INVESTIGATIONS ON THE VIRUS OF HERPES SIMPLEX

THE HERPES SIMPLEX COMPLEMENT FIXATION TEST AND ITS USE IN THE STUDY OF HERPES ANTIBODIES

BY

PEKKA HALONEN

HELSINKI 1955

FROM THE DEPARTMENT OF VIROLOGY, UNIVERSITY OF HELSINKI

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TO BE PRESENTED WITH THE ASSENT OF
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EXAMINATION IN AUDITORIUM XII ON 28TH MAY 1955,
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MERCATORIN KIRJAPAINO

To my wife

Translated by
Eva Palmgren

PREFACE

The present investigation was carried out at the Department of Virology, the University of Helsinki, during the period 1951—1954.

The theme was suggested to me by Dr. Kari Penttinen, M.D., Head of the Virus Department in the State Serum Institute. I am very much indebted to Dr. Penttinen for directing my attention to the field of virology, for many inspiring discussions during the course of the work, and for criticizing the manuscript.

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Mrs. Pirkko Koivunen and Mrs. Airi Åberg assisted me in the technical performance of the work. I wish to express my warm thanks to them. I also wish to thank the staff of the Department of Serology and Bacteriology and of the Department of Virology of the State Serum Institute, who have been helpful to me in various ways.

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Helsinki, December 1954

P. H.

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SURVEY OF THE LITERATURE

HISTORY OF THE STUDY OF HERPES SIMPLEX

The infectious character of herpes febrilis was demonstrated in 1873 by Vidal (156). He injected fluid from a herpetic vesicle into the skin of the host, and obtained a similar lesion at the site of injection. The fluid of the latter was likewise capable of producing vesicles. Experiments in which the infectious agent of the herpes vesicle was successfully transferred to experimental animals were first described in 1919 by Löwenstein (100, 101). Grüter, however, stated that in 1913 he had transferred herpetic keratitis onto the cornea of a rabbit, though his results were not published until 1920 (73, 74). Even in his first investigations of the virus of herpes simplex, Löwenstein disclosed many of its characteristic features. He observed that this virus can be transferred from herpetic vesicles onto the cornea of the rabbit, where it produces typical herpetic keratoconjunctivitis. A similar effect was not obtained with the fluid from herpes zoster vesicles. Furthermore Löwenstein showed that the virus of herpes simplex is easily destroyed by heat, maintains its potency during passage through many rabbit corneas, produces »bodies» in the affected tissues and with difficulty passes through Berkefeld filters.

In 1920 Doerr (42) observed that herpes infection in rabbits occasionally extended to the brain, causing encephalitis. This affinity of the herpes virus for the brain was the main concern of a large number of investigations on herpes simplex published in the nineteen-twenties. It was believed that the causative factor in encephalitis epidemica had been traced. This view found support when Levaditi and Harvier (95) succeeded in isolating a strongly neurotropic strain of herpes from the brain tissue of a patient who had died of this disease. Later this virus was also isolated from brain tissue in several other cases of fatal encephalitis, but, on the other hand, there were many workers who did not succeed in isolating herpes virus in this disease in spite of investigating fairly large series (38, 39, 54, 166). The serological investigations of Andrewes and Carmichael (10) also constituted evidence against Levaditi's theory regarding the herpes virus as the cause of encephalitis epidemica. The role of the virus of herpes simplex in the etiology of this disease is not yet fully understood. It has been the established cause of some fatal cases of encephalitis, but these seem to have

been exceptional, and often they have displayed a clinical picture differing from encephalitis epidemica (von Economo's disease).

In 1921 Blanc and Caminopetros (23) showed that, in addition to the rabbit and the guinea-pig, the mouse can also be used in the investigation of herpes. The mouse has proved to be a very satisfactory experimental animal where accurate determinations of antibody content are involved. In 1929, Gildemeister *et al.* (67) cultivated herpes in tissue cultures. In the nineteen-thirties the use of fertile hen's eggs was adopted in virological research; their usefulness in the cultivation of herpes virus was demonstrated by Goodpasture *et al.* (68a) and by Saddington (128). After one or two days' growth the virus was found to produce typical pock lesions on the chorioallantoic membranes of the eggs. When it was inoculated into the amniotic, allantoic or yolk sac, or directly into the embryo, the latter died within 2 to 4 days, depending on the strain. The extra-embryonic fluids of eggs infected in this way and suitably incubated contained 10^4 to 10^7 living particles (20). Today the virus of herpes simplex is mostly isolated on the chorioallantoic membrane of the embryonated egg. This also constitutes suitable material for the neutralization test (33). The extra-embryonic fluids are employed as antigen in the complement fixation and dermal reaction tests. Thus the embryonated egg has become one of the most widely used and helpful tools in the study of the virus of herpes simplex.

