Columbia SK Group of Viruses.

(Polioencephalomyelitis, Parapoliomyelitis, Encephalomyocarditis)

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Introduction.

The group of Columbia SK (Col SK) viruses, so designated after the name of the member of this group first isolated, are relative newcomers in the field of viral agents. Following discovery of the Col SK strain in 1940 other immunologically similar strains of this virus (MM, EMC, Mengo, F, Ortlieb, AK, etc.) have been isolated from time to time in various parts of the world; if the demonstration of specific antibodies is a reliable criterion of infection, their occurrence may even be said to approach world-wide distribution. Despite intensive studies the role of these viruses as humanpathogenic agents is still obscure and most of the information pertaining to them is based on data derived from animal experimentation. Thus, the synonyms listed in the subtitle refer essentially to pathological responses in Laboratory hosts rather than to a well-defined clinical syndrome in man. From a standpoint of taxonomy, the Col SK group of viruses should be classified between the Coxsackie viruses and the poliomyelitis viruses since all three have the same basic morphological characteristics and share similar cell tropisms. The group is unrelated to the arthropod-borne encephalomyelitis viruses, not only because of differences in viral particle size but also because Col SK virus does not multiply in the infected insect. As experimental agents they are extremely useful in view of their uniform and high virulence, by central or peripheral routes, for a number of rodents, chiefly cotton rats, mice, hamsters and guinea pigs, as well as for certain primates. For this reason they have often been employed as a convenient model infection in the study of poliomyelitis problems, both for investigations on the mechanism of pathogenesis of viral infection and for screening programs in the search for effective antiviral chemotherapeutic substances. In fact, looking retrospectively, it becomes apparent that many of the cardinal recent advances in poliomyelitis research have had their forerunners in similar observations with the Col SK group of viruses. We are referring here: a) to recognition of the pathway of infection from the alimentary tract to the bloodstream and thence to skeletal and cardiac muscle, or to the central nervous system; b) to the demonstration of the ability of the virus to multiply in extraneural tissues *in vivo* as well as in tissue culture media. With respect to tissue tropism, these viruses are, therefore, essentially pantropic, covering the entire range of the neurotropic-viscerotropic spectrum. In the biological sense, they form an intermediate link in the evolution

C. Hallauer et al. (eds.), *Handbuch der Virusforschung*
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from the obligate rodent-pathogenic viruses to the more or less obligate human-pathogenic viral forms. Because of these unique properties, and also because of the relative scarcity of their actual isolation from man or animals, the question may well be raised whether they represent, in effect, an autochthonic viral entity with a sharply defined epidemiology, or whether they should possibly be regarded as transient mutants or variants in a phylogenetic developmental cycle of the poliomyelitis viruses from rodents to man. (See section on classification.)

The Col SK group of viruses, then, occupies a curious position in current research on poliomyelitis and poliomyelitis-like diseases. Unfortunately, our knowledge in several areas is as yet incomplete or even controversial, but further work may be expected to throw more light on the nature of these viruses. Some of the basic points of interest have been brought together by WARREN in 1952, and again in 1953. An extensive review in monographic form was published by KELLER and VIVELL in 1954 to which the reader is referred for further detailed information.

History of Virus Isolations.

Discussions of the isolations of viruses belonging to the Col SK group are fraught with certain difficulties because only few such strains are described in the literature and some isolations have occurred under unusual circumstances. At any rate, the number of viral isolations is definitely not in proportion to the reported incidence of specific antibody levels in human sera. It is true that little systematic search has been made for the presence of the virus in patients, a point which would seem to be of some importance because the infectivity of the virus may be masked by the rapid appearance in the serum of high-titred antibodies. However, the fact remains that large scale tests of recent years by different Laboratories, in which human stools were examined for the presence of Coxsackie viruses by direct transfer to suckling mice, have failed to turn up another isolation of Col SK virus, except for the few positive results obtained in Europe. Routine fecal isolation of the poliomyelitis viruses or of other enteric viral agents in various types of tissue culture media offers no basis of comparison since little is known about the growth requirements of Col SK virus in such substrates beyond the fact that some strains of the group will grow and produce cytopathogenic effects in the same media. Yet, information is lacking whether any of the so-called ECHO viruses (enteric cytopathogenic human orphans) do or do not overlap in serological activity with Col SK virus, assuming that the latter, like some of the poliomyelitis virus strains, may exist in its characteristic antigenic form without fully developed rodent-pathogenicity.

The peculiar situation with which one is confronted has led to doubt in some quarters about the authenticity of some of these isolations and it has been suggested that these viruses may have originated in laboratory animals employed for their isolation, or else were picked up by chance contamination with existing strains carried in the Laboratory. We shall therefore present in more detail the circumstances under which the known strains were actually isolated.

1. Col SK Strain (JUNGEBLUT and SANDERS 1940).

Discovery of the Col SK strain is of particular interest historically since it followed shortly after the first adaptation in 1939 of poliomyelitis virus (Lansing strain) from monkeys to rodents by intermediate cotton rat passage (ARMSTRONG 1939). Before this time it was almost axiomatic that pathogenicity for

animals other than monkeys would exclude a given viral agent from being poliomyelitis virus. For several years thereafter attempts to repeat ARMSTRONG's fundamental work with other strains of poliomyelitis virus led to success in a few cases only. In fact, not until 1955 did it become clear through the work of SILVERBERG, HABEL and SHELEKOW that freshly isolated Type II strains can almost routinely be transferred to mice by direct passage from tissue culture media, if the intraspinal technique of inoculation is employed. Isolation of the Col SK strain must be viewed against this early background in order to fully appreciate the controversial position of this virus.

The virus originated in experiments at Columbia University (JUNGEBLUT et al, 1939-1940) in which human SK poliomyelitis virus was transferred from early monkey generations (11th, 14-16th, 18th) to mice after intermediary cotton rat (*Sigmodon hispidus littoralis*) passage; direct inoculation of mice with the monkey material failed to produce any symptoms (see Fig. 1a-c). The human

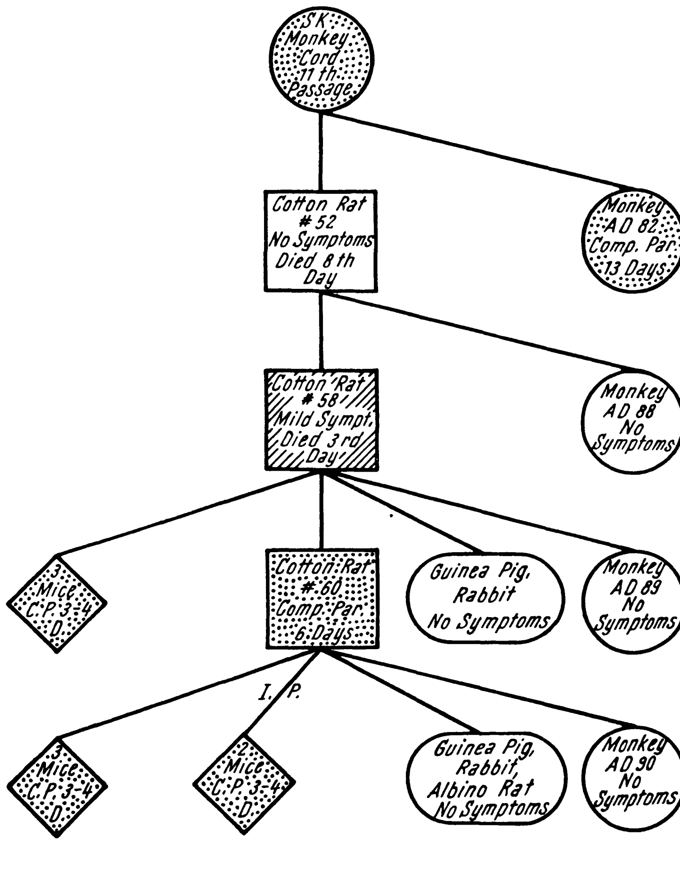

Fig. 1a. Genealogy of SK mouse virus (1st isolation). (C. W. JUNGEBLUT and M. SANDERS, J. Exp. Med. 72, 407, 1940)

virus had been isolated by TRASK, VIGNEC and PAUL at Yale University (1938) from the faeces of an abortive case of poliomyelitis (Daniel SK), and the freshly isolated strain was identified as poliomyelitis virus on the basis of serological tests and because of its inability to infect animals other than monkeys. The observation

by JUNGEBLUT and SANDERS that the simian SK virus, in later monkey passages, was pathogenic for rodents could subsequently be confirmed by two other laboratories. However, confusing results were obtained in that the respective murine

