RECENT ADVANCES IN CLINICAL VIROLOGY

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RECENT ADVANCES IN CLINICAL VIROLOGY

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PREFACE

This book contains twelve reviews of varying relevance to the practice of clinical virology. Clinical virology is not synonymous with medical virology because it includes the virology of veterinary medicine as well as human medicine, and practitioners of both kinds have recently been rediscovering this, to their mutual benefit. Quite apart from the practical considerations involved in cooperation over zoonoses such as rabies and psittacosis, the collaboration between physicians and veterinarians over strictly parallel diseases such as infantile gastroenteritis has proved profitable to both, so that, for example, Dr Flewett's subtitle '. . . an essay in comparative virology' applies not only to his written chapter but also to the work, by himself and various veterinarians, which he describes in it. Nevertheless, the major emphasis of this work is upon human viral diseases, their unravelling, their pathogenesis, their prevention and their treatment. A book like this to some extent dictates its own contents list, and it was at first surprising to find that at least half of it turned out to be concerned in some way with the central nervous system, and yet, on the other hand, this is perhaps not so surprising, since the nervous system is one of the places to which a virus may escape from what Sir Christopher Andrewes once called 'the troubles of a virus'. The central nervous system is a difficult system of the body for the investigator of human disease and it is not surprising that it has been slow to give up some of its secrets. Even the greater freedom of access and experimentation allowed in animals has not supplied all the answers, and it is interesting that the realm of plant virology may give clues here by some possible parallels between the scrapie agent and the plant viroids. Both scrapie and the transmissible human spongiform encephalopathies like Creutzfeldt–Jakob disease may prove to have very low molecular weight nucleic acid agents as their cause, akin to the plant pathogens known as viroids, although these animal pathogens have yet to be characterised, and such a view is avant garde, if not positively tendentious.

The advances catalogued are of various kinds. There are newly discovered viruses for long recognised diseases, such as transmissible infant gastroenteritis. This is an interesting example of what is becoming an increasingly common phenomenon, namely the recognition and characterisation of a virus by electron microscopy before its culture in the laboratory. There are advances

in the understanding of the relation of well-known viruses to long recognised diseases, such as the once unsuspected role of measles virus in subacute sclerosing panencephalitis. Other advances have been thrust upon us by new situations, particularly the increasing use of immunosuppression, for example in patients after organ transplantation, and in patients kept alive (e.g. by haemodialysis) who would otherwise not have survived. There are advances which have resulted from the appearance of entirely new diseases caused by entirely new viruses. Lassa fever is just such a disease, although the virus concerned is a member of a well-characterised group, the arenaviruses. Marburg disease was a new disease, but it had a brand new virus too. To return to scrapie and Creutzfeldt–Jakob disease, this may apply to them also, in that they appar to be transmissible by filterable agents which may involve virtually a new concept in virological, or infravirological, thinking. Finally, there are continuous advances in the means of prevention of virus diseases, and three of the chapters are concerned with the development and improvement of vaccines. The chapter on cytomegalovirus, in particular, draws attention to the many difficulties inherent in producing a new vaccine.

The diffuseness of the literature on virological disease will be obvious from the bibliographies at the end of the chapters, and I hope that the syntheses achieved by the contributors will be of value to all those concerned with the investigation, diagnosis, treatment and prevention of virus diseases in man and in animals.

Hammersmith
July 1976

A. P. WATERSON

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CONTENTS

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HERPES ENCEPHALITIS

Maurice Longson *Andrew S. Bailey*

The etymologist might argue that the words herpes encephalitis should be held to include all 'inflammations of the substance of the brain' (Sydenham Society Lexicon, 1881) caused by any one of the herpesvirus group of agents, but usage reserves the name for those diseases of the central nervous system caused by the virus of herpes simplex (*Herpesvirus simplex*). This usage is adopted for the purpose of the present review, which therefore is *not* concerned with encephalitis caused by *Herpesvirus varicellae*, *Herpesvirus simiae* (B virus), cytomegalovirus or Epstein–Barr virus.

