is responsible for these infections. The infection is thus similar to the process of vaccination and is consequently checked by the antivariolar inoculations.

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HERPES SIMPLEX

Herpes simplex is an eruptive disease of the skin and mucous membranes, occurring frequently on the face and genitalia.

The Virus. The resistance offered to various chemical agents by the herpes virus is variable; this variability appears to depend largely on the virulence of the strain. It is, however, comparatively resistant to 50 per cent. glycerine and low temperatures, but it is readily destroyed by temperatures over 50° C. The virus is rather large, and consequently does not readily pass through bacterial filters; estimated by graded collodion membranes, its size has been found to lie between 0·1–0·15μ. The virus has been cultivated *in vitro* in the presence of living cells.

Infectivity. Herpes simplex is a common disease in man, and it has been divided clinically into two main groups: (*a*) symptomatic, and (*b*) idiopathic. The symptomatic form is the commonest and occurs during the course of some other diseases, such as pneumonia, colds, influenza, jaundice, malaria,

cerebro-spinal meningitis, and also after the inoculation of some foreign protein, as the T.A.B. vaccine. The source of infection is not definitely known ; in the majority of cases infection from outside sources appears highly improbable. Levaditi, Harvier and Nicolau (1922) have demonstrated the presence of the virus in the saliva of apparently healthy individuals. It is thus probable that many individuals are carriers of the virus, which becomes activated on stimulation from varied sources.

The disease is very mild. The onset is sudden, and occurs in the form of intense local irritation with a burning sensation : this is succeeded by a crop of vesicles from which serous fluid exudes ; this rapidly dries up to form a crust, which falls off, leaving no scar.

The disease does not occur spontaneously in animals. It was first transmitted to laboratory animals by Grüter (1924), who in 1910–11 had produced a specific keratitis in rabbits by inoculation of herpetic material on to the scarified cornea of these animals. This observation was confirmed in 1919 by Lowenstein. Levaditi and Harvier (1920) showed that intracerebral inoculation of a strain of herpes virus, which they had obtained from a case of human encephalitis, produced a meningo-encephalitis in a certain number of rabbits. These workers consequently considered that two distinct strains of virus existed, one being dermotropic and the other neurotropic. Flexner and Amoss (1925), however, have shown that the affinity of the virus for the various tissues was purely a question of virulence of

the virus. The so-called neurotropic strain was shown to be more virulent than the dermotropic strain. It has also been found that encephalitis in rabbits may follow corneal intranasal or cutaneous inoculation of an active or neurotropic strain of the virus. In these cases the virus travels to the central nervous system by means of the axis-cylinders of the nerves supplying the affected region (Goodpasture, 1925).

While the rabbit has been the main experimental animal, the guinea-pig and monkey have also been employed. The monkey was until recently considered insusceptible to intracerebral inoculation of the virus. Zinsser (1929) and McKinley and Douglass (1930), however, succeeded in producing an encephalitis in monkeys of the Cebus olivaceus species. These workers found that the lesions produced in the subacute and acute forms closely resembled those found in human encephalitis; they, however, experienced much difficulty in transmitting the disease from monkey to monkey.

The virus has been isolated on several occasions from the brains and naso-pharyngeal washings obtained from cases of epidemic encephalitis. Some workers have suggested in consequence that the herpes virus is ætiologically related to epidemic encephalitis; others, however, consider that the virus, in these cases, was present in the central nervous system merely as a passive invader. This matter will be discussed in the consideration of the latter disease (p. 157).

Immunity. The development of immunity in man

is very doubtful. If present, it is only temporary, and occurs as a local tissue immunity ; even this is frequently absent, as a second crop of vesicles may follow the first crop in the same situation after a very short interval. Antiviral bodies have been demonstrated by means of the neutralization test in the serum of many normal individuals (Andrewes and Carmichael, 1930) ; the *rôle* played by these bodies is very uncertain. It is probable that the lesions are too superficial to be influenced to a large extent by the presence of antiviral bodies in the serum.

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YELLOW FEVER

Yellow fever is an acute febrile disease occurring usually in tropical regions, especially in West Africa, where it is found in both endemic and epidemic forms.

The Virus. Studies of the activity of the virus have been restricted by the difficulty in obtaining an experimental animal. Much important work was carried out by a United States Army Commission at the beginning of the present century, when, by the use of human volunteers, Reed, Carroll, Agramonte and Lazear (1900–01) found that the infecting agent was filterable and was conveyed by mosquitoes. Further progress was not, however, made until Stokes, Bauer and Hudson (1928) demonstrated that the disease could be reproduced in Asiatic monkeys (*M. rhesus*), and they were consequently able to investigate the virus in more detail. The virus, as most other viruses, is comparatively resistant to the action of 50 per cent. glycerine, drying and low temperatures, but is readily destroyed by heat over 55° C. and formaldehyde. The virus, as present in the blood of an infected monkey, has been found to pass through Berkefeld " V " and " N " candles, but, as present in infected mosquitoes, it only passed through the " V " candle with difficulty. The size of the virus has been estimated by the use of " Gradocol " membranes to lie between 0·017–0·028μ.

The claims of a filterable virus as the ætiological agent have not been allowed to pass unchallenged. Many organisms have been described as the causative agent of the disease. These claims have never been substantiated. Noguchi (1919) isolated a spirochæte which he termed *Lept. icteroides*, but it has since been shown that this organism is identical with *Lept. icterohæmorrhagiæ*. The disease which Noguchi investigated was thus infective jaundice, and not

yellow fever. More recently Kuczynski (1930) has claimed that a diphtheroid, *B. hepatodystrophicans*, is the causal agent of the disease. The observations on which this claim was made have not, however, been confirmed by other workers, who consider this organism to be merely a contaminant.

Infectivity. Yellow fever is transmitted from man to man by the agency of mosquitoes, usually *Ædes ægypti* (or *argenteus*) or allied species. The question of the insect vector has been discussed in detail by Buchbinder (1930). Lowe and Fairley (1931) have recently pointed out that infection may also follow the handling of infected material, as in carrying out post-mortem examinations. The virus apparently penetrates the unbroken skin or enters through minute superficial abrasions.

In white people the disease normally runs a typical course. After an incubation period of three to six days there is a sudden onset of fever with intense frontal headache and muscular pains lasting three to four days, during the course of which the pulse rate drops considerably. Then the temperature falls to around 98° F. with the appearance of jaundice, albuminuria and hæmorrhages into the intestinal tract. Death may occur in the early stages, but it is more common on the fifth or sixth day. The mortality rate varies from 20–90 per cent. The usual findings at autopsy are jaundice with petechial hæmorrhages into the skin and mucous membranes, fatty and necrotic changes in the liver and kidneys, together with congestion of the spleen. Cowdry and Kitchen (1929) found nuclear inclusions in the

human liver cells similar to those described in the experimental condition by Torres (1928).

The disease can be reproduced in monkeys (*M. rhesus*) either by subcutaneous or intraperitoneal inoculation of infected material or by allowing the animals to be bitten by infected mosquitoes. Using a virulent strain the disease in these animals is almost invariably fatal; the clinical picture resembles that of the severe human type. Theiler (1930) produced an encephalitis in mice by intracerebral inoculation of the virus. After repeated intracerebral passage in the mouse the virus became less virulent for the monkey, although an encephalitis could be produced on intracerebral inoculation. Other common experimental animals, except the guinea-pig, have proved comparatively refractory to inoculations of both the viscerotropic and neurotropic strains.

The virus is present in the blood of human cases during the last few days of the incubation period and the first three or four days of fever. Mosquitoes feeding on patients during this period become infected, but do not usually become infective for man for at least twelve days. The virus does not seem to multiply in the mosquito, but after the virus has been taken into the alimentary tract some time elapses before it can be demonstrated in the saliva. The length of time of the so-called extrinsic incubation period varies with the atmospheric temperature ; if this is 22° C., the time before the insects become infective is three or four weeks, at 37° C. it is four days, while at a temperature of 20° C. or lower the

insects never become infective. The virus, however, is not destroyed at these low temperatures. Once a mosquito has become infected it probably remains so indefinitely, but no evidence has been obtained to indicate that the virus passes from one generation to the next through the eggs. It is estimated that the mosquito, in biting a yellow fever patient, takes in approximately 1,000,000 to 2,000,000 lethal doses of the virus.