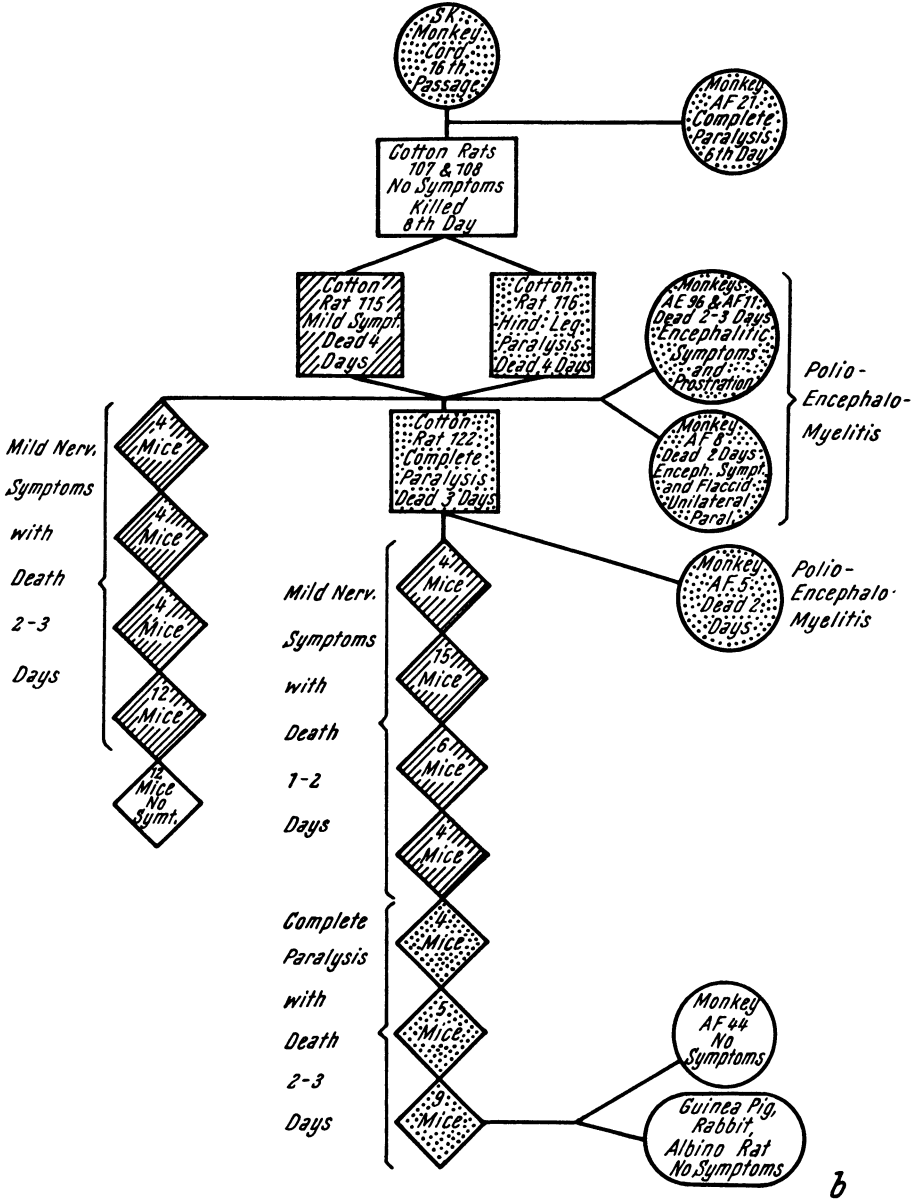

Fig. 1b. Genealogy of SK mouse virus (2nd isolation). (C. W. JUNGEBLUT and M. SANDERS, ibidem.)

strains showed divergent properties. Thus, TOOMEY and TAKACS (1941), injecting the 13th monkey generation of SK virus into cotton rats, obtained on one occasion a low-titred strain, similar to the Lansing virus, whereas another cotton rat adaptation resulted in a strain with high rodent-pathogenicity, similar to Col SK

virus (TOOMEY and TAKACS 1942). Experiments carried out by MELNICK and associates (MELNICK 1946; LAWSON and MELNICK 1947; MELNICK and WARD 1948), in which the 15th, 20th, 21st and 22nd monkey generations were transmitted to cotton rats, yielded only a low-titred murine strain. This strain has since been designated as Yale SK virus (Y-SK) to distinguish it from the Columbia isolate. Further comparison of both strains, Col SK and Y-SK, made it clear that there were other differences between the two agents with respect to their serological reactivity and pathogenicity for rhesus monkeys.

The paradoxical situation in which two strains, Col SK and Y-SK, with different biological and serological properties, were apparently isolated from the same material requires some comment. While viruses may develop new biological properties as they adapt themselves to new hosts, they usually preserve their immunological specificity

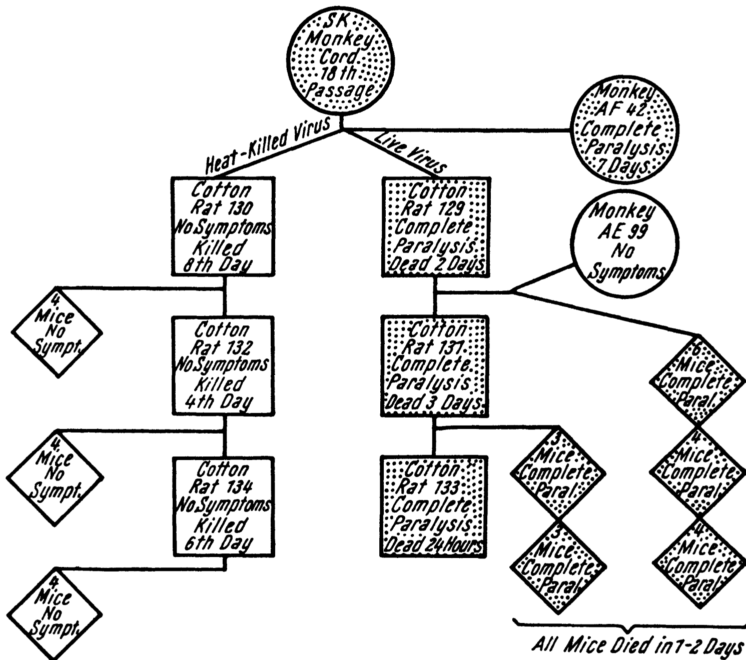

Fig. 1c. Genealogy of SK mouse virus (3rd isolation). (C. W. JUNGEBLUT and M. SANDERS, ibidem.)

in that process. However, examples are not wanting of changes in antigenic structure occurring through mutation or selection of variants, either spontaneously or as the result of animal passage (see JUNGEBLUT, 1952, p. 19). A seemingly simple solution has been offered with the suggestion that Col SK virus was not really adapted from simian SK virus but originated in the wild cotton rats used for the adaptive process (DICK 1949; MELNICK 1950; WARREN 1952). This concept of a latent cotton rat virus activated by passage is not in harmony with the fact that cotton rats, whether infected by central or peripheral routes, are maximally susceptible to Col SK virus and that this high susceptibility existed already on first isolation of the virus. Furthermore, at the time these experiments were carried out, cotton rats from the same source (Venice, Florida West Coast) were employed on a large scale in unsuccessful attempts to adapt a considerable number of other monkey strains of poliomyelitis virus. Adaptation of SK virus, on the other hand, could be demonstrated in three successive experiments (see Fig. 1a-c), yielding in every instance comparable strains;

control set-ups in which heat-killed SK virus was passed serially through blind cotton rat passage gave completely negative results. There is, therefore, reasonable assurance that the murine virus had not generated in carrier cotton rats. This viewpoint is further supported by the following facts: 1) spontaneous paralysis has never been observed in wild or laboratory-bred cotton rats, 2) virus has never been isolated from cotton rat faeces during an interval of 17 years since isolation of Col SK virus, 3) normal cotton rat serum failed consistently to inactivate the virus not only at the time of isolation but again, one year later, in tests repeated with cotton rats supplied from the same source. The absence of antibodies against Col SK virus in the serum of healthy, wild-caught cotton rats was once more clearly shown in subsequent work by WARREN et al. (1949). Thus, no experimental or epidemiological proof can be found for this speculation and in 1953 DICK also comes to the conclusion "that cotton rats *(Sigmodon hispidus)* are unlikely to play any part in the epidemiology of this group of virus infection in North America". It is difficult, however, to offer a satisfactory explanation of the facts in the case. One possibility would be that the simian SK strain was not a homogenous virus but represented a mixed virus population, containing two variants in an interfering system. Some merit may be found in such a view: 1) because human SK virus, on its first isolation in the monkey, exhibited rather unorthodox properties with respect to peripheral infectivity and serological behavior, 2) because the human virus was propagated, in its early passages, by alternating injection of rhesus and cynomolgus monkeys; this may have permitted varying degrees of survival of Col SK and Y-SK virus, the former being only pathogenic for cynomolgus monkeys, the latter being pathogenic for both cynomolgus and rhesus monkeys (see later observations with the AK strain), 3) because interference between Col SK and Y-SK virus can readily be demonstrated, resulting in domination of one strain over the other; the selection of one or the other virus in the cotton rat may have depended on quantitative variation in the interference system. The most logical assumption seems that the patient, from whom the orginal stool sample was obtained, was actually suffering from a double infection with two different viruses. Support for such a hypothesis would come from the fact that Coxsackie viruses and poliomyelitis viruses are often encountered together in the stools of patients diagnosed as cases of poliomyelitis. While separation of the two viruses is sometimes feasible, as in the case of the Dutch E. S. strain and probably also of the Col/Y-SK virus complex, it is not inconceivable that under certain conditions a recombination or synthesis of the two types of virus could occur, yielding an agent with new properties. Whatever the correct answer may be, it is now clear that the original conclusion, namely that the SK strain of human poliomyelitis virus had been adapted to mice, must be abandoned or at least modified to the extent that only part of the human viral agent was actually transferred to rodents in these experiments.

