VIRUSES AND VENEREAL DISEASE*

BY

S. P. BEDSON

Some General Characters of Viruses

Before considering in any detail the venereal diseases of virus origin I propose to devote a short time to the characters of viruses in general. These agents differ quite considerably in some ways from other micro-organisms causing disease, and it might be helpful to a better understanding of the specific problem of viral venereal disease if it were viewed against this more general background. Despite the great amount of work which has been done on viruses it is still not possible to say definitely what is their nature, though most authorities nowadays consider that they are micro-organisms. Apart from this doubt as to their nature a great deal of exact information concerning viruses has been accumulated over the last twenty years and it is known that they possess in common certain characters which mark them off from other living agents of disease.

The Size of Viruses.—Of these characters one of the most important is size. Viruses are all very small, much smaller than the smallest bacterium, and it is due to this that they are filterable. In virus work the unit of measurement in the $m\mu$, or millionth part of a millimetre, and it is known that viruses vary in size from 10 $m\mu$ to about 300 $m\mu$, the smallest bacterium being some 700 $m\mu$ in shortest measurement.

Three methods exist for measuring viruses. One is by filtration through a series of filters of graded porosity, so constructed that their pore-size shows little deviation from the mean and that factors other than size which determine whether or not particles will go through—adsorption for example—are reduced to a minimum. The gradocol collodion filters devised by Elford comply with these requirements and are in general use for this purpose. Another method of estimating the size of viruses is by submission to centrifugal force and calculation of the rate at which they are thrown down. A third

method is by direct measurement on photomicrographs, and this raises the question of the visibility of viruses by means of the microscope. Although claims to have stained and seen certain viruses with the microscope were made over 40 years ago, and it is now established that the larger ones can be seen by these means, the belief that the viruses are all microscopically invisible—that they are ultra-microscopic—is still quite widely held. Many large viruses, those of the psittacosis-lymphogranuloma group and the pock viruses for instance, can be readily stained and seen, in fact their demonstration by ordinary microscopy is at times used diagnostically. But photomicrographs made with stained preparations are not really suitable for the purpose of measuring the size of virus particles since the size is altered in the process of staining. Photographs of unfixed and unstained virus taken by dark-ground illumination with visible or, better still, ultraviolet light, are suitable for measuring the large viruses; the use of ultraviolet light, of course, requires a special quartz optical system. The estimates obtained by these three methods have shown a very reasonable degree of concordance. Latterly the introduction of the electron microscope, with its immensely greater powers of magnification, has brought even the smallest viruses within the range of visibility and photography. This has not only made possible the measurement by photography of all viruses whatever their size, but has also produced interesting information concerning their shape and structure. For some reason which is not immediately apparent the measurements of viruses obtained by means of the electron microscope have been in excess of the estimates previously made by the other methods.

The Cultivation of Viruses.—Another character of viruses is their inability to multiply apart from living cells. At first this was a great handicap in virus work as the only method available for growing viruses was injection of the living animal and this limited the study of viruses very largely to those for which a suitable animal was available. However,

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as soon as it was shown that tissues could be cultivated *in vitro* the possibility of using this technique in virus work was explored and found to be practicable. Though successful, the first attempts to grow viruses in this way were mainly of academic interest, since the tissue cultures of those days were made with minute fragments of tissue and were consequently incapable of producing virus in any quantity. Out of this work, however, have been evolved two methods which have made possible large-scale cultivation of many viruses in the laboratory. One of these is based on the observation that actively multiplying cells are not essential for virus culture; it is sufficient that they should be alive. And it is now known that many viruses affecting man and other animals will grow well in a medium consisting of tissue mince, commonly chick-embryo mince, in Tyrode's solution, with or without the addition of serum. Vaccinia virus, for example, has been grown in sufficient quantity by this method for large-scale vaccination of man against smallpox. The other method of virus culture makes use of the incubated egg of the domestic fowl or duck. Many viruses will multiply in the cells of one or other of the embryonic membranes, chorio-allantoic, allantoic, amniotic, or yolk sac. Perhaps one of the most valuable methods of inoculating the incubated egg is that in the chorio-allantoic membrane, since many viruses (those of the pock group, for example) produce visible lesions when growing in these cells, and not only does the number of lesions bear a direct relationship to the number of virus particles sown on the membrane, but the lesions themselves are often characteristic of the virus producing them. We have thus in the developing egg a simple and relatively cheap method of cultivating many viruses which also provides, in the case of some viruses, the possibility of estimating the number of virus particles and even of differentiating one virus from another, for example, vaccinia from smallpox.