To begin with, the mechanism of herpetic infection seemed confusing, since it was found that herpes vesicles are only encountered in subjects who have neutralizing antibodies to herpes (10). It was a well-known fact that virus diseases are generally transmitted to individuals who lack the specific antibody. Hence it seemed doubtful whether herpes really was an infectious disease. Burnet's theory regarding the »natural history» of the herpes virus represents the current view on the infective mechanism of herpes simplex and the behaviour of this virus in its natural host, *i.e.* man. In a series of serological investigations (32, 33, 34, 35, 36) Burnet showed that children seldom have herpes antibodies; he found that the development of these is, as a rule, associated with stomatitis, and that herpes virus may then be isolated from the mouth of the patient. He stated that primary herpes simplex infection in childhood often produces severe systemic illness with high fever, usually taking the form of stomatitis. Furthermore he assumed that those who have suffered from a primary herpes infection become carriers of herpes virus, which subsequently remains mostly latent, but under the influence of various stimuli may become active and produce vesicles. Later it has been found that a primary infection may involve the genitals, the skin of the face and body, and the eyes. The occurrence of a primary herpes infection has been serologically demonstrated even in the absence of any clinical evidence (8, 112).

Different strains of herpes simplex, isolated in different clinical entities, have proved to be antigenically largely similar. The principal difference observed is that some strains display a tendency to affect the central nervous system of experimental animals and others to produce cutaneous signs. Hence it was suggested that some strains of herpes simplex are

neurotropic and others dermotropic. Later is has been demonstrated, however, that the difference is often inherent in the host animal rather than in the strain of virus. Thus a strain that has caused fatal encephalitis in man may produce chiefly cutaneous signs in the rabbit and slight or no symptoms from the central nervous system (57). Nonetheless, serological differences between different strains of herpes simplex have been demonstrated both by the neutralization and the complement fixation test (57, 143, 162).

On microscopical investigation of tissues infected with herpes virus, various kinds of »bodies» have been found, eosinophilic intranuclear inclution bodies being the most significant. Today these are regarded as very typical of the virus of herpes simplex, although Lipschütz, who in 1921 was the first to describe them (97, 98), stated that he had seen similar intranuclear bodies in the cornea of a rabbit infected with the fluid from herpes zoster vesicles. It is now believed that the intranuclear inclusions consist of living virus and are not cellular degeneration products due to the infection, as has also been suggested. They are round or oval and generally occupy the whole of the nucleus. Intranuclear inclusions have diagnostic significance, inasmuch as their presence constitutes histological confirmation, for instance in cases of fatal encephalitis, that infection has been due to the virus of herpes simplex.

In the last few years a large number of investigations on the virus of herpes simplex have been published, the main purpose of which has been not so much to throw light on this agent and the diseases possibly due to it, as to gain further insight into the nature of viruses (1, 2, 6, 14, 21, 37, 60, 107, 108, 109, 121, 137, 148, 161).

DISEASES CAUSED BY THE VIRUS OF HERPES SIMPLEX

It is a feature common to the various conditions caused by the herpes virus, that in primary infection there is usually a severe systemic illness in addition to a local lesion, whereas, in the recurrent forms, the general reaction is mild, even though local lesions may be severe. In cases of primary infection, where no antibody to herpes is present in the acute stage, the patient usually has high fever and looks very ill. Prognosis is mostly favourable, however, although many fatal cases are mentioned in the literature.

Herpes Simplex and Herpes Febrilis.[1] — These names are mostly used for vesicles occurring on the lips or on the face in association with or independently of fever. Initially a reddish papule appears on the skin or the mucous membrane, which in a few hours becomes filled with transparent fluid. This contains herpes virus in abundance. When the vesicle has ruptured, a crust is formed, which heals in some days without leaving any scar. In herpetic individuals such vesicles develop in connection with

[1] Principally the classification suggested by van Rooyen and Rhodes (154) and Scott (134) has been followed in denominating the diseases.

various stimuli such as a cold, diseases accompanied by high fever, menstruation, emotional upsets, artificial fever therapy, gastric disorders, *etc.* In subjects with this disposition vesicles may even be induced under hypnosis (78, 153).

Herpes Genitalis. — Herpes vesicles occasionally occur on both the male and the female genitals, and they may be propagated by sexual intercourse (12, 80, 141). In primary infection herpes virus may also cause vulvovaginitis and urethritis (46, 93, 142).