Immunity. An attack of the disease is followed by a high degree of immunity and second attacks are rare. Antiviral bodies can be demonstrated in the sera of recovered cases by means of the neutralization test, the complement-fixation and precipitation tests. The bodies have also been demonstrated in the sera of the natives of infected regions who have never displayed any signs of the disease. These individuals have probably undergone subclinical infection in childhood and consequently are protected against further attacks. The virus has been demonstrated in the blood of natives during mild febrile attacks. The recent development of the neutralization test in the mouse has allowed the investigation for the presence of antiviral bodies to be carried out on a large scale, and the distribution of yellow fever has been found to be much wider than supposed. The test is performed either by the intraperitoneal injection of mixtures of the serum and an active neurotropic virus into mice, that have received slight brain trauma by the intracerebral inoculation of starch, or by direct intracerebral inoculation of the serum-virus mixtures.

Convalescent serum has only been employed therapeutically on a small scale, but good results have been claimed. An antiviral serum has also been prepared by the inoculation of the horse. The value of these sera has not however received a serious trial in the field.

Active immunity has been produced in monkeys by the inoculation of formolized or phenolized virus. The virus is liberated from an infected liver or spleen by the use of 9 per cent. salt solution, gross particles are removed and 1 or 2 per 1,000 formaldehyde added (Hindle, 1928). This vaccine is innocuous, but the results of its trial as a prophylactic measure in man have not as yet been striking. The living neurotropic virus, together with human or horse immune serum, is now used, and the results so far have been satisfactory (Sawyer, Kitchen and Lloyd, 1932). There are, however, limitations to the application of this method; in the first place the vaccine could not be used in districts where the introduction of living virus has been prohibited, and secondly, it might be difficult to secure sufficient immune serum to satisfy a large demand. The use of immune horse serum should overcome the second difficulty.

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PSITTACOSIS

Psittacosis in man is a comparatively rare disease in which the association with infected parrots is a prominent feature. Until the widespread epidemic of 1929–30, the disease was almost unknown in this country. It has, however, been recognized, and the association with parrots recorded for a long time in other parts of the world. An extensive outbreak occurred in Paris in 1892, during which Nocard isolated a salmonella organism from infected parrots.

The Virus. Until the recent epidemic in this country the causal agent of psittacosis was considered to be the salmonella organism, *Bact. aertrycke*, isolated by Nocard in 1892. Bedson, Western and Simpson (1930), however, carried out an extensive investigation which indicated that the infection was caused by a filterable virus. These workers succeeded in transmitting the disease to budgerigars by the inoculation of tissue extracts from infected parrots, and later from the organs of human cases. These observations were soon confirmed: Pesch (1930), using green parrakeets, passed the disease

through several generations using tissue extracts, filtered through Seitz filters, from five human cases ; Gordon (1930) reproduced the condition in budgerigars by intraperitoneal inoculation of citrated blood and of tissue extracts from human cases and infected parrots ; Rivers and Berry (1931) produced a meningo-encephalitis in mice, guinea-pigs, rabbits and monkeys by intracerebral inoculation of the virus. The participation of a salmonella organism in the disease process was definitely excluded. Bedson and Western (1930) demonstrated that the virus possessed the following properties : it only passed through Chamberland L1 bis, L2 and Seitz E.K. filters with some difficulty ; it was relatively resistant to low temperatures and glycerine. They found, however, that tissue preserved in glycerine soon lost its virulence and that it was more satisfactory to place the tissue in buffered phosphate and keep in the cold.

Elementary bodies have been demonstrated in the lesions (Coles, 1930). Bedson and Bland (1932) studied the development of these bodies, and found that the virus passed through a complicated growth cycle, which suggested affinities to the mycetozoa, or possibly the microsporidia. This question has been considered previously (p. 45). Lillie (1930) considered these bodies to be similar to the Rickettsiæ.

Infectivity. There is little doubt that the disease was introduced into this country by Brazilian parrots. The majority of cases gave a definite history of contact with sick parrots, from a few of

which the virus has been isolated. In a few instances transmission from man to man must be considered a possibility. Man is apparently very susceptible to infection with the virus, and the greatest precaution is necessary in carrying out experimental work, as several cases of laboratory infection have been recorded.

The symptomatology is rather indefinite. After an incubation period of eight to fifteen days the onset is usually abrupt. The initial symptoms are headache and general malaise, accompanied by high temperature. The lungs are frequently involved, being the seat of a varying degree of irregular consolidation. Other signs are variable, and no typical diagnostic feature is present. Convalescence is slow and tedious. The mortality rate varies in the different outbreaks; Sturdee and Scott (1930), in a study of the recent epidemic, found that about 20 per cent. of cases were fatal.

The pathological findings are somewhat indefinite; changes associated with a general infection are generally seen, together with a peculiar inflammatory condition of the lungs resembling an influenzal pneumonia.

Immunity. It has been found by reinfection tests that budgerigars or mice, recovered from experimental infection, acquired a certain degree of immunity. This immunity is difficult to demonstrate by serological methods; the neutralization test has been unreliable, but complement-fixation tests have proved satisfactory. Active immunity has also been produced in mice by the inoculation of a 10 per cent.

suspension of spleen from infected mice to which 1/1,000 formaldehyde has been added.

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VERRUCÆ

Verrucæ or warts are benign papillary growths. The symptoms are negligible; complaints are usually made on cosmetic grounds. The disease has consequently received little attention.

Wile and Kingery (1919) ground up warts and passed the resulting emulsion through a small Berkefeld filter; the filtrate, bacteriologically sterile, was inoculated intradermally into human subjects and produced similar growths after an incubation period of four to seven weeks. This observation was confirmed by Kingery (1921), who later successfully passed the lesion of the first generation after an incubation period of some six months. Successful passage experiments had been performed previously

with the emulsion of the lesions ; Jadassohn (1896) obtained many positive results by inoculation of emulsions into the skin of the hands.

The growths commence as a hyperkeratosis, while later there is proliferation of the papillary tufts. All the epithelial layers are more or less thickened. Lipschutz (1924) found basophilic intranuclear inclusions in the cells of the lesions.

With regard to immunity no experimental evidence is available. These growths frequently disappear spontaneously. There appears to be some individual susceptibility to the virus ; immunity in these persons after recovery is only of short duration.

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MOLLUSCUM CONTAGIOSUM

Molluscum contagiosum is a contagious epithelial growth of benign nature. The condition affects humans, usually children ; the infection is fequently transmitted by the handling of infected material, such as towels. The growths occur round the face or genitalia ; they are usually discrete and few in number. The disease is trivial and causes little discomfort ; the lesions tend to disappear after several months.

Juliusberg (1905) considered that the disease was

caused by a filterable virus. He passed an emulsion of the lesions through a Chamberland filter and stated that the condition was reproduced after an incubation period of fifty days. Wile and Kingery (1919), after filtration of an emulsion of the lesions through Berkefeld filters, reproduced the disease on intradermal inoculation in man after an incubation period of twenty-five days. Findlay (1930) stated that he also passed the causative agent through Berkefeld " V " and Chamberland L1 filters, and on inoculation reproduced the disease after an incubation period of five weeks.

The lesion consists of a nodule produced by the local proliferation and swelling of the cells of the skin. The central oval cells contain the so-called molluscum bodies. Lipschutz (1911) described minute bodies in the vacuoles of the epithelial cells which he termed "*strongyloplasmata hominis.*"

Little is known about the immunity response in this disease.

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FOOT AND MOUTH DISEASE

While man is comparatively immune to foot and mouth disease, rare but definite cases have occurred

(Pape, 1921). The disease manifests itself by the presence of a sharp fever with vesicular eruptions, which heal rapidly, on the buccal mucous membrane and on the hands and feet. The diagnosis can only be determined by transmission of the disease to susceptible animals, such as the guinea-pig, and by the demonstration of cross-immunity with a definite strain of the foot and mouth virus (Arkwright, 1928).

The infection is transmitted from infected animals; spread from man to man has not been recorded.

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RIFT VALLEY FEVER

In 1930 a disease causing a heavy mortality in newly-born lambs appeared in the Rift Valley, Kenya Colony. This disease, on investigation, was found to be caused by a filterable virus and was termed Rift Valley fever (Daubney and Hudson, 1931). The virus was demonstrated in the blood, liver and spleen of infected animals by the intravenous inoculation of healthy sheep. Cattle were also susceptible, as an outbreak was observed in a dairy herd. Man appears to be comparatively resistant to the virus. Several of the workers investigating the condition experienced a sharp febrile attack with joint pains, rather similar to dengue fever. The condition tended to clear up in three to four days. It was also found that the

majority of natives engaged in herding sheep during the epizootic had experienced similar attacks. Transmission was considered to occur by the agency of mosquitoes.