Inclusion Bodies.—The third character of viruses which should be mentioned is that many viruses when they infect cells and grow in them, produce changes in the cells which are readily recognized by ordinary histological methods. These changes take the form of apparently homogeneous and acidophilic masses of varying size, either in the cytoplasm or nucleus, which are known as inclusion bodies. Not every virus has been shown to produce inclusion bodies, and those that do so, characteristically give rise to either cytoplasmic or nuclear inclusions; few viruses produce both kinds, but smallpox virus is said to do so.

There has been considerable dispute in the past

as to the nature of these inclusions, but it is now known that the cytoplasmic ones are virus colonies, masses of virus particles embedded in a gelatinous matrix known as inclusion material. It is this inclusion material which is responsible both for the apparent homogeneity of inclusions and for their affinity for acid dyes like eosin. By analogy one would expect that nuclear inclusions were also virus colonies, but it is not known whether this is so or not. Incidentally, there is nothing inherently improbable in this since it has been shown that a rickettsia, *R. rickettsii*, of Rocky Mountain spotted fever, when growing in cells in tissue culture, preferably does so inside the cell nuclei.

These then are some of the characters of viruses, and the picture of them that I have attempted to give you is that of very minute micro-organisms incapable of multiplying except in the interior of living cells, where, in all probability, they deviate to their own use the enzyme systems of the cell in order to reproduce.

Venereal Disease of Virus Aetiology

Turning now to the more specific question of venereal disease due to agents of this kind, the first viruses to consider are those of the psittacosis-lymphogranuloma group. This comprises a number of large viruses which not only closely resemble one another morphologically, but also give further evidence of their close relationship in the way they interact serologically. These viruses are some 250-300 $m\mu$ in size, or even larger at times, since when multiplying they show a sequence of developmental forms, the largest of which may even exceed the staphylococcus in size. Unlike other viruses they stain by the methods of Castaneda and Machiavello originally devised for the staining of rickettsia, in fact some authorities consider that this group of viruses is more closely related to the rickettsia than to the other viruses. The group contains the psittacosis viruses, which comprise a number of closely related viruses occurring naturally in a number of bird species including psittacines, pigeons, domestic fowls, ducks, various species of finch, and sea birds, the viruses of feline pneumonitis and of mouse pneumonitis, and several viruses producing pneumonitis in man—Louisiana, Illinois, and San Francisco (S.F.)—which appear to have man as their natural and, possibly, only host. As the name of the group implies, the virus of lymphogranuloma venereum is included, and the viruses of trachoma and inclusion conjunctivitis, which resemble one another closely, also find a place here.

LYMPHOGRANULOMA VENEREUM (L.G.V.)

L.G.V. Virus.—The most important virus of the

psittacosis-lymphogranuloma group for us to consider is that of lymphogranuloma venereum. It has the characters of the group, stains well by Castaneda's method and is readily seen with the ordinary light microscope. Experimentally it will infect the mouse, guinea-pig, and monkey, and will grow fairly readily in the incubated hen's egg, more particularly when injected into the yolk sac. Man is its sole natural host, and infection is transmitted by contact which, in the vast majority of instances, is venereal. It is not definitely established that infection in man is ever inapparent but there is some evidence in support of this and it would be surprising if sub-clinical infection with L.G.V. virus did not sometimes occur.

Laboratory Diagnosis.—I do not propose to mention the clinical aspects of this disease; in fact, it would be presumptuous for me to do so. Instead, I shall pass immediately to a consideration of those laboratory investigations which may aid the clinician to reach a diagnosis in those cases where he is in doubt.