Eczema Herpeticum. — In 1887 Kaposi (86) described a varicelliform eruption, sometimes occurring in infantile eczema patients as a grave complication. This disease has been referred both to bacterial and to virus infection. From some patients vaccinia virus has been isolated, or there has been a history of recent exposure to vaccinia virus. In 1941 Seidenberg (140) isolated herpes simplex virus from the vesicles of a patient with Kaposi's varicelliform eruption. Later a large number of papers have been published in which it has been shown, either by means of isolation of virus or serologically, that all cases of Kaposi's eruption in the series concerned, or some of them, have been due to herpes virus (15, 28, 49, 52, 72, 89, 103, 125, 146, 157). For cases of Kaposi's varicelliform eruption caused by herpes virus the name eczema herpeticum has been adopted. Not all cases of eczema herpeticum have been primary herpes infections, antibody to herpes having been found even in the acute stage of the disease in some patients from whom herpes virus has been isolated (47). In adults, Boake, Dudgeon and Burnet (26) and Pette (117) observed recurrent eruptions, due to herpes virus and resembling Kaposi's varicelliform eruption.

Herpes Stomatitis. — The acute, infectious gingivostomatitis of children is generally regarded as a primary infection with the virus of herpes simplex. It is characterized by systemic illness with high fever and severe inflammation of the mouth. In its gravest forms it is also accompanied by vesicles elsewhere on the mucous membranes and the skin. In many cases of stomatitis, and according to certain investigators in all, antibody to herpes develops during the course of the illness, and then herpes virus may be isolated from the patient's mouth (8, 22, 31, 34, 35, 41, 61, 69, 75, 99, 136). In 1949 Rogers *et al.* (122) showed that in adults acute gingivostomatitis may also be a primary herpes simplex infection with the same clinical picture as in children. The recurrent form of stomatitis has also been referred to herpetic infection (87, 136, 154), but according to Dodd and Ruchman (40) and Blank *et al.* (25) this agent is not the causative factor in recurrent stomatitis and recurrent aphthous ulcers. Scott (134) regards recurrent herpes stomatitis as a possibility, though he believes it to be extremely rare.

Diseases of the central nervous system due to the virus of herpes simplex. — In 1941 Smith, Lennette and Reames (145) isolated herpes virus from the brain tissue of a child who had died of encephalitis; on histological examination intranuclear inclusion bodies were found in the cerebral lesions. These writers also surveyed the records on encephalitis in which virus had been

isolated; the virus had definitely been identified as that of herpes in some 10 cases. Encephalitis in which herpes virus has been isolated has subsequently been described by Fisher and Patrick in 1947 (51), Fastier and Alexander in 1950 (48), Paillard, Wildi and Wirth in 1950 (113), Quilligan and Wilson in 1951 (120), Draheim and De Rodaniche in 1952 (43), Florman and Mindlin in 1952 (56), and by Tongeren and Jong in 1952 (155).

Furthermore cases of encephalitis where herpes virus had been isolated and, in addition, intranuclear inclusion bodies had been found, were reported in 1944 by Zarafonetis *et al.* (165), in 1946 by Whitman, Wall and Warren (159), whose series included two adults, and in 1951 by Ginder and Whorton (68). In 1951 Wildi (160) isolated herpes virus from the brain tissue of a child who had died of encephalitis, and demonstrated histologically the presence of inclusions, which he did not, however, regard as typical of herpes.

The presence of intranuclear inclusion bodies in encephalitis was reported by Dawson (38, 38a), Akelaitis (5), Kinney (88), Swan (149), Malamud, Haymaker and Pinkerton (104), Greenfield (71), and France and Wilmers (59).

An increase in herpes antibodies in patients with encephalitis, meningoencephalitis or aseptic meningitis was reported in 1951 by Afzelius-Alm (4), in 1952 by Scott *et al.* (138), in 1953 by Adair, Gauld and Smadel, (3) and in 1955 by Penttinen *et al.* (115).

In 1943 Armstrong (11) isolated herpes virus from the cerebrospinal fluid of a patient displaying mild symptoms of choriomeningitis. He assumed that herpes virus is in rare instances the cause of lymphocytic meningitis. In 1942 Janbon, Chaptal and Labraque-Bordenave (82) also isolated herpes virus from the cerebrospinal fluid of a patient with meningitis.

Furthermore it has been shown in a number of papers that the occurrence of herpes vesicles is associated with various symptoms of the central nervous system. Mention may be made of the case described in 1929 by Pette (117), where a patient displayed recurrent signs of acute meningitis invariably accompanied by severe herpes facialis over the whole of the face.

According to Braley (29) the virus of herpes simplex is one of the causative factors in *acute keratoconjunctivitis*, *dendritic ulcers*, *keratitis disciformis* and *chronic keratitis bullosa*.

In 1952 six cases of *herpetic oesophagitis* were reported by Fingerland, Vortel and Endrys (50), who concluded that herpetic ulcers of the oesophagus are perhaps commoner than has been assumed. In 1950 Ruchman and Dodd (126) described *herpetic rhinitis*. *Infantile herpetic hepatitis* was recorded by Quilligan and Wilson (120), by Zuelser and Stulberg (168) and by McDougal *et al.* (105), in association with fatal herpetic infection. In certain cases of *erythema multiforme* the herpes virus has been credited with some etiological significance (7, 90, 106, 123, 163).