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CHAPTER VII

DISEASES PROBABLY CAUSED BY FILTERABLE VIRUSES

MEASLES

MEASLES is an acute febrile disease of extreme infectivity occurring usually in children in epidemic form. The infection is probably carried in the naso-pharyngeal secretions and is transmitted from man to man by direct contact. While the disease is very common in children, all susceptible individuals coming in contact with cases may be attacked.

Statistical studies of the epidemiology of measles indicate that subclinical infection is probably not infrequent (Stocks, 1930). The development of the disease after infection takes the following course: after an incubation period of about fourteen days the onset of the disease is characterized by fever, sore throat and coryza, with the appearance of white spots, termed "Koplick Spots," on the buccal mucous membrane. On the fourth day the rash, which develops very quickly, appears and takes the form of papules, which commence around the neck and spread all over the body; these are usually discrete, but they may be confluent. The tongue is thickly coated. After three to four days the rash and constitutional symptoms subside and desquama-

tion commences. The mortality rate varies according to the incidence of certain complications, the most frequent of which are broncho-pneumonia, meningitis, otitis, nephritis and endocarditis.

The post-mortem findings of fatal cases are more the result of the various complications than of measles itself. In the actual disease the mucous membrane of the upper respiratory tract is the seat of a catarrhal inflammation.

Ætiology. Experimental work has been severely handicapped by the failure to obtain an experimental animal, in which the typical disease can be produced. Monkeys have been employed by many workers with varying degrees of success. Efforts to produce the disease in other laboratory animals have not been very successful. Nevin and Bittmann (1921) and Grund (1921), on unsatisfactory experimental evidence, claimed to have transmitted the disease to rabbits ; Purdy (1925), however, did not confirm their results. McCartney (1930) also tried to reproduce the disease in a large variety of animals, including guinea-pigs, rabbits and rats, but only obtained negative results.

The most convincing evidence so far obtained is that of Blake and Trask (1921), who infected the upper respiratory tract of ten monkeys (*M. rhesus*) with human nasopharyngeal washings, collected in the early stages of the disease, and reproduced a febrile condition and a rash resembling measles in eight of the ten animals ; Berkefeld filtrates of the washings also produced the same results. They also passed the condition through a series of monkeys by

intravenous inoculation of the whole citrated blood, which was examined bacteriologically and found to be sterile. Degkwitz (1927) confirmed the results obtained by Hektoen in 1905, by showing that, when susceptible individuals are inoculated with blood, collected within a short time of the development of the rash, a typical attack of measles occurred. The same result was also obtained with blood, diluted and passed through a Berkefeld filter.

These observations suggest that the ætiological agent of measles is a filterable virus. This view is not however universally accepted, as other workers have isolated certain bacteria from the tissues and claim these as the causal agents.

Caronia (1924) claimed that he had cultivated anaerobically the causal agent of measles from the blood and filtrates of nasopharyngeal washings by the use of the Noguchi medium. He obtained a faint granular deposit consisting of minute Gram-negative diplococci. The claims of relationship of these bodies with the disease have not been confirmed by a large number of workers (McCartney, 1927, and others).

More serious claims have been made for the green-producing cocci, which have been isolated from the blood and nasopharyngeal swabs of measles cases by Tunnicliff (1917, etc.), Ferry and Fisher (1926), and others. The organism isolated by Tunnicliff was anaerobic in primary culture, but grew well aerobically on subsequent subculture; Ferry and Fisher found that the coccus isolated by them was definitely aerobic from first isolation. These workers

claimed that the respective cocci were the ætiological agents of the disease and prepared antistreptococcal sera by the inoculation of horses. These sera have been employed therapeutically in human measles, but not with very convincing results. Other workers do not accept the view that these cocci are ætiologically related to the disease. Long and Cornwell (1927) prepared blood cultures from twenty-six cases of measles, and only obtained cocci, similar to those described above, in four instances; these were regarded as contaminants. Smith (1929) made a detailed study of the various green-producing cocci found in the nasopharynx, and as a result considered that cocci similar to those isolated by Tunnicliff were normal inhabitants of the upper respiratory tract and had no relationship with measles. The ætiological relationship between the green-producing cocci and measles is thus far from conclusive.

Degkwitz (1927) stated that he had cultivated the virus of measles by cultivating blood from early cases of the disease in symbiosis with a pneumococcus or streptococcus in human blood-plasma and buffered salt solution. Virus cultures were carried through sixteen generations, and Degkwitz claimed that susceptible individuals inoculated subcutaneously with the cultures gave a typical reaction, which was said to be specific. This work requires confirmation before it can be accepted without reservation.

Immunity. Measles is followed by a high-grade of immunity; second attacks are extremely rare. Owing to absence of satisfactory laboratory animals,

the presence of antiviral bodies in convalescent serum is difficult to demonstrate. It is however highly probable that these bodies are present, as convalescent serum has proved successful both as a prophylactic and a therapeutic measure. Pooled adult serum has also been successfully employed.

Serum Therapy. Sera have also been prepared in animals by the inoculation of the various organisms claimed as the causal agents of the disease. The results of the use of these sera have not been satisfactory : Gunn (1928) compared the results obtained by the use of several sera; the degree of protection afforded is shown in Table III. The various sera were inoculated into susceptible children within the first few days (two to eight) after exposure to infection and the subsequent development or absence of the disease noted.

TABLE III

	Number injected.	Per cent. apparently protected.
Human convalescent serum .	69	95·7
Tunnicliff's horse serum .	21	42·9
Ferry and Fisher's horse serum	12	0·0
Degkwitz's sheep serum .	10	40·0
Control group (no serum) .	23	21·7

As it has been found that the inoculation of normal horse serum alone affords a certain degree of

protection, the results, quoted above, indicate the marked superiority of convalescent serum over the other preparations.

When the serum is administered a short time after infection, the attack is usually greatly decreased in severity and the patients subsequently acquire a definite active immunity. Some authorities consequently consider that, in individuals over three years of age, when the prevalent type of the disease is mild, the administration of serum should be postponed until the infection has developed. As the passive immunity produced by the inoculation of the serum soon passes off, the advantages of obtaining an acquired immunity are obvious.

The question of serum therapy has recently been the subject of an extensive investigation by the London County Council (1933), in which the total number of observations recorded was 2,362. This total consisted of 1,475 observations on the use of serum obtained from healthy adults, who had had some time previously a definite attack of measles, with the results of 680 inoculations of convalescent serum and 207 uninjected persons to form controls.

The adult serum was employed to produce both complete protection and attenuation of the disease, and, after reviewing the results, the following conclusions were made :—

Protection. (*a*) Complete protection is advised for (1) all debilitated patients, (2) all suffering from any serious intercurrent illness, (3) all children under three years of age.

(*b*) A minimum dose of 10 c.c. is given, and from

the age of three years the dose, in c.c., is reckoned by multiplying the age in years by four.

(*c*) The serum must be given as soon after exposure to infection as possible, preferably within five days.

(*d*) All injections should be given intramuscularly.

(*e*) The duration of the passive immunity conferred is roughly three to four weeks.

Attenuation. (*a*) After the age of three years the aim should be to produce a modified attack of measles wherever possible. This is achieved by injecting the serum, in the doses given above, between the sixth and ninth day after exposure to infection.

(*b*) The same results may also be obtained by halving the dose of serum and giving the injections within the first five days.

(*c*) The immunity following such a modified attack appears to be permanent.

A comparison of the results obtained by convalescent serum and adult serum indicates that while convalescent serum is a more potent prophylactic agent, the difference is of statistical significance only in respect of children under five years of age.

The results show conclusively that adult serum, as well as convalescent serum, merits a high place in any future policy of measles control.

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GERMAN MEASLES

German measles is a mild infectious disease characterized by the appearance of a rash usually without any prodromal symptoms. This condition has a very low mortality rate, while the symptoms are very mild. In view of these facts very little experimental work has been performed. The disease has never been satisfactorily reproduced in animals; the nature of the causative agent is thus very uncertain. The disease is considered here owing to its close clinical relationship with measles.

After a variable incubation period of fourteen to twenty-one days, a shotty papular rash appears, accompanied by a moderate degree of pyrexia, enlargement of the cervical glands, and frequently a sore throat. The rash fades with or without desquamation, and except for a persistence of the enlargement of the cervical glands the patients recover completely without the appearance of complications.