Microscopy.—With such a large virus which stains differentially (Castaneda) it might be expected that the examination of smears made from morbid material would be useful diagnostically. And although this is worth doing in the case of bubo pus, it is difficult to recognize the virus with any degree of certainty unless it is present in considerable quantity. Another worth-while microscopical investigation is the examination of sections of material obtained by biopsy from the various granulomatous lesions which occur in the tertiary stage of L.G.V. The lesions produced by this virus consist essentially of collections of reticulo-endothelial cells which start in the vicinity of small vessels and undergo colliquative necrosis beginning at the centre. There is an infiltration of the surrounding tissue with lymphocytes, histiocytes, and plasma cells, and giant cells are also found. Although a certain number of polymorphonuclear and eosinophile leucocytes are found amongst the infiltrating cells, there is no true pus formation and it is the necrosis of the cells in the granulomatous nodules which is responsible for the pus-like material obtainable particularly from the involved inguinal lymph nodes. The L.G.V. lesion is thus a granuloma, resembling other granulomata but differing sufficiently in some respects to make a histological examination of biopsy material of some diagnostic help. It is possible at times to demonstrate the virus in sections of L.G.V. lesions but the greatest caution must be used in identifying virus in such preparations.

Demonstration of L.G.V. Virus by Animal or Egg Inoculation.—Microscopy is thus of limited use in

the laboratory diagnosis of L.G.V. and recourse to other methods is necessary. One of these is to attempt transmission of the infection to animals or to chick embryos. Material for this purpose is either bubo "pus" or biopsy material. Mice are susceptible to L.G.V. virus injected intracerebrally as well as by other routes, and so is the monkey, though the latter animal is not generally available and is too expensive for routine use. The guinea-pig is also susceptible, and has been used at times for separating bacteria from virus in material containing both. The inoculation is made intradermally in the groin and the inguinal glands are removed two or three days later if they become involved; the virus is said to invade the glands before the bacteria. Eggs which have been incubated for 5 to 6 days can be infected by this virus when introduced into the yolk sac, and the usual procedure in attempting to demonstrate L.G.V. virus in morbid material is to inject some of it into mice intracerebrally, and some into eggs by the yolk-sac route. The presence of bacteria in such material may necessitate filtration or the use of streptomycin to which the virus is insusceptible but which may prevent the bacteria from multiplying. Infection having been established in mice, or eggs, or both, the virus can be studied and identified. This, of course, takes time, and in my limited experience the successful isolation of virus, even from material in which it can be seen by microscopy, is disappointingly infrequent. However, this is contrary to the published findings of others, and wherever possible an attempt to demonstrate the virus by these means should be made.

L.G.V. Complement-Fixation Test.—Inevitably the laboratory has to rely largely on indirect methods for the diagnosis of lymphogranuloma venereum, and, amongst these, the demonstration of specific antibodies by the complement-fixation test is of considerable value. In this connexion it should be pointed out that the psittacosis lymphogranuloma group of viruses possess in common a group antigen, in virtue of which they cross-react serologically to a high degree. They do possess specific antigens, as Rake and Jones (1944) were the first to demonstrate, but unfortunately these specific antigens are very labile, whereas the group antigen is most stable and in the complement-fixation test tends to dominate the picture. In the antigens in general use in the L.G.V. complement-fixation test it is the group antigen which is operative and in consequence the reaction is no more than group specific. However, the sera from cases of L.G.V. contain antibody to the specific antigen in addition to group antibodies, and the latter can be removed

by absorption with group antigen. If a complement-fixation test is made with such an absorbed serum and fresh unheated L.G.V. virus as antigen instead of the usual heated or phenolized preparations which possess little but group antigen, the reaction becomes specific (Bedson, Barwell, King, and Bishop, 1949). Unfortunately, this procedure is hardly suitable for routine purposes, mainly because of the unpredictable content of the labile specific antigen in freshly-made suspensions of unheated active virus. In addition to this disadvantage, the danger involved in the use of live virus as antigen is a further reason for not adopting this specific complement-fixation test as a routine. One relies, therefore, on the heated or phenolized virus as antigen in the routine complement-fixation test in L.G.V., bearing in mind that the result is only group specific and interpreting it in the light of the other findings. And although complete fixation with a serum dilution of 1 in 32 or over is suggestive of active infection, the demonstration of a four-fold or greater rise in titre is better evidence of this.