2. MM Strain (JUNGEBLUT and DALLDORF 1943).

In the fall of 1942 five cases of poliomyelitis occurred within a sharply circumscribed half square mile area of White Plains, Westchester County, N. Y. The cases followed each other in rapid sequence between the end of September and the beginning of October. They were the only cases reported in the town for the current year, though 2 cases of poliomyelitis had been reported in this locality during the previous year. The outbreak involved 2 adults and 3 children; two patients died of bulbar paralysis and 3 recovered with extensive residual paralysis. Postmortem examination of both fatal cases revealed severe and typical poliomyelitic lesion in the cord and medulla.

Intracerebral injection of a rhesus monkey with brain and cord material from one of the fatal cases (T. D.) failed to produce any symptoms. However, prostrating paralysis — with typical cord lesions — occurred in another rhesus monkey following intracerebral injection with a suspension from cord and medulla of the second fatal case (Martin Mahoney). Intracerebral transfer of this monkey

cord to another monkey failed to transmit paralysis but caused death in a Syrian hamster on the 20th day after injection. At the same time the original human material was also injected intracerebrally into other laboratory animals, i. e. rabbits, guinea pigs, albino mice, cotton rats, and hamsters. None of the injected animals showed any definite signs of disease, except for one hamster which appeared sick on the 18th day after injection and was found dead the next morning. Intracerebral transfer of this hamster's brain to another hamster caused in the latter animal complete flaccid paralysis of both hind legs within 5 days. Further intracerebral passage of the paralyzed hamster's brain to new hamsters, cotton rats, albino mice, rabbits and rhesus monkeys was followed by prostrating paralysis, within 2 to 3 days, in all injected small rodents; the monkey developed a transient fever but remained free from recognizable paralysis and no symptoms were observed in the rabbit. From this point it was possible to transmit the disease serially through passages of hamsters and mice, using either intracerebral or intraperitoneal routes of injection. MM virus showed cross neutralization with Col SK virus indicating the similarity of both agents. At the time of the isolation of MM virus all experimental animals were kept in strict quarantine and the chances that the virus had been picked up by Laboratory contamination with Col SK virus are remote. The alternate possibility that the virus had originated in the hamster as a latent virus is not supported by any subsequent experience.

3. EMC Strain (HELWIG and SCHMIDT 1945).

Between November 1944 and October 1945 there occurred three unexplained deaths among one gibbon and two chimpanzees on exhibit at the Anthropoid Research Station in Dania, Florida East Coast. All three animals had been in close contact with visitors. Death occurred unexpectedly, after only short illness, with symptoms of cardiovascular collapse. In all three instances the autopsy showed massive myocarditis and marked pulmonary edema but no pathological changes in the central nervous system. No attempt was made in the first case to recover the etiological agent. In the second case the chest fluid and a saline suspension of ground spleen were injected intracerebrally or intraperitoneally into mice, and the virus was thus recovered. In the third case mice inoculated with pleural fluid failed to yield any virus. The infections specimen of the second case caused flaccid paralysis and myocarditis in mice; hamsters and guinea pigs were also susceptible to the virus and presented similar paralytic or cardiac symptoms. Histological studies by SCHMIDT (1948) of the spinal cords of paralyzed mice showed extensive destruction of all the outer neural elements but the ganglion cells were apparently not involved by this myelitis. Foci of encephalitis could be seen in the mouse brain with perivascular cuffing of adjacent vessels, but no encephalitis was encountered in the guinea pig. Extensive involvement of the heart muscle was also present, with necrosis of fibers and round cell infiltration. This virus was later shown to have cross-immunological relationships with Col SK virus and was called EMC virus (Encephalomyocarditis virus) because it produced encephalitis and myocarditis in experimental animals.

The infection with this virus appears to be endemic at the Anthropoid Research Station in Dania, Fla., as shown by subsequent studies of WARREN (personal communication). In August 1953 a 2½ year old chimpanzee caged at the same place developed fever and dyspnoea and died 17 days later with terminal signs of pneumonia. At autopsy the chimpanzee was found to have a massive pleural effusion and the heart was considerably enlarged. Histological examination of the heart revealed an acute endo- and myocardial process in several areas; no important pathological changes were observed in any of the other tissues examined. Small portions of tissue

obtained from the lung, liver, spleen, heart muscle and kidney were emulsified and inoculated into albino mice but all injected animals remained well. While no infectious agent could be isolated, the serum taken on the 7th day after onset of illness neutralized 32,000 LD_{50} doses of EMC virus. Approximately 2 weeks after the death of this animal, sera were collected: a) from 9 additional chimpanzees in cages near the deceased animal; b) from 14 wild rats *(Rattus norvegicus)* which were abundant in the vicinity of the animal cages; c) from 11 persons who had been employed at the animal farm for varying periods of time. All sera, together with the frozen viscera from 6 of the rats, were examinated at the Army Medical Service Graduate School in Washington, D. C. The sera were tested for neutralizing antibody against EMC virus (intraperitoneal mouse test) and only those neutralizing at least 1000 LD_{50} doses of virus were considered positive; the rat viscera were inoculated into albino mice. No virus was' isolated from the rats. Results of the neutralization tests were as follows: 2 of 9 chimpanzee sera and 6 of 14 rat sera were positive, all of 11 human sera were negative.

Further isolations of EMC virus from the same area in Florida were reported in 1956 by KISSLING et al. In June 1952 specimens of lung, liver, spleen, kidney and stomach contents from a baboon which had died of unknown cause at the Anthropoid Ape Research Station in Dania, Fla. were sent to the Communicable Disease Center of the U.S.P.H.S. in Montgomery, Ala. by the Florida State Board of Health. The baboon had died 24 hours, after the first visible signs of illness; the animal had been at the primate farm for 3 or 4 years. Pooled suspensions of these viscera were inoculated into albino mice and yielded in the second mouse passage a high-titred viral agent which was specifically neutralized by EMC antiserum. A second isolation of a virus with essentially the same properties was made in February, 1953, from the brain of a monkey which had been housed at the same place in Dania. EMC virus was isolated a third time from a squirrel brain submitted by the Florida State Laboratory in February 1954. This squirrel, which originated in the neighboring Palm Beach County, Fla. had appeared sick and bit a child who handled it. Therefore it was captured to have it examined for evidence of rabies infection. This inadvertent finding represents the first isolation of EMC virus from a wild animal indigenous to North America, although antibodies to EMC virus have previously been demonstrated in wild rats in the Southern United States by WARREN and co-workers.

The source of infection at Dania is still obscure. The original observers emphasized the intimate contact of the primates with man and there is ample evidence that typical poliomyelitis may develop in chimpanzees after natural contact with human patients or carriers. However, an epidemiological survey of the incidence of poliomyelitis in Florida between 1944 and 1946 fails to reveal any immediate and convincing temporal or local relationships with reported human cases. Also, no human illnesses pointing to EMC infection or serum antibodies were found among the personel at the station. A different clue is offered through the work of WARREN and associates (1949) who demonstrated repeatedly the presence of specific virucidal antibodies in the serum of wild rats *(Rattus norvegicus)* trapped in the vicinity of the primate farm. This observation suggests a local reservoir of the virus in wild rats. It is not clear, however, whether the anthropoid apes received the virus from the rats or whether the rats were infected by the apes, and the point may be argued either way.