One attack produces a high grade of immunity against the disease. There appears to be no cross-immunity between measles and this disease; these two diseases consequently are probably quite distinct conditions.

MUMPS

Mumps is an acute specific febrile condition characteristically involving the parotid glands. The disease is very widespread, occurring in most parts of the world, and is most frequently a complaint of childhood. Infection is usually the result of direct contact with a case. The disease is frequently endemic, and it is probable that the virus persists in the community through the agency of carriers.

The incubation period varies considerably, but is usually between fourteen to twenty-one days. The first signs of the condition are vomiting, headache, muscular pains and pyrexia, followed after a short interval by swelling of one or both parotid glands; involvement of the other salivary glands may also be present. The disease rapidly subsides unless such complication as orchitis, pneumonia, endocarditis and local suppuration supervene. The blood during the acute stages shows a definite lymphocytosis with reduction in the number of polymorphonuclear leucocytes. The mortality rate is very low; death, when it occurs, is almost invariably the result of complications.

Ætiology. Many attempts have been made to transmit the disease to various animals. Granata (1908) injected the filtrate after passage through a

Berkefeld filter, of the saliva obtained from a case in the acute stages intradermally into rabbits and produced pyrexia ; when the inoculation was made into the parotid gland a non-suppurative parotitis resulted. Saliva, treated in the same manner, from normal individuals, did not produce these effects. Nicolle and Conseil (1913) inoculated the parotid glands of three monkeys with fluid obtained from puncture of the parotid glands of patients during the acute stages of the disease and produced a febrile condition lasting a few days after an incubation period of sixteen to thirty days ; in one monkey a unilateral parotitis developed. Gordon (1914) obtained nasopharyngeal washings from ten cases of mumps and freed them from bacteria by passage through a Berkefeld filter ; a small amount of the filtrate was injected intracerebrally into separate monkeys. At the end of three to four days five monkeys showed meningitic symptoms ; four died, and in one of these an acute interstitial parotitis was present. Further transmission experiments were unsuccessful. Wollstein (1916) found rabbits and monkeys unsatisfactory, and consequently made an extensive study of the experimental disease in cats. She obtained saliva from acute cases of mumps, filtered it, and then injected it into the testes and parotid glands of these animals. Swelling was produced in these organs after five to nine days ; this condition was also reproduced on subsequent passage through the cats. Wollstein (1918) later obtained evidence of the presence of the virus in the blood of severe cases by the use of a similar technique.

The results just mentioned, especially those of Wollstein, are very suggestive of the " virus " origin of the disease. Kermogant (1925), however, obtained results which indicated to him that the causative agent was a spirochæte. By the use of the centrifuge he concentrated the virus from the buccal washings of patients in the deposit, which he employed for the intrabuccal inoculation of monkeys. On cultivation anaerobically he obtained a spirochæte growing in symbiosis with a minute Gram-negative bacterium ; inoculation of the culture reproduced the disease in monkeys. He also found that while filtration through Chamberland L_2 and L_3 candles removed the bacterium, the filtrate was still active on inoculation, and by cultivating it with the bacterium he obtained a culture of the spirochæte. He was, however, unable to demonstrate this organism in the tissues affected during mumps. The exact relationship of this organism with the disease is thus still uncertain.

Johnson and Goodpasture (1934) have recently published further evidence supporting the virus-theory of ætiology. Saliva obtained from definite cases was injected into the parotid glands of *M. rhesus* monkeys through the duct of Stensen ; filtrates of the saliva, after passing through Berkefeld " N " filters, were also used. As controls the saliva of normal persons was injected—these results were invariably negative. A disease comparable to mumps in man was induced in the test animals. As the examination for demonstrable micro-organisms, including spirochætes, and also the herpes virus, was

negative, it was concluded that the causative agent of mumps must be a filterable virus.

Immunity. An attack of mumps is succeeded by a high degree of immunity, as evidenced by the rarity of second attacks. It has consequently been suggested that convalescent serum should be employed for prophylaxis and treatment of the disease.

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DENGUE FEVER

Dengue fever, a mosquito-borne disease occurring in the tropics, is characterized by a short fever with severe pains in the bones, followed by the appearance of a rash. Epidemics, which may reach alarming proportions, tend to occur at intervals. The mortality rate is, however, very low. The time and extent of the epidemics depend to a large degree on the distribution and activity of the main distributing agent—the mosquito *Ædes ægypti* or *argenteus*. There is no evidence of spread by contact or fomites. The *rôle* played by the mosquitoes has been fully reviewed by Siler, Hall and Hitchens (1925).

After an incubation period of three to six days, there is a sudden onset, the initial signs being headache, fever and pain in the bones. The temperature subsides after two or three days with remission of all symptoms. There may, however, be a secondary rise of temperature to 102° F. on the fifth or sixth day, when an indefinite type of rash appears. The symptoms again subside and convalescence, which may be very prolonged, ensues. The similarity of the clinical picture to that found in the recently described Rift Valley fever has already been mentioned (p. 139).

As in the absence of complications the disease is never fatal, the nature of the lesions has received scanty attention, and no characteristic changes have been recorded.

Ætiology. Man only is definitely known to react to the virus ; results with laboratory animals have been either negative or inconclusive (Blanc, Caminopetros and Manoussakis, 1928). Ashburn and Craig (1907) transmitted the disease to human volunteers by the inoculation of blood from cases of dengue, and also reported one case out of nine volunteers who were bitten by infected mosquitoes (*Culex fagitans*). These volunteers had, however, previously passed through an epidemic of the disease, and, although they did not display any manifestations of the disease, they probably acquired at that time some degree of immunity to the disease. Cleland, Bradley and McDonald (1916 and 1919) transmitted the disease by infected mosquitoes, and also found that the disease could be reproduced in human

volunteers by the inoculation of blood removed from patients in the early stages of the disease. Evidence that the causative agent is filterable has been obtained by Ashburn and Craig (*loc. cit.*), Kligler and Ashner (1928), and others.

These findings indicate that the ætiological agent is probably a filterable virus, and, as the disease is transmitted by mosquitoes, the identity of the causative agent with that of yellow fever has been suggested. The evidence at present available does not, however, appears to favour such a view. Both diseases are chiefly transmitted by the same mosquito, *A. ægypti*, but the clinical courses of the two conditions are very dissimilar. Duval and Harris (1924), by the use of the Noguchi technique, cultivated "*globoid bodies*" from infected blood and claimed that these bodies were related to the ætiological agent. These results have not been confirmed. Sellards and Siler (1928) found Rickettsia bodies in the gut of infected mosquitoes; these bodies were not found in control mosquitoes. The relationship of these bodies with the disease is uncertain.

Immunity. An attack of dengue is followed by an immunity of varying duration (two months to several years).

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PAPPATACI FEVER

Pappataci or sand-fly fever is a tropical disease transmitted by sand-flies, and is characterized by its sudden onset and short duration. The disease is endemic in tropical countries, where the distribution is governed mainly by the habits of the sand-fly (genus *Phlebotomus*). These insects tend to remain localized round their breeding places—old rubble walls, cavities and similar places.

The onset of the disease is sudden; within twenty-four hours of the insect bite, fever, irritability, muscular pains, congestion of the conjunctiva and general lassitude are found. The blood at this stage shows a leucopenia with an absolute decrease in the number of polymorphonuclear leucocytes. In some cases an erythematous rash may appear. After two days there is a drop of temperature, and there may be a tendency to epistaxis and vomiting. There is, as a rule, quick recovery.

As the disease is never fatal the morbid anatomy has not been investigated.

Ætiology. Laboratory animals are not susceptible to this disease (Birt, 1910). Human

volunteers have been consequently employed for experimental purposes. Doerr (1908), by subcutaneous inoculation of small amounts of blood, obtained from infected persons, reproduced the disease in susceptible persons after an incubation period of three to seven days. By filtration he also found that the infective agent was present mainly in the plasma and not the corpuscles of the blood. Kligler (1928) showed that the causative agent would pass through a Berkefeld " N " filter. These observations are indicative of a filterable virus origin of the disease.

Whittingham (1923) obtained cultures of leptospira from the blood removed on the first day of fever, and he considered that these organisms were concerned in the ætiology of the disease. Kligler and Ashner (1928) and many other workers have not been able to confirm these observations. It is considered probable that the disease investigated by Whittingham was not pappataci fever but one of a similar nature which was described by Fletcher (1928).

Immunity. An attack of the disease is usually succeeded by a high degree of immunity.