Frei Test.—Another indirect test of infection with L.G.V. virus is the demonstration of skin sensitivity by the intradermal injection of inactive virus. This test, first introduced by Frei, can be made with virus obtained from human lesions (bubo pus), infected mouse brain, or infected yolk sacs; the last is now used almost exclusively. The virus is inactivated by heat (100° C. usually) and a control made from normal yolk sacs is used to detect the rare egg-sensitive individual. The test and control materials are injected intradermally at separate sites in the forearm in a dose of 0.1 ml., and readings are taken at 48 hours and, if possible, again 2 to 3 days later. An inflammatory nodule develops at the site of the injection of the Frei antigen in those who are sensitive to the virus; the swelling should be at least 5 mm. in diameter to be considered positive and no reaction should be given to the control antigen. The nodule persists usually for 2 to 3 weeks. This test, though useful, has its limitations. As in the complement-fixation test, the active antigen in Frei test material is the group antigen, so that a positive reaction merely indicates hypersensitivity to a virus of the psittacosis lymphogranuloma group. A positive Frei test can, at times, be obtained in atypical pneumonia due to psittacosis virus (Eaton, Beck, and Pearson, 1941), and a preparation made from psittacosis virus will give a positive reaction in L.G.V. (Bedson and others, 1949). In addition to this disadvantage, it is recognized that skin sensitivity resulting from infection with L.G.V. virus persists after clinical recovery; it has been found positive 30 years or

more after infection, and its duration may at times be lifelong. By itself, therefore, a positive Frei test means no more than that infection with L.G.V. or a related virus has occurred; its significance is similar to that of the Mantoux test in tuberculosis. In conjunction with the complement-fixation test, however, it is of very definite value; a positive Frei test and a positive complement-fixation test at a serum dilution of 1 in 16 or over are indicative of active infection and strongly support a diagnosis of L.G.V. in a patient whose clinical findings are at all suggestive of this disease. Barwell (1949) has shown that an acid extract of L.G.V. virus gives a positive skin reaction in cases of L.G.V., and that in a limited series of tests the reaction was specific, but a more extensive trial will be necessary to assess the value of this test.

INCLUSION BLENNORRHOEA

Another virus belonging to the psittacosis-lymphogranuloma group, which is concerned in venereal disease though to a very much less extent than L.G.V. virus, is the virus of inclusion conjunctivitis. This virus has the morphology and staining reactions of the group. It is a parasite of man and is capable experimentally of producing infection in the baboon; it has not been possible to get it to grow either in the egg or in tissue culture. In man it is found in the genital tract; infection in the female is symptomless, but in the male it gives rise at times to a low-grade urethritis. Infection is passed by venereal contact. This virus may also produce a conjunctivitis in the new-born infant, infection having been picked up during birth from the genital tract of the mother, or in older individuals, to whom the infection comes, in many cases at any rate, from the water of swimming baths contaminated by the bathers. Laboratory diagnosis of infection with this virus is confined to the microscopy of suitably stained smears (Giemsa, Castaneda) made from scrapings of the lesions (urethra; cervix; conjunctiva). The interest of this virus to the venereologist concerns the part which it may play in the causation of so-called non-specific urethritis. That this virus does produce an urethritis in the male is established (Thygeson, 1948), but it is not known how widespread infection with this virus is in Gt. Britain, nor are there sufficient observations on which to base an estimate of the part played by it in non-specific urethritis. A short series of 25 cases examined in my department (A. J. King, unpublished) suggests that inclusion-conjunctivitis virus is not a common cause of this condition. These cases were all seen on the second or third day of the disease, when smears were prepared from urethral scrapings. If the discharge was sufficiently

copious some was collected and stored in sealed ampoules in the dry-ice box for future reference if necessary. A specimen of serum was taken at the time, and a second 4 to 6 weeks later; these were titrated for psittacosis-lymphogranuloma group antibody by the complement-fixation test. On the occasion of the second bleeding a Frei test was made, with the idea that since the Frei antigen was group specific and inclusion conjunctivitis belonged to the group, infection with it might make the tissues hypersensitive to the group antigen. The results of this small investigation were entirely negative, there being no evidence that any of these cases was due to inclusion conjunctivitis virus.

HERPETIC INFECTION

The third and last virus infection I wish to mention is herpes. This virus can produce lesions on the genitalia and infection with it can be transmitted venereally. Before going any further, I should like to emphasize that the disease herpes simplex or herpes febrilis has no connexion with herpes zoster. The two conditions are produced by unrelated viruses, and it is the unfortunate use of the term herpes in relation to zoster which is largely responsible for the confusion.