Clinical Presentation

It is beyond the scope of this chapter to discuss in detail the clinical presentation of herpetic infection of the central nervous system; there are many excellent reviews of the subject (Bergouignan et al, 1968; Liversedge, 1973; Oxbury and MacCallum, 1973; Illis and Gostling, 1974). Whether *H. simplex* can exist as a latent infection of the central nervous system (Nagington et al, 1976) in a manner similar to the latency known to occur in the peripheral nervous system (Baringer and Swoveland, 1973) remains a distinct possibility. This apart, *H. simplex* infections of the central nervous system can, at least in theory, present in one of five different clinical forms:

(a) *Minor, or subclinical infections* which may in time lead to chronic nervous disease (Nahmias and Dowdle, 1968), psychiatric disorders (Lycke, Norrby and Roos, 1974) and psychopathy, as described by Cleobury et al (1971) who, using immunological methods, have produced figures which would appear to suggest that aggressive psychopathy could in some way be associated with a *Herpesvirus simplex* infection. Much more epidemiological work will be required before these tenuous suggestions can be confirmed.

(b) *Herpes meningitis* is a not infrequently described illness, but one which at least in the present authors' experience, and in that of many others (*Discussants*, 1973) appears to be exceedingly rare in the United Kingdom. It is a feature of what can be called *true* herpes encephalitis, that the virus cannot usually be isolated from lumbar cerebrospinal fluid (CSF). It is also a feature of the disease that meningeal symptoms are inconsistent, if not rare. It is however reported that *Herpesvirus simplex* can on occasion, and sometimes in

epidemic waves, cause a true benign aseptic or lymphocytic meningitis, with minimal involvement of the brain substance and frequent recovery of the causative agent from lumbar fluid (Armstrong, 1943). It is of more than passing interest to note that herpes meningitis is described in the literature written at the turn of the century (Ravaut and Darré, 1904) and that virus isolation from clinical cases was reported by Ravaut and Rabeau in 1921. Later, Afzelius-Alm (1951) described an epidemic of 56 cases in Gothenburg. A fascinating feature of herpes meningitis is the alleged association with genital herpes and infections caused by type II strains of *H. simplex*. This association was precisely the point emphasised by early French authors (Ravaut and Rabeau, 1921), and now, half a century later, a number of reports from Australia (Duxbury and Lawrence, 1959), the United States (Terni et al, 1971; Morrison et al, 1974; Harford, Wellinghoff and Weinstein, 1975) and Scandinavia (Sköldenberg, Jeansson and Wolontis, 1973), are adding credence to the possibility. Some investigators (Harford et al, 1975; Cappel and Klastersky, 1973) also report the isolation of *H. simplex* type I. A disquieting feature of the problem is the occasional isolation of the virus from lumbar CSF collected from subjects without clinical evidence of disease (Flexner and Amos, 1925; Zurukzoglu, 1937; Longson, 1970; Harford et al, 1975). Further reports of herpes meningitis are awaited with interest and it will be important to observe whether this illness is associated with the appearance during convalescence of specific anti-*H. simplex* immunoglobulins in the CSF and a consequent reduction in the serum : CSF ratio of such antibodies (see below).

(c) *Mild diffuse encephalitis* with good prognosis may occur. A series of cases was reported from Glasgow (Ross and Stevenson, 1961). Any appraisal of this condition is most difficult and comes up against the problem of the criteria of diagnosis. It would appear that virus cannot be isolated from CSF, and the mildness of the illness precludes the obtainment of brain biopsy tissue. Some cases may be diagnosed by immunofluorescent tests on lumbar fluid lymphocytes, but many investigators consider these techniques to be suspect (see below). Until recently, therefore, the diagnosis rested on a retrospective detection of a four-fold (or greater) antibody rise in a patient's serum. Unfortunately, such a test cannot in any circumstances, by itself, be used to diagnose nervous system infection by *H. simplex* (*Brit. med. J.*, 1972; Longson, 1975). Herpes simplex (herpes febrilis, cold sores, fever blisters, herpes labialis) has, since the days of Hippocrates, been associated with infective diseases of the most varied aetiology (Beswick, 1962), and a neurotropic role cannot be ascribed to the virus of herpes simplex simply because antibodies have appeared in the blood, even if there has been uncontroversial encephalopathy. Notwithstanding these words of caution, it may well be that the detection of specific *H. simplex* antibodies in the CSF of patients with mild, diffuse encephalitis confirms the existence of benign cases (McKendrick, 1976).

(d) *Severe diffuse meningoencephalitis with grave prognosis* will be considered together with form (e), focal encephalitis.