4. Mengo Strain (DICK, SMITHBURN and HADDOW 1948; DICK 1948).

This virus is of special interest because there is direct evidence that, apart from being pathogenic for laboratory animals, it is also pathogenic for man. The agent was originally isolated in 1946 by direct transfer to mice from the CNS of a spontaneously paralyzed rhesus monkey which had been captive in the outside monkey quarters of the Yellow Fever Research Institute in Entebbe,

Uganda (Africa). A month later the same virus was isolated, on two occasions, from pools of mosquitoes *(C. taeniorhynchus)* captured in the vicinity of the Institute or at some distance from Entebbe. Subsequently two more isolations succeeded, one from the blood of a wild-caught apparently healthy mongoose, the other from the blood of a baboon which had been sick with a febrile illness without showing any signs of paralysis. All viral strains were serologically identical among themselves and were cross-neutralized by Col SK and EMC virus antisera. Finally, (November 1946) there was also reported a case of human infection with this virus in one of the laboratory workers. (DICK et al 1948.) The clinical history of the patient's (DICK) disease, beginning after an incubation period of 5-8 days, refers to symptoms of a mild encephalomyelitis (fever, vomiting, headache, irritability, delirium, deafness, hyperaesthesias, photophobia), but symptoms of transient paralysis were also present, involving the muscles of deglutition and the upper fibers of the right trapezious muscle. Had the case occurred in the midst of a poliomyelitis epidemic it would probably have been listed neurologically as a case of polioencephalomyelitis, with a presumable localization of the lesion in the upper cervical region of the cord and involvement of the nuclei of the 8th, 9th and 11th cranial nerves. The virus isolated from the patient's blood during the acute stage (1st day of illness) produced fever and muscle weakness in rhesus monkeys and fatal encephalomyelitis in mice and in guinea pigs. The identity of the human strain was established through neutralization tests with the patients serum and the original virus isolation from the paralyzed rhesus monkey. The agent received the name Mengo virus because of its occurrence in the Mengo district of Uganda. During December 1951 three more strains were isolated from monkeys in the open runs of the compound which had died suddenly without previous neurological or other symptoms. The multiplicity of Mengo virus isolations in Entebbe, Uganda, compare with the repeated EMC virus isolations in Dania, Florida, and indicate that the virus in some localities must have an extensive extrahuman reservoir in wild animals.

5. AK Strain (VERLINDE, BEEM and KLARENBEEK 1952).

The adaptation of this strain of poliomyelitis virus from monkeys to rodents has many points of similarity with the descendance of the Col SK strain so that the circumstances under which the mouse-pathogenic AK virus was obtained by the Dutch authors will be discussed *in extenso*. The simian AK strain was isolated in 1949 by VERLINDE and NIHOUL (1950) from the stools of a 2-year old girl with the clinical diagnosis of poliomyelitis, on the 6th day after onset of paralysis. The strain was carried by VERLINDE, BEEM and KLARENBEEK (1952) through 17 serial passages either in cynomolgus or in rhesus monkeys. Paralysis and characteristic anterior horn lesions developed regularly following intracerebral, intramuscular or intratonsillar inoculation; the incubation period varied from 4-27 days, with an average of 9 days. Rhesus cord from the 16th passage induced paralysis in only 1 of 3 rhesus monkeys and the virus died out in the next rhesus passage. Attempts to produce paralysis in mice with earlier monkey cord passages were unsuccessful but the 17th monkey passage proved pathogenic for mice by intraspinal, intracerebral or intramuscular injection; the mouse-pathogenic strain proved avirulent for rhesus monkeys and had a very low virulence for cynomolgus monkeys (see fig. 2). The high mouse infectivity titer (10^{-8}), the short incubation period (4 to 5 days), the hemagglutinating capacity, and the loss of monkey pathogenicity of the murine strain raised doubts in the minds of the investigators as to the possibility of accidental con-

tamination with one of the established viruses of the Col SK group. Therefore, the mouse inoculations were repeated several times under very carefully controlled conditions. Mice inoculated with monkey cord of the 17th passage of the AK strain were caged in a place where no animals had been kept before;

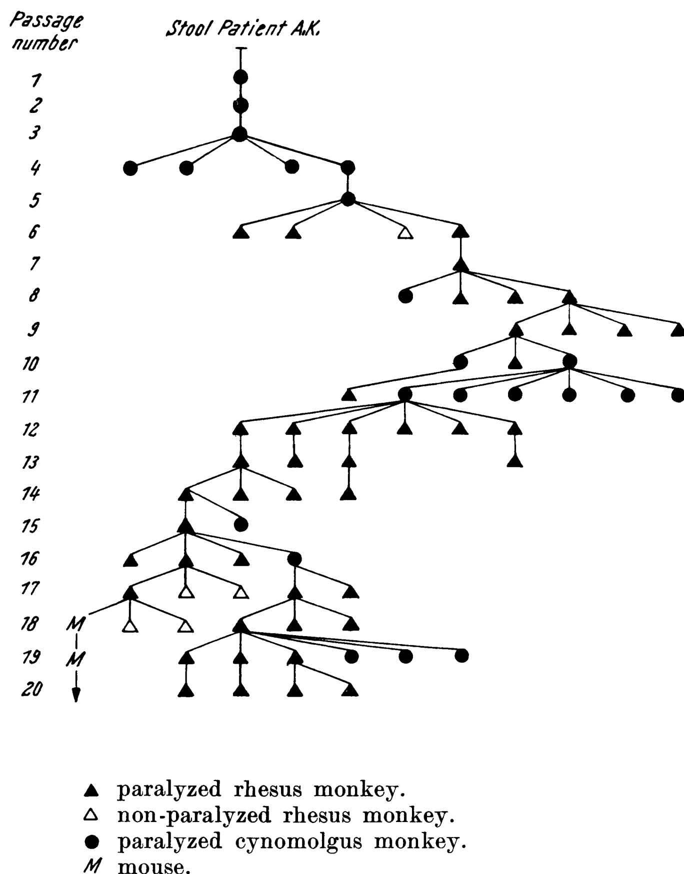

Fig. 2. Derivation of the AK strain (J. D. VERLINDE and A. KLARENBEEK, Ant. v. Leeuwenhoek **19**, 324, 1953).

for control purposes, mice inoculated with earlier monkey passages of the AK strain (10th to 16th) or with the Brunhilde strain were placed in an animal house together with mice infected with viruses of the Col SK group and no precautions were taken to prevent cross infection. Paralysis did not develop among any of the control mice, but mice inoculated with the 17th monkey passage of the AK strain came down regularly with paralysis. Thus, there seems to be circumstantial evidence that the mouse-pathogenic agent was already present in the 17th monkey passage. A new monkey-line was then started from the 16th passage which was carried until the 21st serial passage. All these passages were only pathogenic for monkeys, except for the 19th passage which again proved also virulent for mice. The murine strain derived from the 19th monkey passage had the same properties as that which had originated from the 17th passage, with the only difference that it had maintained its monkey-pathogenicity. The

apparent similarities in the isolation of the murine AK strain with that of Col SK virus were pointed out by the Dutch authors. The immunological properties of the AK strain which were studied in great detail will be discussed elsewhere.

Later studies of the AK strain by VERLINDE and MOLRON (1954) throw new light on the nature of this virus. Thus, whereas Lansing antiserum unequivocally neutralized AK virus in rhesus monkeys, no such effect could be obtained in cynomolgus monkeys unless the Lansing serum was mixed with an equal amount of Col SK antiserum. The mystery can be resolved if one assumes that the AK strain possesses both a Lansing and a Col SK component, or is a mixed virus containing both agents. For, since cynomolgus monkeys are susceptible to both, Type II poliomyelitis and Col SK virus, Lansing serum or Col SK serum alone will fail to produce a complete neutralization effect of such a complex virus. On the other hand, full neutralization in rhesus monkeys can readily be obtained with Lansing serum alone, because these monkeys are, as a rule, resistant to Col SK virus while they are susceptible to Lansing virus. Attempts to separate the two viruses by neutralization with limiting dilutions of Lansing serum or Col SK serum failed. Since the two components proved inseparable it was thought that in the early monkey passages of the AK strain Type II poliomyelitis virus had suppressed the Col SK virus by effective interference and that in the 17th monkey passage interference was reversed, Col SK virus suppressing the poliomyelitis virus. This hypothesis was put to experimental test by intracerebral serial passage in rhesus monkeys of an *in vitro* prepared mixture of Col SK virus and Type II poliomyelitis virus (AYCOCK strain). The results obtained (Table 1)

Table I. *Comparison of serial Rhesus Monkey passages of a mixture of Type II Poliomyelitis Virus (Aycock) and Col. SK Virus with the 16th passage of the AK Strain*

(J. D. VERLINDE and J. H. MOLRON, Ant. v. Leeuwenhoek 20: 129, 1954)

Passages	Aycock Monkey Virus + Col. SK Mouse Virus	AK Strain 16th Monkey Passage
1st Passage	1 rhesus paralyzed ↓	1 rhesus paralyzed, 2 rhesus negative ↓
2nd Passage	2 rhesus negative, mouse positive ↙ ↓	2 rhesus negative, mouse positive ↙ ↓
3rd Passage	rhesus negative, mouse positive	rhesus negative, mouse positive

show a striking similarity between the naturally occurring loss of virulence of the AK strain for rhesus monkeys and the experimentally induced interference which leads to suppression of the AYCOCK strain and dominance of Col SK virus. Similar tests carried out with another freshly isolated strain (E. S.) which had the joint characteristics of Type II poliomyelitis virus and of Col SK virus will be described later.

German Strains: 6. F Virus (KOCH and BIELING 1950) 7. Ortlieb Virus (VIVELL 1950).