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EPIDEMIC ENCEPHALITIS

Epidemic encephalitis or encephalitis lethargica is an infectious disease of the central nervous system involving mainly the grey matter of the brain. This disease has only been recognized as a distinct clinical entity since 1917, when v. Economo described the condition, which he had observed in a small outbreak in Vienna; lethargy was here a prominent symptom and the disease was consequently termed "Encephalitis Lethargica." Shortly before this Cruchet, Montier and Calmette (1917) had recorded forty cases of "subacute encephalomyelitis" occurring during the winters of 1915–16 and 1916–17 in certain districts of France. These cases have since been recognized as probable cases of "epidemic encephalitis." Since 1917 epidemics of varying severity have been recorded in most parts of the world. The incidence of the disease is greatest in young adults, but the mortality rate is greatest at the extremes of life. There is a tendency to a seasonal distribution, as the majority of cases have occurred during the cooler months of the year. The incidence of the disease appears to have decreased in recent years. In this country the peak year was 1924, when 5,036 cases were reported, whereas in 1932 only 564 were recorded.

Epidemic encephalitis displays a remarkable variety of symptoms. This is not altogether surprising, as the disease process may involve any part of the upper central nervous system. As a result of the marked diversity of clinical signs many

authors have attempted to classify the disease according to the different manifestations exhibited, but unfortunately these attempts have not proved entirely satisfactory. However, the symptoms may be divided into two groups: (1) those due to a general infection; (2) those due to a localization of the lesions in the central nervous system. For a detailed account of the disease reference should be made to the excellent monographs on the subject by Parsons (1922), Hall (1924), and v. Economo (1931). The onset may be sudden or gradual. When sudden, headache, vertigo, syncope, convulsions, delirium may appear, singly or in combination. Such an occurrence is not very common, the onset being usually more gradual, when there may be general malaise, headache, fever, vomiting, lethargy by day, with nocturnal insomnia and visual disturbances, such as blurred vision and diplopia. Other signs due to localized cerebral lesions may be present. Myoclonus, choreiform movements, and tremor are comparatively frequent. Hiccup is also common, and certain authors have considered that there is a separate disease, " Epidemic Hiccup "; the evidence for such an assumption is not satisfactory, and the accepted opinion at present is that " hiccup " constitutes a mild form of epidemic encephalitis (Brain and Strauss, 1930).

The cerebrospinal fluid shows only a slight change—faint increase in globulin content with a slight mononuclear pleocytosis. The mortality rate is high, death occurring in about 30–60 per cent. of cases. In those cases recovering, the

occurrence of sequelæ is of great importance, and these are found in about 30–50 per cent. of cases. These after-effects succeed various intervals of apparent recovery, and may be very severe. The most important are " Parkinsonism," mental changes and ocular disturbances.

The lesions produced in the disease process have been carefully investigated by Buzzard and Greenfield (1919), Da Fano (1921), and others. Macroscopically the changes are very slight ; on section of the brain congestion and minute hæmorrhages may be observed. Microscopically the lesions are usually most pronounced in the upper part of the mid-brain, the pons and the medulla. The main changes are : engorgement of the vessels with mononuclear infiltration in the Virchow-Robin spaces, infiltration of the nervous tissue with small round cells, found particularly in the grey matter, degeneration of the nerve cells with occasional neuronophagia, but not to any marked degree.

Ætiology. As no definite causative agent has been isolated, many theories have been formulated as to the ætiology of the disease. Some workers have suggested that epidemic encephalitis is a cerebral form of poliomyelitis, but tangible evidence to support such a claim has never been produced. The theories meriting consideration are :—

(1) **Distinct Virus.** Loewe, Hirshfeld and Strauss (1919) inoculated monkeys and rabbits with brain tissue and filtered nasopharyngeal washings from human cases and produced definite hæmorrhagic lesions in the brains of a few of these animals

suggestive of encephalitis. As a result of these experiments they claimed that the disease was caused by a filterable virus. In view of the more recent research it is considered that, when the inoculations were successful, these workers were probably dealing with the virus of herpes (*vide infra*). By the use of the Noguchi medium globoid bodies were later cultivated from apparently infected material.

McIntosh (1920) claimed that he had transmitted the disease to monkeys ; his experiments, however, were unfortunately only carried out on a small scale, and are consequently of doubtful value. Many workers, including Dible (1925), have been uniformly unsuccessful in their efforts to transmit the disease to laboratory animals.

Kling, Davide and Liljenquist (1921) stated that they had transmitted the disease to rabbits through several generations. Subsequent examination of the brains of these animals revealed the presence of the *Encephalitozoon Cunculi* (Levaditi, Nicolau and Schoen, 1924). As these sporozoa are a frequent cause of spontaneous encephalitis in rabbits (McCartney, 1924), the results obtained by the use of these animals are of uncertain value.

(2) **Relationship with the Virus of Herpes.** The herpes virus has been isolated on a few occasions from the brain tissue and the nasopharyngeal washings obtained from cases of encephalitis (Levaditi and Harvier, 1920 ; Doerr and Schnabel, 1921 ; Bessemans and Boeckel, 1923 ; Perdrau, 1925 ; and others). Many of these workers consequently con-

sidered that the herpes virus was the causal agent of the disease. Levaditi elaborated a theory which postulated that the strain of herpes virus responsible for epidemic encephalitis was one which had a special affinity for the nervous system, and was termed the neurotropic strain. This, he maintained, was quite distinct from the usual dermotropic strain.

On the other hand, many workers, including Flexner and Amoss (1925), were uniformly unsuccessful in their efforts to isolate the herpes virus from tissue obtained from encephalitis patients. Moreover, Flexner (1923) isolated a strain of herpes virus, which was capable of producing a meningo-encephalitis in rabbits, from the cerebrospinal fluid of a case of cerebral syphilis. This indicated that the herpes virus could be found in the central nervous system as a secondary or even a passive invader. This explanation was given by Flexner and his colleagues to account for the presence of the virus in the few cases of epidemic encephalitis described by the other workers. The American workers were also unable to find any difference, other than in virulence, between the two strains described by Levaditi.

Other facts which do not support the relationship of the herpes virus with epidemic encephalitis are: (1) inclusion bodies, which are characteristic of herpes, have never been demonstrated in lesions of human encephalitis (Parker, 1924); (2) the herpes virus has been isolated from the nasopharyngeal washings of normal individuals (Levaditi, Harvier and Nicolau, 1922); (3) facial herpes has not been

observed with any frequency in cases of encephalitis ; (4) Zinsser and Tang (1929), and Andrewes and Carmichael (1930), by neutralization tests on the herpes virus with serum obtained from normal individuals and recovered encephalitis cases, were unable to establish any indication of relationship between the herpes virus and encephalitis. It is thus seen that the identity of the virus of herpes and the causative agent of encephalitis is by no means settled.

The recent production of an encephalitis in Cebus monkeys, in which the histological picture resembled that found in herpes encephalitis, by the intracerebral inoculation of the herpes virus, may reopen the question (McKinley and Douglass, 1930).

(3) **Relationship with Influenza.** This question has been raised owing to the fact that the pandemic of influenza in 1918 coincided with the general onset of encephalitis (Flexner, 1928). There is, however, little for, and much against, such a relationship. The two diseases are clinically quite distinct ; influenza is extremely contagious, encephalitis is rarely so ; the lesions of cerebral complications of influenza are quite distinct from those of encephalitis ; subsequent epidemics of the two diseases have been quite independent in point of time.

(4) Various bacteria, particularly streptococci, have been isolated from the brains of encephalitis patients at autopsies. Claims have been repeatedly made that these organisms are ætiologically related to the disease. These claims have not been substantiated by the majority of workers.

The present position of the ætiology of the disease was reviewed by the Matheson Commission (1932), and the following opinion expressed: "Again it is necessary to conclude that the question of the ætiology of epidemic encephalitis is still unanswered."

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AUSTRALIAN X DISEASE

In 1917 and 1918 a peculiar acute inflammatory disease of the nervous system was observed in Australia, the exact nature of which is still uncertain. The condition also appeared again in that country in 1925 (Kneebone and Cleland, 1926). The disease appears to have been strictly limited to Australia.

The onset was frequently abrupt with the appearance of convulsions and pyrexia passing on to a state of coma; eye symptoms were frequently absent. Death supervened rapidly in most cases, the mortality rate being 80–90 per cent.

The pathological investigations were unfortunately rather incomplete. The main changes were observed in the brain and consisted of marked congestion of the veins with perivascular infiltration; changes in the nerve cells were slight.