(e) *Focal encephalitis with grave prognosis*, or herpes encephalitis of the so-called 'neurosurgical' type, or acute necrotising encephalitis (Van Bogaert, Radermecker and Devos, 1955), or acute polioclastic encephalitis (Greenfield, 1950) represents, together with the diffuse form described in paragraph (d) above, what may be called true herpes encephalitis. Whereas originally the focal type of infection was the more easily diagnosed disease, and therefore the more often reported, in recent years, both types of pathology are readily recognised and one form is probably merely a frequent variant of the other.

The remainder of the present review will be restricted essentially to these severe forms of herpes encephalitis.

Epidemiology

It is often stated that herpes encephalitis represents the commonest form of sporadic encephalitis which occurs in temperate climates. This statement requires some clarification. It is exceedingly difficult to obtain any accurate information about the frequency of the disease. In England and Wales, there are three sources of data—the figures returned by the Registrar General, the epidemiological information compiled by the Epidemiological Research Laboratory of the Public Health Laboratory Service and the data cards sent to the Herpes Encephalitis Working Party.[1]

It is not the purpose of this review to enter into details of the available figures, and most of these have already been published elsewhere (Longson, 1975). In brief, it would appear that about 175 deaths each year (during the period 1967 to 1971) could be ascribed either to 'viral encephalitis' or to 'encephalitis and encephalomyelitis'. (The diagnostic pitfalls in the separation of these two categories make it unwise to attempt.) On the other hand, the Registrar General's returns, show that during 1967 to 1971, about 20 people died each year because of *H. simplex* infection. Fatal infections with this virus are represented in the main by neonatal herpes and herpes encephalitis (transplantation surgery was rare in the years under consideration). Neonatal herpes is infrequent in England and Wales (Juel-Jensen and MacCallum, 1972; Longson, 1974; Tobin, 1975) and the Registrar General's figure of 20 *may* thus represent an indication of the number of deaths attributable to diagnosed cases of herpes encephalitis. During the same five-year period 1967 to 1971, the Epidemiological Research Laboratory was

[1] Professor Hume Adams, Glasgow; Dr D. B. Brownell, Bristol; Dr P. H. Buxton, Liverpool; Dr D. G. T. Davies, Glasgow; Dr T. H. Flewett, Birmingham; Dr T. C. Hall, USA; Dr L. S. Illis, Southampton; Dr B. E. Juel-Jensen, Oxford; Dr L. A. Liversedge, Manchester; Dr Maurice Longson, Manchester; Dr F. O. MacCallum, Oxford; Dr G. D. W. McKendrick, London; Dr J. M. Oxbury, Oxford; Mr Julian Peto, Oxford; Dr Marc Rappel, Brussels; Dr M. V. Salmon, Smethwick; Dr C. E. C. Wells, Cardiff; Dr I. M. S. Wilkinson, Cambridge.

informed of no less than 242 cases of herpes encephalitis, but when the case records of the patients were critically reviewed, only 57 of these cases were retained (Longson, 1975). On the other hand, a retrospective study by the Herpes Encephalitis Working Party revealed 99 cases of the disease between 1966 and 1972. This figure is certainly an underestimate of the total number of properly diagnosed cases of herpes encephalitis, but the publicity given to the Register and the spontaneous interest which it created across the country, together with the geographical spread of the information received by the registry, probably means that the underestimate is not excessive. An estimate of about 25 to 50 cases of herpes encephalitis per year for the United Kingdom can thus be tentatively put forward. If we calculate on the basis of an expected mortality of about 60 per cent, it could be assumed that there will be about 25 deaths per year from herpes encephalitis, which corresponds well with the Registrar General's return of 20 deaths per year from *H. simplex* infections. On the other hand, the Registrar General returns 175 deaths from encephalitis each year and, until the aetiology of the other 150 deaths can be determined, no statement about the relative frequency of herpes encephalitis can be entertained. In the meanwhile, it is of course tantalising to suggest that many cases of herpes encephalitis go unrecognised. It is certain that the disease is not easy to diagnose, particularly in centres with only remote access to the specialised facilities which will now be described.