In 1949 a number of illnesses characterized by vague symptoms of meningitis, encephalitis, or pneumonic involvement occurred among a group of 33 children in Giessen, Germany. Attempts were made by KOCH to isolate a viral agent by injecting mice or hamsters (ic or ip) with blood, spinal fluid, stool suspensions, or ear and throat washings from these cases. These inoculations as well as subsequent antibody studies with the patient's sera were carried out by BIELING (1949) in Marburg. A viral agent which caused paralysis in rodents was recovered from 4 cases in the group from blood, spinal fluid, or stools; three isolations were carried in serial mouse passages, one isolation could not be transmitted beyond the first generation. The authenticity of these isolates is supported by the fact that specific neutralizing antibodies were present in about 18% of the convalescent human sera. Of the four strains described, Strain F is the best known because it was later studied extensively in other Laboratories. Strain F was isolated from the spinal fluid of a 10 weeks-old infant, with the clinical diagnosis of bronchopneumonia, who died of myocarditis; the anatomical-pathological diagnosis was confirmed by autopsy. The Giessen strains are said to be similar to several strains isolated independently by BIELING (1952) in 1949/50 from cases with meningeal involvement in Marburg, some of which had antibodies against Col SK virus. The Giessen-Marburg strains also appear to be closely related in biological and immunological properties to five or six strains isolated in 1950/51 by VIVELL (VIVELL and MAUER 1952) in Freiburg from the spinal fluid of patients with aseptic meningitis, encephalitis, or myelitis; these cases are more fully described by BETKE and KAISER (1952). The most important representatives of this latter group are the Leonhard and the Ortlieb strains. The Ortlieb strain which was isolated repeatedly under carefully controlled conditions from the stools of a fatal case of encephalitis is considered to be the prototype of the Freiburg strains, even though antibody determination in the patient's serum was not possible. Another strain isolated by BELLER and KELLER (1949) as early as 1943 (Li 32) — originally thought to be also a member of this group of viruses — was subsequently shown to be a virus belonging to the Encephalitis B group. (KELLER and VIVELL 1955.) The relationship of Strains F and Ortlieb to the Col SK group was confirmed in the writer's Laboratory on the basis of serological and hemagglutination studies as well as by VERLINDE and HOFMAN (1952). Strains F (Faitz) and S (Senger) were also studied by DICK (1953) who found both to be closely related, if not identical, with Mengo Virus as evidenced by their immunogenic properties and pathogenicity for monkeys.

Further Isolations of Col SK Virus in Holland: (S and W Strains).

During a period of 4 years, in which several hundred stool samples, spinal fluids or throat washings from patients with the clinical diagnosis of poliomyelitis, encephalomyelitis, aseptic meningitis and epidemic pleurodynia were routinely inoculated into suckling or adult mice, three strains of virus belonging to the Col SK group were isolated by VERLINDE and VAN TONGEREN (1953) in Leiden. The first strain was recovered from the stools of a case of aseptic meningitis in a boy, 2 years of age, after a short period of illness. The virus was readily transmissible in suckling or adult mouse passages producing encephalomyelitis after ic or ip inoculation. The brain suspension gave typical hemagglutination to a titer of 1 : 160 which was inhibited by Col SK antiserum to a titer of 1 : 4096.

Table 2. *Isolation of S Virus and of W Virus*

(J. D. VERLINDE and H. A. E. VAN TONGEREN: Arch. ges. Virusforschg. 3: 217, 1953)

Isolation	Animals	Patient S		Controls*	Animals	Patient W		Controls*
		Spinal Fluid	Stools			Throat Washings	Stools	
I	Suckling Mice ↓	$^{0}/_{8}$ sc	$^{5}/_{5}$ 4—5 d sc	$^{0}/_{14}$	Suckling Mice ↓	$^{5}/_{5}$ 2—3 d sc	$^{5}/_{5}$ 2—4 d sc	$^{0}/_{12}$
	Suckling Mice ↓		$^{7}/_{7}$ 3 d sc		Adult Mice ↓	$^{5}/_{5}$ 2—3 d ic	$^{5}/_{5}$ 2—3 d ic	
	Adult Mice ↓		$^{4}/_{4}$ 2 d ic		Adult Mice	$^{7}/_{8}$ 3—6 d ic	$^{8}/_{8}$ 3—7 d ic	$^{0}/_{12}$
	Adult Mice		$^{4}/_{4}$ 2 d ic					
II	Adult Mice ↓		$^{3}/_{4}$ 4—7 d ic	$^{0}/_{8}$	Adult Mice ↓	$^{3}/_{6}$ 3—4 d ic	$^{6}/_{6}$ 3—7 d ic	$^{0}/_{14}$
	Adult Mice ↓		$^{3}/_{4}$ 2 d ic		Adult Mice ↓ (→ Rabbit)	$^{8}/_{8}$ 3—4 d ip	$^{8}/_{8}$ 3—5 d ip	
	Adult Mice		$^{4}/_{4}$ 2 d ic		Guinea Pig ↓		$^{2}/_{2}$ 3—4 d ic	
					Cynomolgus		$^{1}/_{1}$ 4 d ic	
					Rabbit ←		$^{0}/_{1}$ ic	
III	Cynomolgus ↓		$^{1}/_{1}$ 5 d ic	$^{0}/_{1}$	Cynomolgus ↓	$^{1}/_{1}$ 12 d ic	$^{0}/_{1}$ ic	
	Adult Mice		$^{8}/_{8}$ 2—4 d ic/ip		Adult Mice	$^{4}/_{6}$ 3—5 d ic		

* Controls = Normal Spinal Fluid. Normal Stools, or H_2O
Numerator = number of mice paralyzed: Denominator = number of mice injected

Since no specific antibodies to the virus could be demonstrated in the patient's serum the authors considered the isolation as inconclusive and destroyed the strain. Two other strains of the same type were recovered in November 1952 and January 1953: one from the stools of patient E. S., a girl 2 years of age with the clinical diagnosis of paralytic poliomyelitis, the other from the stools and throat washings of patient W., a girl 1 year of age with the clinical diagnosis of encephalomyelitis. The two patients lived in widely separated areas of the country. Results of repeated virus isolations and of neutralization tests with the patients sera are shown in Tables 2 and 3. Both viruses agglutinated sheep

Table 3. *Neutralization of S and W Virus in Mice by Patients Sera*
(J. D. VERLINDE and H. A. E. VAN TONGEREN: ibid)

Virus	Serum	Neutralization Index (Log)
S	Patient S 10th Day	0.8
	Patient S 29th Day	2.2
	Patient S 43rd Day	2.4
	Father S	0
	Mother S	0
	2 Sisters S	0
W	Patient W 10th Day	1.0
	Patient W 25th Day	2.2
	Father W*	3.2

* History of minor illness with mild diarrhea shortly before child took ill.

red cells and human group 0 cells and the hemagglutinating capacity was inhibited by Col SK virus antiserum. Interestingly enough, the two virus strains did not produce precisely the same type of illness in cynomolgus monkeys. Thus, strain S induced flaccid paralysis and the cord showed lesions indistinguishable from those found in experimental poliomyelitis; cerebral pathology was limited to the motor cortex and the roof of the cerebellum. On the other hand, the symptoms produced by Strain W were those of encephalomyelitis; lesions were present both in the anterior horns of the cord and disseminated throughout the brain, including the cerebral cortex, the brainstem and the roof of the cerebellum. Since the children recovered completely, the authors are careful to point out that it must remain uncertain whether or not the viruses were actually responsible for involvement of the central nervous system in the patients.

In later work with the ES strain VERLINDE and MOLRON (1954) came to the conclusion that this isolate was apparently a mixture of Type II poliomyelitis virus and of Col SK virus, for the following reasons: The patients material proved pathogenic for mice and for cynomolgus monkeys and cord-virus from the first cynomolgus passage could be transmitted to cynomolgus and rhesus monkeys as well as to mice; however, the murine strain possessed the characteristics of Col SK virus whereas the simian strain, when typed by neutralization against the three prototype antisera, appeared to be related to Type II poliomyelitis virus. Both components of the ES strain could be separated by neutralization of second passage cynomolgus cord with either Lansing or Columbia SK hyperimmune serum.

The results of this study suggest that Col SK virus, like Coxsackie viruses, may occasionally be isolated from human material in combination with poliomyelitis virus, causing a mixed infection with both viruses. It is conceivable that the same occurred not only upon isolation of the original Col SK virus and the Yale-SK virus, but also with MM virus which died out in serial rhesus monkey passage.

Col SK Virus Isolations from South American Monkeys.