Ætiology. The indications from the limited amount of experimental work performed are that a separate distinct virus was concerned in the ætiology of the disease. Cleland and Campbell (1919), by intracerebral inoculation of bacteria-free nervous tissue from typical cases, transmitted the disease to

monkeys (*M. rhesus*) and sheep; a certain number of the latter (some 50 per cent.) were, however, found to be insusceptible to the disease. Kneebone and Cleland (1926) also transmitted the disease to sheep.

While the sheep transmission experiments were not absolutely decisive, they appear to be sufficient to indicate that the causative agent was different from that of epidemic encephalitis and poliomyelitis. Flexner (1923), however, considered that the agent might possibly be an aberrant form of the poliomyelitis virus; this seems to be a rather remote possibility.

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HERPES ZOSTER

Herpes zoster or shingles is an acute infection characterized by a vesicular eruption of the skin accompanied by pain around the infected region. Zoster is not a highly infectious disease; small epidemics have been described, but they are extremely rare.

The vesicular eruption is frequently unilateral, and is often preceded by local hyperæsthesia, itching or pain. The pain is referable to one or more nerve roots of the nerves supplying the involved skin region. The vesicles rapidly become pustular, dry up and form crusts in a few days. The regional

lymph glands are frequently enlarged. Recurrence is rare. Zoster may be divided into two main classes: (1) idiopathic; (2) symptomatic, occurring during the course of other infections or intoxications.

The most important changes are found in the central nervous system, where the lesions are distributed in the posterior root ganglia and posterior horns, and consist largely of perivascular infiltration with scattered areas of mononulcear infiltration (Wohlwill, 1924). The skin lesions are similar to those found in herpes simplex.

Ætiology. Efforts to transmit the disease to laboratory animals have not been successful (Cole and Kuttner, 1925, and others). Marinesco and Draganesco (1923), however, claimed that in a few instances corneal scarification and inoculation of rabbits gave rise to areas of infiltration and opacity; transmission in series was not possible. The nature of the causative agent is therefore still undecided. Bacteria have been definitely excluded as the ætiological agents. As claims have been made of successful transmission of the disease to children by the inoculation of the vesicular fluid (Kundratitz, 1925), the process appears to be of an infectious nature.

The indications are that a filterable virus is involved, although filtration experiments have not yet succeeded, but whether this virus is definitely specific or is related to some other virus is still open to question. The general opinion is that the viruses of herpes simplex and herpes zoster are quite distinct (Levaditi, 1926). The arguments upholding

this view are: (1) herpes simplex is easily transmitted to laboratory animals, while zoster is not; (2) herpes zoster does not recur; recurrence is a prominent feature of herpes simplex.

The same certainty does not hold so far as the relationship with the virus of varicella is concerned. The diversity of opinion on this matter may be gathered from the opinions of two authors of recent reviews of this question: Bedson (1930) concluded from evidence available that the virus of all types of zoster is the same as that of chicken-pox. McKinley (1929), on the other hand, considered that herpes zoster and varicella are independent diseases caused by different agents. Evidence in support of the former hypothesis appears to be accumulating—by complement-fixation tests it has been found that the serum of patients, recovered from varicella, reacted equally with antigens prepared from both varicellar and zoster lesions (Netter and Urbain, 1931); the inoculation of susceptible children with zoster material resulted in the development of chicken-pox, while the inoculation of children recovered from chicken-pox produced negative results. If the two viruses are related, it is obvious that a considerable modification in infectivity must have taken place. The varicella virus is extremely infectious and attacks infants, whereas the infectivity of the zoster virus is relatively low and the disease is frequently seen in adults.

Immunity. *H. zoster* is apparently succeeded by a comparatively high degree of immunity, as second attacks are uncommon.

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VARICELLA

Varicella or chicken-pox is an extremely contagious disease frequently found in children and characterized by a cutaneous eruption.

After an incubation period of ten to twenty-one days, the onset is characterized by slight prodromal symptoms as pyrexia, vomiting and muscular pains. Within twenty-four hours the eruption appears on the body, and at the commencement it is papular, but rapidly becomes vesicular and then pustular, followed by drying up and scab formation. Complete recovery is the rule in the absence of complications, which are extremely rare.

Little attention has been paid to the general pathology of this condition. The most interesting feature is the appearance of nuclear inclusion bodies in the epidermal cells and endothelial cells of the corium. These have been studied in detail by Gins (1918), Rivers (1926), and others.

Ætiology. Little is known of the nature of the causative agent of this disease. Transmission experiments with laboratory animals have not been very successful, but Rivers (*loc. cit.*) reported the production of lesions, with the presence of intranuclear inclusions in the testicles of vervet monkeys, as a result of intratesticular inoculation of emulsified chicken-pox lesions. Transmission experiments by the intradermal inoculation of children have, however, been successful; Greenthal (1926) obtained positive results by the intracutaneous inoculation of vesicular fluid. The vesicular fluid in all cases was bacteriologically sterile, and the indications are that this disease is due to a filterable virus. Reference has already been made to the probable relationship with herpes zoster.

Immunity. An attack of varicella is succeeded by a high degree of immunity ; second attacks are rare. The use of convalescent serum has been suggested for the treatment of the disease (Gunn, 1932).

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INFLUENZA

Influenza is an acute contagious febrile disease characterized by its sudden onset and the production of extreme prostration. Influenza is present all over the world, and epidemics of varying dimensions are

always occurring. Periodically epidemics of alarming proportions may develop, such as the pandemic of 1918–19. The spread appears to be mainly from direct contact. This question is considered in detail in the Ministry of Health Report (1920).

The incubation period is short, lasting a few days only. The onset is almost invariably abrupt and is signalled by the appearance of headache, nausea, fever, with vague muscular or joint pains; sore throat, catarrh and intestinal disturbance may also be present. The chief symptoms tend to vary in the different outbreaks, being as a rule either intestinal, respiratory or muscular. In the absence of an epidemic the determination of this disease is very difficult. Influenza itself is rarely fatal, but complications of a serious nature are very common, especially those affecting the respiratory organs, and account for the high mortality rate of the disease.

In uncomplicated cases there is usually hyperæmia of the trachea and bronchi with inflammatory swellings of the nose and neighbouring sinuses. In fatal cases œdema of the lungs and, or, patches of bronchopneumonia are frequently found.

Ætiology. Influenza has not been transmitted to any of the lower laboratory animals with regularity, and consequently research on the ætiology of this disease has been severely hampered. As nothing of a conclusive nature has been obtained the question of ætiology is still under dispute. Three of the main agents, which have been considered in this respect, will be briefly discussed.

(1) *H. Influenzæ.* Many years after Pfeiffer (1893)

had isolated this organism from cases of influenza, it was generally accepted as the ætiological agent of this disease. During the pandemic of 1918–19 the *rôle* of this organism became the subject of close investigation; conclusive evidence, however, that it was the direct causative organism of the disease could not be obtained. A few samples of the many investigations performed at this time are: Mandelbaum (1918) examined sputum and nasopharyngeal washings from many cases without isolating *H. influenzæ*; Loewenhardt (1920) isolated this organism from the sputum of 122 out of 160 cases. The figures recorded in America gave a higher proportion of positive results, probably through the selection of more suitable media for the cultures; Park (1919) obtained 80–100 per cent. positive results from examinations made during different outbreaks; Pritchard and Stillman (1919) found 83–93 per cent. positive. By examination of the respiratory organs from cases at autopsy, *H. influenzæ* has been recovered with great frequency (Fildes and McIntosh, 1920). However, it has also been shown that this organism can be readily isolated, by using suitable media, from the throats of normal individuals (Fleming and McClean, 1930; Ministry of Health Report, 1930). Thus the appearance of this organism in the nasopharynx of influenzal patients is in itself of doubtful value in considering the ætiology of the disease.

Some experimental evidence, suggesting that *H. influenzæ* is the causal agent of the disease, has been derived from the swabbing of the throats of

human volunteers with cultures of this organism (Cecil and Steffen, 1921). The resulting infections, while resembling influenza, were not absolutely characteristic of the epidemic disease. Wahl, White and Lyall (1919), however, had previously found that the introduction of living *H. influenzæ* on to the nasopharyngeal mucous membrane of a few healthy individuals failed to produce any abnormal symptoms.

Thus while it is probable that *H. influenzæ* plays an important *rôle* in epidemic influenza, whether this is primary or secondary cannot be determined on the evidence available. There is no doubt that the influenza bacillus is not invariably a saprophytic organism; it is frequently the causative organism of post-influenzal broncho-pneumonia.