Diagnosis

Herpes encephalitis is recognised, in life, as a result of a multidisciplinary effort in which the infectious diseases physician, neurologist, neurophysiologist, neurosurgeon, neuroradiologist, neuropathologist and virologist all have an important, and indeed, a determinative role. An early and uncontroversial diagnosis of the illness cannot however be made without the examination of brain material. Later, serological tests may provide a definite answer, but, during the first 14 days of the disease, only brain biopsy can provide the source of certain information unless, of course, the causative virus can be identified in lumbar CSF (see below). Until non-invasive tests become established, it will be necessary to obtain brain biopsy tissue, or ventricular fluid, if a positive diagnosis is to be achieved during the acute encephalitic phase. The Herpes Encephalitis Working Party has put forward the following diagnostic criteria: recognition of the virus in CNS material (using either cultural or immunological techniques) *or* strong histopathological evidence of acute necrotising encephalitis (preferably with the recognition of Lipschütz inclusions and/or ultramorphological evidence of *Herpesvirus* capsids in brain tissue) *together with* serological evidence of *H. simplex* infection.

Initially, the patient is admitted to hospital because of a disturbance of affect or of consciousness, there may be loss of memory, mutism or a change in behaviour. Sometimes a more catastrophic episode such as coma or epilepsy

may focus attention on the brain disease. Admission to a neurosurgical unit because of a suspected space-occupying lesion is a classical presentation, but not necessarily as frequent as is often supposed. Many problems of differential diagnosis require careful evaluation, not least being the possibility of tuberculous meningitis or of a brain abscess or tumour. The whole aspect of clinical diagnosis has recently been reviewed (Illis and Gostling, 1974) and does not require further elaboration here. In the authors' experience, the severity of the encephalitic illness has invariably been remarkable. According to most authors, brain oedema and raised intracranial pressure probably represent the cardinal problem in herpes encephalitis and the decision of whether or not to perform a lumbar puncture must be tempered accordingly.

Lumbar cerebrospinal fluid

The examination of CSF may be most important in the process of differential diagnosis, but it is rarely of value in the positive recognition of herpes encephalitis per se. Pressure may or may not be raised (Adams and Miller, 1973), there is a highly variable lymphocytic pleocytosis ranging from as few as no cells per millilitre to as many as 1000 cells/ml (Longson, 1970; Oxbury and MacCallum, 1973) and, although importance has been attached to the high number of red cells which may be present in the fluid (Miller, Hesser and Tomkins, 1966), this feature is too inconsistent for it to be of much diagnostic significance. The CSF may in fact be absolutely normal (Oxbury and MacCallum, 1973). Except perhaps in *H. simplex* type II infections (Johnson, 1972), infectious virus can rarely be isolated from lumbar fluid (Longson, Liversedge and Wilkinson, 1973) and a negative virus culture from this material is thus of no help. According to Dayan and Stokes (1973), *H. simplex* antigens can be recognised in CSF lymphocytes by immunofluorescent tests, but this technique is as yet unproven (Tomlinson and MacCallum, 1973a) and in the hands of some workers, quite unreliable (Longson et al, 1973; Lennette, 1973). Other possible tests on lumbar fluid include the culture of CSF lymphocytes prior to the immunofluorescent recognition of virus antigen (Lindeman et al, 1974), the identification of Lipschütz-type inclusions in CSF lymphocytes (Gupta et al, 1972) and the analysis of the immunoglobulin content of the fluid (Rappel et al, 1971). None of these tests can be considered as of proven value, but some may merit further appraisal.

Fluid collected from the patient during the acute phase of the illness should be conserved for subsequent antibody assay (see below).

Electroencephalography

The electroencephalographic (EEG) changes seen in patients with acute necrotising encephalitis were first described by Radermecker (1956) and were subsequently emphasised by Upton and Gumpert (1970). Whether the now well-known features of the recording (Illis and Gostling, 1974), which include

periodic discharges at 1 to 2 s intervals and a slow delta-type rhythm of 2 to 3 Hz are of diagnostic value (Cobb, 1975) or not (Illis and Taylor, 1972) is a matter of dispute, but what is clear is that the EEG is an important procedure in the effort of diagnosis (Smith et al, 1975). In herpes encephalitis, the record is never normal (Adams and Jennet, 1967); it may provide the neurosurgeon with important information which will help him to select his biopsy site and, if carefully interpreted, can yield results of remarkable accuracy, at least in children (Noronha, 1975).

Neuroradiology

Neuroradiological investigations share with electroencephalography equal importance in the diagnostic 'work-up' of herpes encephalitis. It is, of course, an essential step in the process of differential diagnosis, where it can greatly assist in the identification of cerebral masses but, contrary to the EEG, a normal radiological appearance does not necessarily exclude the possibility of herpes encephalitis. The radiological record can be invaluable to the neurosurgeon when he selects the site of his biopsy.