Two strains of virus belonging at least antigenically to the Col SK group, were isolated in 1950-51 by Roca-Garcia[1] in Colombia, South America, from night monkeys *(Aotus trivirgatus)*. The animals had been trapped in the vicinity of the town of Villavicencio and were delivered at the Carlos Findlay Institute, Bogota. The first virus isolation was from a night monkey which arrived at the Laboratory in July 1950 in moribund state. On autopsy, the lungs were found congested and, although other organs showed no macroscopic abnormality, histopathological studies revealed myocarditis. The virus was recovered by injecting mice intranasally with lung suspension. A second strain originated from a lot of three night monkeys received in February 1951. Two days later one of these animals was found dead although it had shown no previous signs of illness. No macroscopic lesions were found at autopsy, but histopathological studies of the organs revealed an encephalitis. Virus was isolated in mice following injection with suspensions from brain or lung, and a pool from liver, spleen and kidney. The virus was pathogenic for mice by intracerebral or peripheral inoculation, causing paralysis within 3-19 days depending on the route of infection and the amount of virus used. Virus was present in blood (viremia from the first to the seventh day, with a peak on the second and third day) as well as in lung, liver, spleen, kidney and adrenals, with the highest virus concentrations in the adrenals and brain; virus was also excreted in the stools from the first to the tenth day after inoculation. Guinea pigs, young hamsters, white rats and rabbits injected with the virus showed no signs of illness but circulating virus was demonstrable in each case for several days and specific antibodies were found in the serum after one month; infected white rats also excreted virus for a period of several days. When tested for monkey pathogenicity, the virus produced no illness in rhesus monkeys but circulating virus was present in the blood for several days and all monkeys developed neutralizing antibodies. Four susceptible Aotus monkeys were injected by various routes with first passage mouse brain virus. Virus was present in blood samples from all animals following inoculation. Three monkeys died on the fourth day and virus was recovered from their brains; one monkey survived but developed specific antibodies. By cross neutralization tests in mice the virus was identified as belonging to the Col SK group since Mengo immune serum neutralized the agent and post-inoculation sera from the experimental animals neutralized the strain. The virus was not examined for hemagglutinating capacity. Attempts to infect *Aedes aegypti* by feeding mosquitoes on baby mice infected with the virus or by bathing mosquitoe larvae in virus suspensions gave negative results. Unfortunately, none of these strains were preserved for further study.

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[1] We are greatly indebted to Dr. Manuel Roca-Garcia (Lederle Laboratories, Pearl River, N. Y.) for a full account of these isolations.

Morphology of Virus.
(Electronmicroscopy, Ultrafiltration, Ultracentrifugation)

Determinations of the physical constants of a virus by electron-microscopy, ultrafiltration, ultracentrifugation, ultraviolet light absorption etc. depend for their reliability on the use of purified preparations. Unfortunately, many of these tests with Col SK virus were carried out with only partially purified material, either from animal passage or tissue culture origin, and it is doubtful whether any of these preparations reach the degree of purity and homogeneity that has been achieved for poliomyelitis virus with the help of modern methods of purification. For that reason the data may be open to revision.

Col SK virus has been estimated by SANDERS and JUNGEBLUT (1942) to have a particle size between 10 and 15 mμ by ultrafiltration of infected tissue culture fluid. The endpoint of filtration was 30 mμ and the calculation is based on Elford's formula which computes the ultrafiltration measurement as being equal to one third to one half the diameter of the largest retaining pore of the membrane. Using physical and chemical methods of extraction and concentration (isoelectric precipitation, salting out, ultracentrifugation) from infected mouse brains, BOURDILLON and MOORE (1942) and BOURDILLON (1944) obtained highly

Table 4. *Potency of Purified Col. SK Mouse Virus Preparations.*
(J. BOURDILLON: Arch. Biochem. 3: 285, 1944)

Material	Volume	Approximate Protein Concentration	Titer (Log of MLD. per Inoculum)							
			Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8
I Original Suspension	400 cc	0.2 %	7.0	7.5	ca. 7.5	7.2	< 6.5	7.5	7.2	7.3
II Soluble Fraction of Acid Precipitate from I	40 cc	0.05 %	8.0	8.5	ca. 8.5	8.7	8.2	> 8.5	7.8	8.8
III Soluble Fraction of Ultracentrifuge Sediment from II	1 cc	0.01 %					9.5			9.7

potent virus preparations which possessed an appreciable degree of physical homogeneity as determined in the analytical ultracentrifuge and which reacted in precipitin tests with specific viral antiserums. It is of interest that similar precipitin reactions have quite recently been described by SMITH et al. (1956) when purified preparations of poliomyelitis virus were brought together with type-specific antisera. The degree of purity achieved by BOURDILLON in eight different purifications is indicated by a definite increase in virus titer with a simultaneous decrease in total protein concentration as illustrated in Table 4.

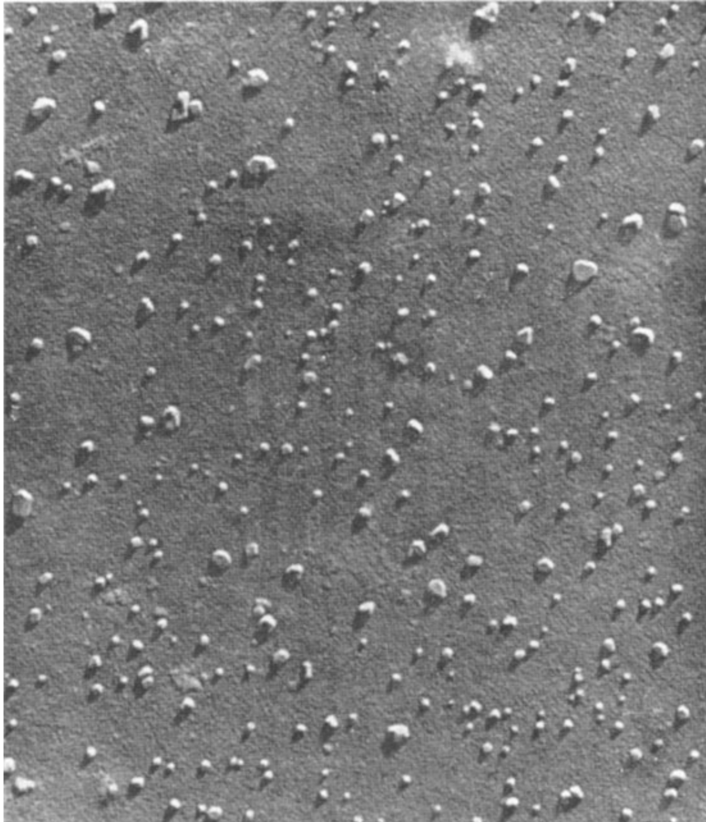


Fig. 3a. Electronmicrogram of purified Col SK Virus. Preparation purified by adsorption and elution with red cells. Magnification 29,700 ×. The irregularly shaped large forms are salt crystals remaining in the preparation. The larger-size spherical particles, which measure 26-32 mμ individually, are considered to be the virus. The nature of the smaller-size spherical particles, measuring 12-14 mμ, is unknown.

A dialyzed preparation examined between crossed polarized sheets showed double refraction, suggesting the presence of elongated molecules, some of which may have formed into aggregates approaching the crystalline state. Runs in the ultracentrifuge with a highly purified and concentrated preparation showed, after eliminating a slowly sedimenting component, only one boundary which had a sedimentation rate of about 130 S, with comparatively little spreading. The figure of 130 S is not too far apart from a figure of 160 S reported by GARD and PEDERSON (1941), and later again by LEYON (1951), for THEILER virus. By comparison, the sedimentation coefficient of a 0.02 per cent suspension of

purified MEF poliomyelitis virus particles is given by SCHWERDT and SCHAFFER (1955) as 154×10^{-3} cm/sec/dyne/gm. Assuming for Col SK virus a molecular weight of 10 million (which corresponds roughly with a sedimentation constant of 130 S), the high infectivity-protein ratio of the purified preparations leads to the conclusion that something of the order of 100 molecules represents one infectious dose. When tested in the TISELIUS apparatus, the isoelectric point of the virus was p_H 6 and its approximate electrophoretic mobility was 2.0×10^{-5} at p_H 7.1, ionic strength 0.1, or 5.3×10^{-5} at p_H 8.4, ionic strength 0.1. Schlieren diagrams permitted no correlation between any visible fraction and virus mobility. The major component in purified MEF material, with which infectivity was

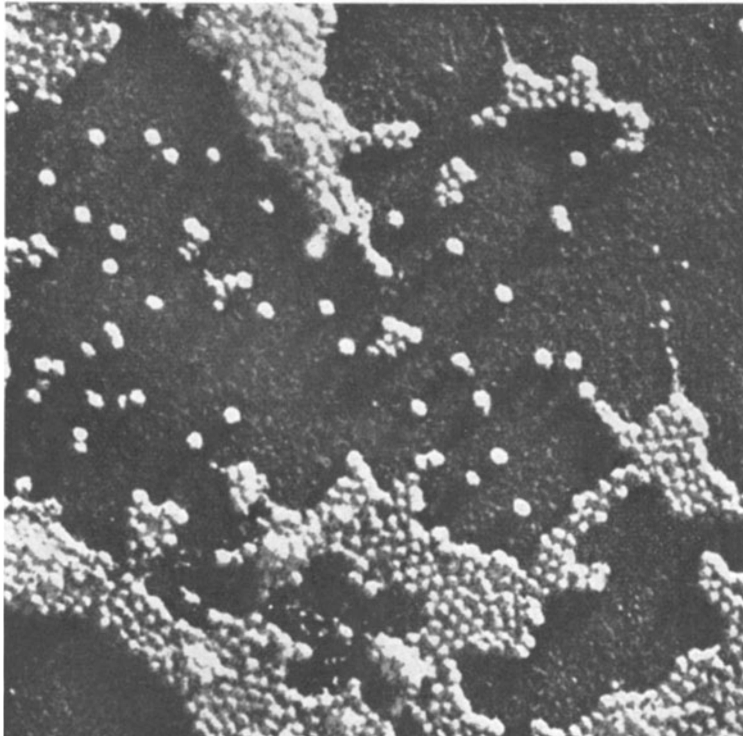


Fig. 3b. A purified preparation of THEILER's virus, strain FA, gold-shadowed, magnification 60,000 × (From: H. LEYON and S. GARD: Biochim. et Biophys. Acta 4, 385, 1950.)

found to be associated, moved with the low mobility of -0.5×10^{-5} cm^2 volt sec. The few data on the electrophoresis of viruses thus far reported suggest that viruses have p_H mobility curves of the same type as most proteins.