(2) *Bacterium Pneumosintes.* Olitsky and Gates (1921), using the Noguchi-culture technique, isolated a minute, Gram-negative cocco-bacillus from nasopharyngeal washings obtained from cases in the earliest stages of influenza. This organism was found to pass through the ordinary bacterial filters, and was cultivated, when first isolated, under anaerobic conditions only. The organisms on intratracheal injection in rabbits gave rise to hæmorrhagic lesions of the lungs. As these workers failed to isolate this organism from the nasal secretions and nasopharynx in other conditions, they concluded that *Bact. pneumosintes* was ætiologically related to influenza. Subsequent work has failed to confirm these observations (Garrod, 1928; Mills, Shibley and Dochez, 1928); whether this failure can be explained

by the technical difficulties involved is uncertain. The evidence at present available appears insufficient to indicate that this organism plays a causal *rôle* in influenza ; it would appear that *Bact. pneumosintes* is a member of the normal flora of the upper respiratory tract.

(3) *Filterable Virus.* The doubt cast on the connection of *H. influenzæ* with the disease directed attention to the possibility that a filterable virus might be the ætiological agent. The results, here as in other cases, have been inconclusive. The inoculation of filtered nasopharyngeal washings from influenza patients produced febrile disturbances in human volunteers (Nicolle and Lebailly, 1918, 1919 ; De La Rivière, 1918). Evidence of a more convincing nature has recently been obtained in this country by Smith, Andrewes and Laidlaw (1933). These workers were able to transmit in series a febrile disease in ferrets by the intranasal instillation of filtrates of throat washings obtained from influenzal patients. This disease was produced by five out of eight throat washings from influenza patients obtained in the early stages of the disease ; throat washings from normal individuals gave negative results ; the serum of influenza convalescents was also found to be capable of neutralizing the virus. As a result of their experiments these workers suggested that influenza in man is caused by a filterable virus, infection by which facilitates the invasion of the body by bacteria, so giving rise to the various complications.

The ætiology of influenza must, however, be still

considered unsolved until the experiments suggesting the participation of a filterable virus have been extended and confirmed. In the absence of an epidemic it is difficult to obtain material owing to the uncertainty in the diagnosis of sporadic cases.

Immunity. An attack of influenza confers a doubtful immunity of rather short duration. The occurrence of two or more attacks in the same person is not infrequent.

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ACUTE CORYZA

Acute coryza or common cold is an acute infectious disease affecting the mucous membrane of the upper respiratory tract. Infection usually takes place by direct contact from man to man. The symptoms, although rather trivial, frequently give rise to much local discomfort. Catarrhal inflammation of the nasal and nasopharyngeal mucosa, frequently followed by a muco-purulent discharge, is found.

Ætiology. Although the condition is common and the lesions readily accessible, the ætiology of the common cold is not definitely established. Numerous attempts have been made to incriminate a definite bacterium, but without much success. Examinations of the bacterial flora of the affected parts in different outbreaks have yielded very discordant results, and also do not indicate that any single organism is the sole primary agent of the disease (Shibley, Hanger and Dochez, 1926; Ministry of Health Report, 1930; Kneeland, 1930).

Attention has consequently been directed to the claims of a filterable virus. Kruse (1914), and Foster (1918), as a result of experiments performed on human volunteers, suggested that the primary infection was due to a virus. These claims were disputed for a considerable time, and it is only in recent years that this question has received serious consideration. Robertson and Groves (1924) sprayed the nasopharynx of 100 healthy volunteers with the filtrates of nasopharyngeal washings of 11 cases of uncomplicated acute coryza. Only one volunteer

developed coryza, while 95 remained quite well; these workers consequently considered that their results did not indicate the participation of a filterable virus in the disease-process.

The recent work of Dochez and his colleagues (1931) provides apparently conclusive evidence that the primary agent of the common cold belongs to the group of filterable viruses. Nasopharyngeal washings from cases of coryza, passed through Seitz filters, were inoculated intranasally in chimpanzees, and in twenty-four to forty-eight hours the apes began to manifest signs of upper respiratory infection. Similar successful inoculations were also carried out on human volunteers. This virus was also passed through several generations in tissue-culture. These workers consequently considered that in the common cold primary infection was due to a filterable virus, which provoked an increased activity of the potential pathogenic bacteria present in the nasopharynx. The secondary infection was responsible for the purulent inflammation of the nasopharynx and the sinuses. This hypothesis has received support from the recent work of Kneeland and Davies (1932), who investigated the occurrence of colds in an infant population. It was found that in the mild types there was no alteration in the bacterial flora of the nasopharynx, while in the severe forms, however, there was always a marked predominance of a pathogenic bacterium, usually a pneumococcus or *H. influenzæ*. Further support is forthcoming from the results obtained by the inoculation of mixed bacterial vaccines. Many workers have reported

that, while the incidence of the uncomplicated common cold has not been reduced to any appreciable extent by the prophylactic injections of catarrhal vaccines, the duration of the attacks has been shortened and the occurrence of the secondary phenomena considerably reduced.

Immunity. Acute coryza is apparently only succeeded by a low grade of immunity, as repeated attacks are extremely common.

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CHAPTER VIII

DISEASES POSSIBLY CAUSED BY FILTERABLE VIRUSES

TRACHOMA

TRACHOMA is a specific contagious disease characterized by inflammation and hypertrophy of the conjunctiva. The disease is usually found in the poorer districts where hygienic conditions are primitive. The eye secretion is considered to be infective and constitutes an important agent in the transmission of the disease.

The incubation period has not been definitely ascertained, but the indications are that it is of long duration. The onset is insidious and in some cases may escape notice. Three forms are described—papillary, granular and mixed; in most cases the symptoms are both subjective and objective. The subjective symptoms usually consist of photophobia, lachrymation, itching, pain and visual disturbance, either singly or in combination. The objective signs are swelling of the lids, drooping of the upper lid, redness and thickness of the conjunctiva of the tarsus and fornix, due to hypertrophy, with the appearance of granules. The granules and papillæ disappear, but the conjunctiva does not return to normal but becomes cicatrized. The formation of

scar tissue constitutes an important diagnostic point. Relapses are frequent, and local complications frequently ensue.

The changes mainly found in the conjunctiva are those of proliferation with diffuse and local areas of mononuclear infiltration.

Ætiology. Prior to 1927 little of a definite nature was known about the ætiology of trachoma; transmission experiments to laboratory animals had been very inconclusive. Inclusion bodies in the conjunctival epithelial cells, first described by Halberstaedter and Prowazek in 1907, have been found repeatedly, and, as bodies of a similar nature had been described in several diseases due to filterable viruses, such an ætiology was considered possible for trachoma. However, Noguchi (1925), as the result of a careful and elaborate investigation, isolated a small Gram-negative bacillus from material removed from the eyes of American Indians suffering from trachoma. By subconjunctival inoculation of this organism, which he termed *Bact. granulosis*, he produced a condition resembling trachoma in monkeys (*M. rhesus*). As a result of his findings, Noguchi considered that this organism was ætiologically related to the disease. Attempts to repeat this work in many laboratories were uniformly unsuccessful; McCartney and Mayou (1930), moreover, considered that the conjunctival reaction produced by Noguchi by the inoculation of the organism was not absolutely typical of trachoma. Recently Weiss (1933) has been able to confirm Noguchi's work, and he suggested that three factors were mainly responsible

for the failure of other workers to obtain successful passage experiments. These were :—

(1) The infrequency of isolation of *Bact. granulosis*, probably due to the technical difficulties involved ;

(2) The loss of virulence of the organism when cultivated in artificial media ;

(3) Many individuals of the species of monkey (*M. rhesus*) employed in experimentation are naturally resistant to infection.

In order to overcome the natural resistance of the monkeys, the conjunctiva was irritated with cement prior to the inoculation with the organism. Attempts made to demonstrate the existence of a filterable virus and also those designed to demonstrate the existence of inclusion bodies were unsuccessful. Weiss consequently concluded that the *Bact. granulosis* was the causative agent of the disease. Further work is, however, necessary before the participation of a virus can be definitely excluded.

Immunity. Recovery from trachoma is usually followed by a high grade of immunity.

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ACUTE DISSEMINATED ENCEPHALOMYELITIS

Acute disseminated encephalomyelitis is a disease of the central nervous system, which may occur

either spontaneously or in the wake of one of the exanthemata.