The neuroradiological approach to herpes encephalitis comprises contrast angiography with, or without subtraction techniques, scintillation angiography and computer-assisted axial tomography, with or without contrast techniques (EMI Scanner). The classical appearances in contrast angiography of a space-occupying lesion, with displacement of the internal carotid artery and a mid-line shift are well known, although of doubtful specificity (Fastier and Alexander, 1950; Cope and Howieson, 1967; Amin, 1972). These striking features are however probably limited to the focal type of acute necrotising encephalitis, and, if it is not to be missed, more subtle features need to be observed in the diffuse type of infection. Working at the Midland Centre for Neurosurgery and Neurology in Smethwick, England, Pexman (1974), who uses subtraction techniques, has extended the earlier observations of Sheldon (1973). He has shown that after five days of pyrexial illness, cases of herpes encephalitis often reveal persistent filling of some terminal arteries during the capillary phase, together with early venous drainage and the appearance of local areas of hypervascularity, or 'blushes', in affected areas of the brain. Pexman (1974) considers that these findings are highly suggestive of, but not exclusive to herpes encephalitis.

Technetium brain scans are of value in the localisation of any 'hot spot' and the radionuclide images so obtained play a certain role in the early diagnosis of herpes encephalitis, because the distribution of the areas of uptake can be characteristic (Radcliffe et al, 1972).

A more recent, and as yet little explored, neuroradiological technique is computer-assisted axial tomography. Very few cases of *H. simplex* brain disease have been studied with the EMI Scanner, but first observations (Thomson, 1976; Isherwood and Longson, 1976) suggest that the scans may provide useful information in the early stages of the disease. In one case

Isherwood and Longson (1976) observed low density areas predominantly in the white matter, but also as scattered areas in the grey matter. The appearances were consistent with those of severe oedema.

Brain biopsy

The examination of a fragment of brain tissue remains the sine qua non of the diagnosis of herpes encephalitis, at least during the first 14 days of the illness. The biopsy may be taken during the course of a surgical decompression procedure, or may follow an exploratory intervention in the search for a possible space-occupying lesion, or it may be the result of an elective biopsy operation. In any case, the site of the biopsy must be selected with care. Tomlinson (1973) amongst others, has emphasised the unequal distribution of virus in different parts of the organ. The biopsy site will be selected on the basis of clinical, electroencephalographic and radiological evidence, but in accordance with accepted rules of neurosurgical practice. In the absence of any definite localisation, the inferior aspect of the temporal lobe represents the site of choice. The opportunity of brain operation should be taken and ventricular fluid collected for virus culture (Buckley and MacCallum, 1967; Flewett, 1973b).

The physical appearance of the brain during the biopsy procedure can provide valuable information; many authors have described the turgid, inflamed and haemorrhagic tissue seen through the slit in the dura and have considered it to be pathognomonic of *H. simplex* infection. The biopsy tissue may be collected either as a wedge biopsy or as a needle aspirate. In either case, it is merely collected into a dry, sterile container for transmission to the laboratory, where fragments are separated, using aseptic precautions, into various samples for electron microscopy (buffered gluteraldehyde), routine surgical histology (10 per cent formalin) and virological tests (medium '199' or equivalent).

Histology. The appearances are not necessarily specific, unless good fortune leads the microscopist to an area where he can identify Lipschütz inclusions, typical of herpetic infection. The detection of such inclusions can be facilitated if the tissue is fixed in Bouin's or Zenker's fluid, or even in corrosive sublimate. In the absence of inclusions, the appearance will be limited to those of virus encephalitis, albeit of unusual severity in many cases. Oedema, haemorrhages and necrosis are striking features (Haymaker et al, 1958; Adams and Jennet, 1967; Longson, 1970; Adams and Miller, 1973).

Electron microscopy (*Brit. med. J.*, 1972; Salmon, 1973; Norris, 1972; Timperley, Norris and Carr, 1975) can reveal the presence of *Herpesvirus* capsids in some infected cells, but they can prove very difficult to find, because only a minority of cells contain virus inclusions. The problems of scanning severely limit the usefulness of the method, particularly for the urgent diagnosis of a case prior to the use of specific chemotherapy. In any case, the electron microscope cannot distinguish between one *Herpesvirus* and