Electron micrograms made from BOURDILLON's purified mouse brain preparations containing Col SK virus (JUNGEBLUT and BOURDILLON 1943) showed fairly clear and uniform eliptic or spherical particles of 25 to 30 mμ in diameter which were absent in non-infectious control material. Unpurified tissue culture preparations infected with the same virus showed long, threadlike forms measuring 20 by 75 to 5000 mμ which were not found in uninoculated controls; media infected with WEE virus showed no threads but poorly defined small spherical bodies. The filamentous threads in the Col SK tissue culture prepara-

tions, in some instances, seemed to be made up of a series of small spherical bodies, giving a picture of beaded, string-like structures. These forms are not unlike those published by GARD (1943) for the high molecular alpha component which he found in purified THEILER virus or human poliomyelitis virus and which he considered identical with the virus unit; since the same component was also present in normal stools from man, mice or brown wild rats (GARD et al. 1944), GARD thought of it as an incomplete form (intestinal protein), or precursor of the neurotropic virus variant. Purification of Col SK virus by precipitation with methanol and liberation of the virus at a narrow *p*H range was described by SANZ-IBANEZ and TOLEDANO JIMINEZ-CASTELLANOS (1948).

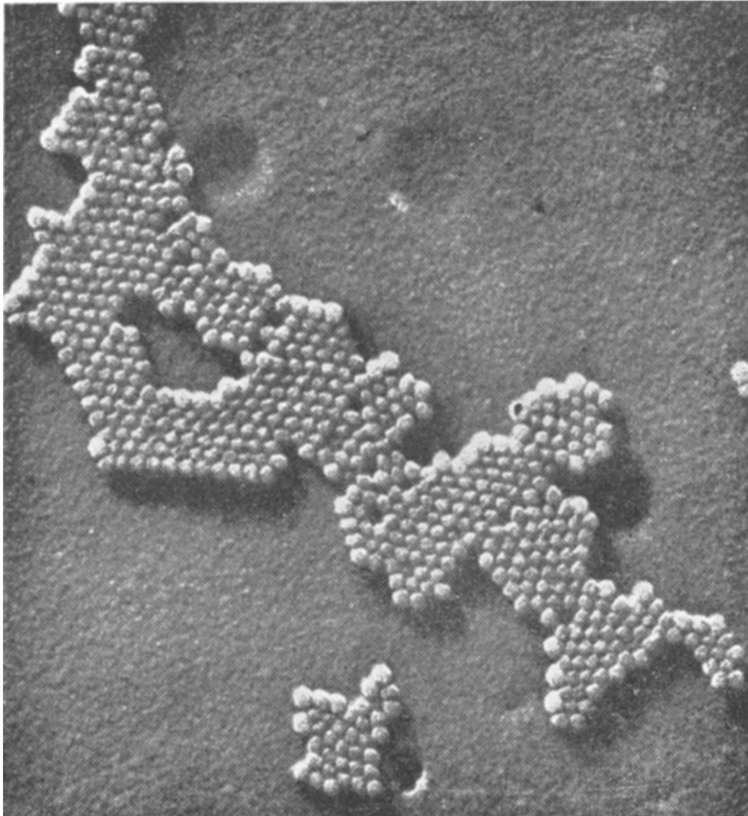


Fig. 3c. Poliomyelitis virus, Type 2 (MEF-1). Two-dimensional crystalline array showing uniform hexagonal close packing. 75,000 ×. (From: A. R. TAYLOR and M. J. MCCORMICK: Yale J. Biol. and Med. 28, 589, 1956.)

With the advent of hemagglutination, new and better methods became available for the purification of Col SK virus. Basing his method of purification on HALLAUER's discovery that virus after adsorption on red cells in low concentrations of monovalent ions elutes completely at higher salt concentrations, HORVATH (1953) prepared brain virus eluates which had four times the hemagglutinating activity of the original mouse brain extracts; these preparations contained either no nitrogen demonstrable by CONWAY's method or had values as low as 30-70 γ per cc as compared with a content of 1000-1500 γ N/cc in the

original material. Chromium-shadowed electron micrograms made from these preparations revealed a fairly homogenous suspension of spherical particles which measured between 26 and 32 mμ (27 mμ average) in diameter (Fig. 3 a). For comparison, pictures of purified MEF virus and of purified THEILER FA virus are reproduced in Figs. 3 b and c which show particles of similar shape and dimensions (22-28 mμ range); a smaller 12 mμ component in purified MEF virus preparations is not associated with infectivity but appears to be at least partially responsible for the complement-fixing activity, which shows overlapping serological reactions between the three types of freshly isolated strains

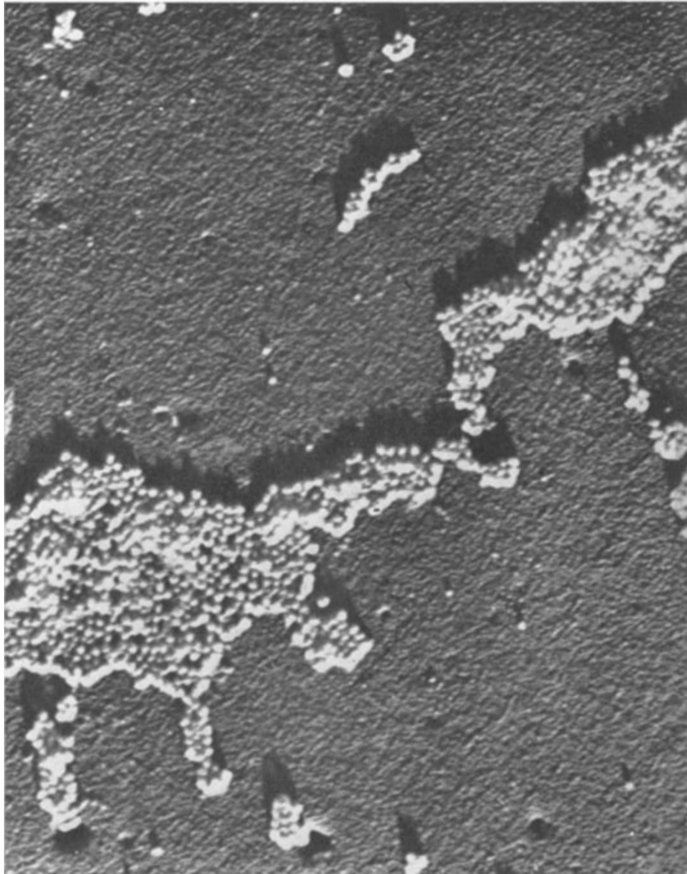


Fig. 3 d. EMC Virus, purified by protamin clarification; virus particles approaching crystalline state. Magnification: 44,500 ×. (Through the courtesy of Drs. S. S. BREESE Jr. and JOEL WARREN. Electron Microscope Laboratory, Army Medical Service Graduate School, Washington, D. C.)

of poliomyelitis virus. Since many technical variables, such as the state of hydration, the effect of metal-shadowing, and the influence of compression affect the ultimate size and shape of small virus particles (TAYLOR and McCORMICK 1956), this comparison permits, of course, only a superficial appraisal of the range of magnitude for the respective viruses.

When studying the ultraviolet absorption properties of Col SK virus preparations, HORVATH found a maximum at 260 mμ for the original extract, but most of this absorption is nonspecific since normal mouse brain extracts yielded

identical UV absorption spectra. A plot of UV extinction against wavelength obtained with a purified preparation is shown in Fig. 4a. Another approach to the problem is to compare UV absorption spectra of crude preparations before and after adsorbing the virus on red cells. The results are shown in Fig. 4b. One can substract the extinction after adsorption on red cells from the values read before adsorption and obtain a differential spectrum as in Fig. 4c. There is little doubt that the virus absorbs UV light in a fashion characteristic of nucleic acids and nucleoproteins; the differential spectrum is even sensitive enough to show progressive increase of the maximum $\varDelta$ E with increasing virus adsorption on red cells. Reference may also be made to the close similarity between the UV absorption spectrum of a purified MEF virus suspension and that of purified Col SK virus (see Figs. 4a and d), the former giving a maximum at 260 mμ and a minimum at 241 mμ, the latter a maximum at 265 mμ and a minimum at 248 mμ.