The disease has received much prominence in the last decade owing to its not infrequent occurrence in children and young adults after vaccination with calf-lymph. This association was first observed in this country by Turnbull and McIntosh (1926), who collected a series of cases and found that the same histological picture was also present in a case, diagnosed in 1912 as poliomyelitis following vaccination. Since 1922 cases have been reported in most civilized countries, particularly in Holland, Germany and Great Britain. While the total incidence of the disease is low it has been sufficiently frequent to merit serious consideration. The following figures indicate the incidence of the disease in England and Holland during the period 1923–27 :—

TABLE IV

	People vaccinated.	No. of cases.	No. fatal.	Case rate.
England and Wales .	4,540,000 (approx.)	93	51	1 : 48,000
Holland .	495,000 (,,)	124	38	1 : 4,000

The relative incidence of post-vaccinal encephalomyelitis was thus approximately ten times greater in Holland than in this country over the same period of time. The disease was present during the period 1927–29 to approximately the same extent ; in this country ninety cases were recorded, of which twenty-

five were fatal. Since 1929, however, there appears to have been a gradual decrease in the incidence of the disease, and at the present time cases are extremely rare.

The age at which the disease was found varied in different countries; individuals over three years appeared most susceptible. The following table gives the age distribution of the cases notified in Holland and England (1923–27):—

TABLE V

Age group, years.	No. of cases.	
	Holland.	England.
0–1	1	9*
1–3	8	3
3–6	98*	2
6–12	16	45
12–	—	34

* = largest number of vaccinations performed on the general population at that period.

It is seen that in England while vaccination was mainly performed on children under six months, the incidence of the disease was low at this stage; this provides strong evidence in favour of early primary vaccination. In Holland the largest number of vaccinations was carried out on children between the ages of three and six, at which period the greatest number of cases of encephalomyelitis occurred.

The onset of the disease is abrupt, usually seven to fifteen days after vaccination, the average time being eleven days. The vaccination usually runs a normal course, and is settling down when headache, vomiting temperature and lassitude leading to drowsiness, and, or, paresis in one or more limbs appear. Encephalitis and myelitic forms may be separate or combined. The mortality rate is high; 30–60 per cent. of cases end fatally after a rapid course. When recovery occurs this takes place slowly, and is usually complete; the sequelæ so common after epidemic encephalitis are rarely encountered.

The characteristic post-mortem changes are found in the central nervous system, and, in contradistinction to the definite neurotropic virus diseases, they are situated in the white matter. The principal lesions are extra-adventitial perivascular infiltration usually associated with perivascular demyelination. The nerve cells are only slightly involved. In view of the nature of the lesions, Marsden and Hurst (1932) have suggested the term "Acute Perivascular Myelinoclasis" for the condition; they state: "the term 'acute disseminated encephalomyelitis' commonly used in reference to these cases, contains nothing to indicate the specific nature of the lesions by which the disease is differentiated from others to which the same title has been applied. In the absence of definite knowledge of their ætiology it is by no means certain that the lesions are primarily inflammatory in nature. In all the encephalitides attributable with certainty to a virus, the primary attack is on the grey matter; this is true also of

those due to the majority of bacterial or inorganic toxins. Moreover, since the disease under discussion, excited by a rather heterogeneous group of factors including vaccinia, measles and antirabic inoculations, is in essentials always the same, it is clear that we are dealing with a single clinical and pathological entity; this should be recognized by the use of a single comprehensive term embracing all the cases whatever their exciting cause. We propose, therefore, to use in this paper the term 'acute perivascular myelinoclasis' as indicating its essential features. . . ."

Ætiology. The ætiology of this condition has been the subject of extensive investigations in recent years, but the results have generally been inconclusive. Most of the work has been carried out with a view to determining the nature of the disease following vaccination, and for detailed accounts the reports of the Vaccination Committee (1928, 1930) should be studied. The main theories formulated are briefly :—

1. *Vaccinia Virus itself directly responsible.*

For : (*a*) The uniform symptomatology, which is quite distinct from the diseases caused by other neurotropic viruses, *e.g.*, poliomyelitis, encephalitis lethargica.

(*b*) The fairly constant "incubation period"—eleven days after vaccination.

(*c*) Nervous involvement has also been recorded during and after attacks of small-pox.

(*d*) Vaccinia virus has been found in a few of the brains of these cases, but no evidence of

the presence of other viruses has been detected.

(*e*) Vaccinia virus can produce encephalitis in rabbits and monkeys under certain conditions.

(*f*) Clinical evidence suggests that each type is caused by special virus.

Against : (*a*) A similar condition has been found in association with measles, influenza, variola and rabies treatment, and the disease may even occur spontaneously. Also, why was the disease not evident before 1922 in similar numbers ?

(*b*) The histological picture is almost identical in all the above conditions ; this is suggestive of a common origin.

(*c*) The vaccinia virus generalizes after introduction into the skin, and in animals the presence of virus in brain is a common occurrence after subcutaneous or interdermal inoculation.

(*d*) Vaccinial encephalitis in animals, that is, the encephalitis produced by the intracerebral inoculation of the vaccinia virus, shows an entirely different histological picture from above ; demyelination has never been found, the essential lesion is a meningo-encephalitis.

(*e*) The same batch of lymph has frequently been used on other people without any untoward effects. While the condition has been produced by the various preparations of the

vaccinia virus, such as vaccine-lymph and neurovaccine.

2. *Theory of Fortuity.* That is, the accidental occurrence of a nervous disease at the time of vaccination.

Against: (1) The almost constant "incubation period" or, to be more exact, the time of occurrence after vaccination.

(2) The lack of similar cases of like nature in districts where cases are present or in the community as a whole in the absence of vaccination.

3. *Activation of a Latent Virus.* This theory is unsatisfactory, as no evidence of the presence of another virus in any of the tissues has been demonstrated; experiments in rabbits and monkeys have all been negative. It is suggested by the supporters of this theory that resistance of body is lowered by the vaccinia virus, and this allows the hypothetical virus to exercise its pathogenic properties.

4. *Activation or Action of a Toxin.* No evidence has been obtained to support this conception, but the similarity of the histological changes to those found in disseminated sclerosis must not be ignored.

It can be gathered from the diverse theories formulated that the ætiology of the disease is still obscure.

Some interesting observations with regard to the prophylaxis and the treatment of the disease have, however, been made.

Prophylaxis. Certain regulations, which have been considered in the description of "small-pox,"

should be observed in the administration of vaccine-lymph (see p. 122). The type of lymph has not influenced the results, but it is hoped that the use of a smaller dose of virus might prove practicable. Attention to these factors has probably played a *rôle* in the decreased incidence of the disease.

Serum Therapy. It has been found recently that the use of antivaccinial serum, obtained from parents previously vaccinated, has been employed with very good results (Hekman, 1930). The explanation of this is not obvious, but it is probable that the results are due to some action of the serum on the vaccinia virus.

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PERTUSSIS OR WHOOPING-COUGH

Whooping-cough is an acute infectious disease of childhood, which is characterized by an initial catarrhal inflammation of the nasopharynx and is succeeded by paroxysmal coughs, which terminate in the characteristic whoop. In some cases the only manifestation of the disease is the catarrhal stage, which closely resembles the common cold.

The disease is not in itself fatal. Death follows the onset of complications, the most frequent of which is broncho-pneumonia.

Ætiology. While the ætiology of whooping-cough is still unsettled, the majority of workers consider that the Bordet-Gengou bacillus is the causative agent of the disease. This organism has been isolated in a high percentage of cases by many investigators, but only in the early stages of the disease. Attempts to reproduce the disease in laboratory animals have, however, been unsuccessful. Other workers have not been able to isolate the bacillus from cases even in the early stages; they are consequently not prepared to accept the ætiological relationship of this organism to the disease.

Rich (1932) put forward evidence which indicated to him that a filterable virus was primarily responsible for the disease. He found that the pneumonia following an attack of whooping-cough was an interstitial pneumonia, which type was also encountered in pneumonia following measles, psittacosis, and influenza—all probable virus diseases. He also demonstrated intranuclear inclusion bodies in the enlarged alveolar and bronchial epithelial cells in five cases. The inoculation of chimpanzees with filtered tracheal washings from cases of whooping-cough also suggested the participation of a filterable virus, which had not been proved distinct from that of the common cold. As a result of these findings, Rich concluded that whooping-cough was primarily a virus disease and that the Bordet-Gengou bacillus was a secondary invader; whooping-cough was thus

a disease analogous to the common cold, distemper and influenza.

Whether this theory will stand the test of time remains to be seen.

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