Virus of Herpes Simplex.—This virus is of moderate size—about 100 m μ —just big enough to enable one to see it with the ordinary microscope. In addition to man, many of the ordinary experimental animals, including the rabbit, guinea-pig, and mouse, are susceptible and can be infected experimentally. It can be cultivated in the egg and in tissue culture; in the egg on the chorio-allantois it produces small pock-like lesions. It gives rise to nuclear inclusions in the cells it infects.

Natural History of Herpes.—Man is the sole natural host of this virus and, presumably as the result of long association, host and virus have become so well adapted to one another that infection rarely produces more than a trivial upset and must frequently be symptomless. Yet infection of man with herpes virus is widespread, and it might be helpful to a better understanding of genital disease due to this virus if I considered briefly with you the man-herpes relationship in general. Herpes in man can be divided into two main groups: primary infections, and relapses occurring in those who carry this virus. Primary infection can occur at any age, but much more commonly it is contracted in the early years of life between the age of 6 months and 5 or 6 years. This in the main is due to two things: fairly intimate contact appears necessary for infection to pass, and with increasing age some physiological insusceptibility to infection seems to

develop. Primary infection, though symptomless at times, may be severe in its clinical manifestations. It commonly takes the form of a vesicular stomatitis accompanied by fever and constitutional disturbances; specific antibody is developed as the result of this infection and recovery, though clinically complete, leaves behind it a carrier state which lasts throughout life. Residual infection can remain in various situations, the mouth, nose, eyes, genital tract, and rectum, and it is in such carriers that relapses occur when, as the result of the action of such factors as fever (natural or artificial), menstruation, or emotional stress, the resistance of the host is temporarily depressed and the virus gains the upper hand. This results in a transient herpetic eruption usually on the skin at muco-cutaneous junctions; there is no systemic disturbance. In the adult population some 20 to 70 per cent. carry herpes virus. The incidence varies with the social strata, being highest in the low income group (Burnet and Williams, 1939), and the carrier state is regularly associated with the possession of herpetic antibody (Andrewes and Carmichael, 1930; Brain, 1932).

Genital Herpes.—In the female, infection with herpes virus takes the form of a vesicular eruption on the labia or mucosa of the outer portion of the vagina; the vesicles readily rupture leaving small painful ulcers covered with a greyish slough. In the male, the herpetic lesions are on the glans, commonly around the corona. These infections may be primary and infection can be transmitted venereally; more commonly they represent the recrudescence of infection in a carrier.

Laboratory Diagnosis

Demonstration of the Presence of Virus.—This can be attempted with fluid collected from unbroken vesicles, which unfortunately are rarely found, or with a suspension of scrapings from the ulcers. Inoculation of eggs on the chorio-allantois and of rabbits by corneal scarification are suitable procedures for the isolation of herpes virus. The presence of bacteria in the starting material can be countered by the addition of penicillin and streptomycin, or by using the guinea-pig and injecting the material intradermally in the skin of the hind feet where the presence of a few bacteria is unimportant. Whatever method is chosen a varying number of passages will be required to establish the virus when it can finally be identified serologically.

Serological Investigations.—Owing to the high percentage of individuals who possess herpetic antibody as the result of being carriers of the virus, examination of the patient's serum for specific

antibody is of limited value in diagnosis. Where a primary herpetic infection is suspected, it is worth examining acute and convalescent serum specimens as well as attempting to isolate the virus, since if the case is, in fact, one of primary herpes the development of herpetic antibody should be demonstrable. The neutralization test and the complement-fixation reaction are both available for this purpose, the latter being not only simpler to do but also equally suitable (Hayward, 1949). Where a recurrent herpes is suspected, only the inability to find herpetic antibody in the serum is of significance; such a finding would strongly suggest that herpes virus was not the cause of the condition.

Non-Specific Urethritis

In conclusion I might refer again to non-specific urethritis. Earlier I made mention of it in connexion with the virus of inclusion conjunctivitis, only to conclude that this virus did not appear to play an important role in the causation of this state. The aetiology of the vast majority of cases of this relatively common disease remains unestablished, though there is evidence that micro-organisms of the pleuro-pneumonia group are quite possibly

concerned in the causation of this condition (Dienes and Smith, 1946; Beveridge and others, 1946; Salaman and others, 1946) and the related syndromes of Reiter and Bechet. Further investigation of these interesting states is much required.

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