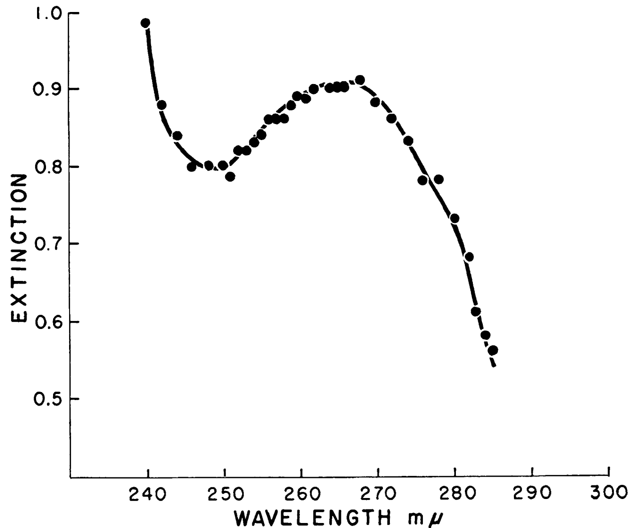


Fig. 4a. Ultraviolet absorption of purified Col SK virus (B. HORVATH: Arch. ges. Virusforschung 5, 228, 1953).

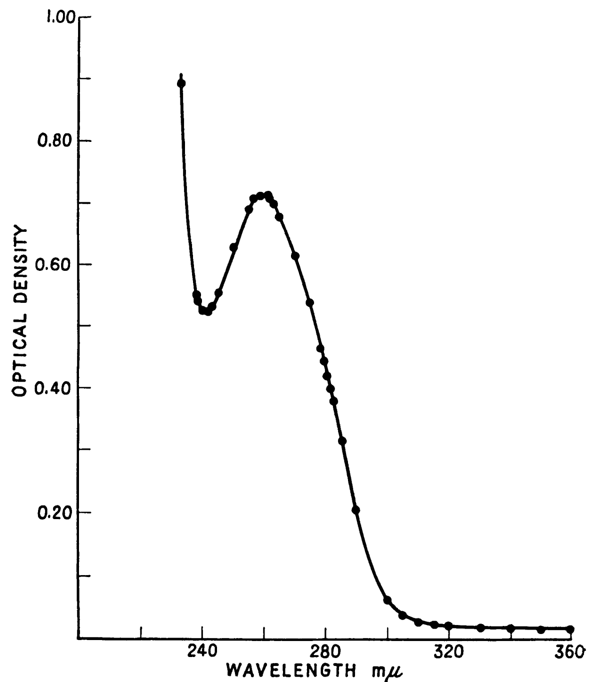


Fig. 4b. Ultraviolet absorption spectrum of purified MEF 1 virus particles suspension in saline. (C. E. SCHWERDT and F. L. SCHAFFER: Annals N. Y. Acad. Sci. 61, 748, 1955.)

Purification of MM virus harvested from infected mouse brains was carried out by GOLLAN (1948) with the aid of methods which use freezing and thawing, alcohol precipitation and dialysis as their main features. The results of a typical experiment (Table 5) suggest that a 43-fold purification was obtained. With further improvement of the methods an even higher degree of purification was

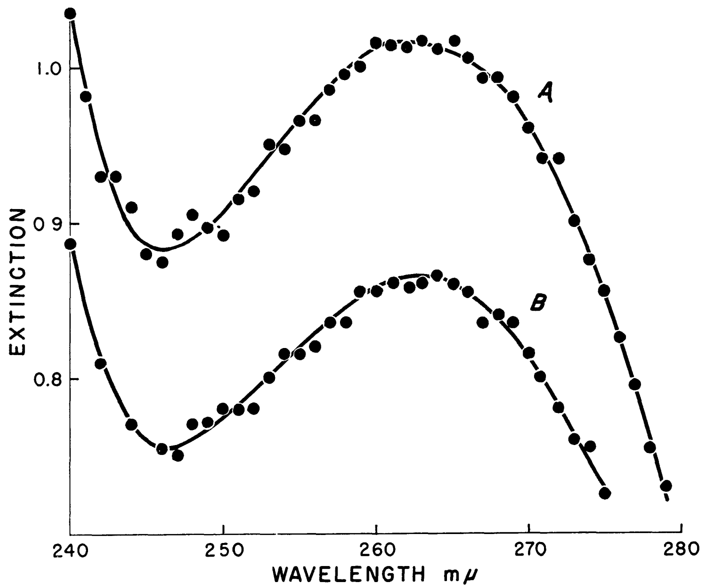


Fig. 4c. Ultraviolet absorption of Col SK virus brain extract (after removal of ether-soluble impurities). *A*: Virus suspension before the addition of sheep red cells. Activity = 128 H. U. *B*.: Supernatant after adsorption of virus on sheep red cells. Activity = 2 H. U. (B. HORVATH: Arch. ges. Virusforschung 5, 228, 1953).

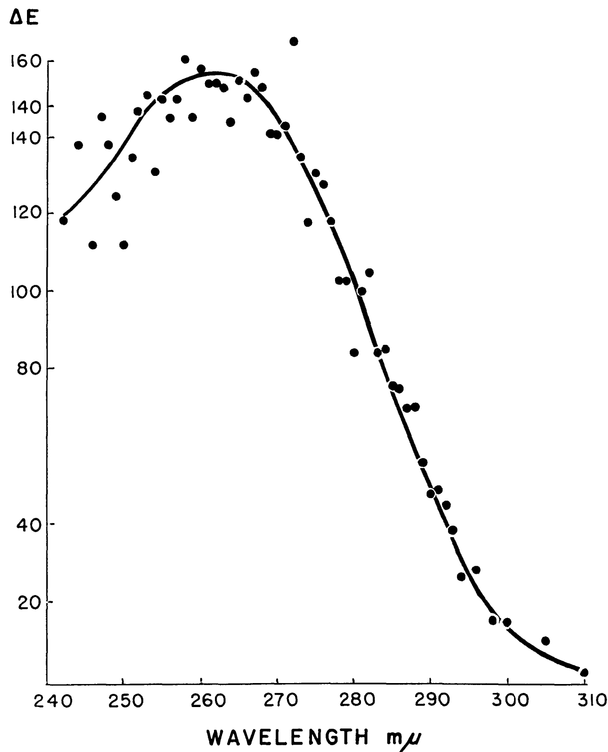


Fig. 4d. Differential spectrum (Difference between curves *A* and *B* in Fig. 4c. Ordinate: Extinction before adsorption of virus minus extinction after adsorption. Abscissa: Wavelength). (B. HORVATH: Arch. ges. Virusforschung 5, 228, 1953.)

Table 5. *Nitrogen Content, Virus Activity, and Virus Yield during Purification of MM Mouse Virus*
(F. GOLLAN: Proc. Soc. Exp. Biol. and Med. 67: 364, 1948)

Material	G Nitrogen/ml mg	LD_{50}/ml	G Nitrogen/LD_{50}	Yield in %
Original Solution	.00347	$10^{7.9}$	$3.4\times10^{-10.9}$	100
Fraction 1	.00270	$10^{7.8}$	$2.7\times10^{-10.8}$	82
Fraction 2	.00063	$10^{9.2}$	$6.3\times10^{-13.2}$	74
Fraction 3	.000397	$10^{9.0}$	$3.9\times10^{-13.0}$	66
Fraction 4	.000207	$10^{9.2}$	$2.0\times10^{-13.2}$	67
Fraction 5	.00014	$10^{9.1}$	$2.4\times10^{-13.1}$	65
Fraction 6	.00008	$10^{8.8}$	$8.0\times10^{-13.8}$	65

Infectivity and Nitrogen Content of EMC Mouse Virus following Protamine Clarification, tryptic Digestion and Ultracentrifugation
(M. L. WEIL, J. WARREN, S. S. BREESE JR., S. B. RUSS and H. JEFFRIES: J. Bact. 63: 99, 1952)

Material	LD_{50}/ml (Log)	Final Concentrate Nitrogen/ml μg	LD_{50}/G Nitrogen (Log)
Purified LOT A	8.0	0.06	15.2
Purified LOT B	8.0	0.06	15.2
Purified LOT C	8.7	0.15	15.5
Purified LOT D	8.9	0 09	15.9

subsequently achieved, the final fraction containing 1.19×10^{-12} g of "virus" nitrogen per cc. Electron micrographic studies of this purified preparation by GOLLAN and MARVIN (1948) showed uniform particles in the range from 10-12 mμ, with an average diameter of about 12 mμ. These findings are in agreement with the early measurements of purified LANSING poliomyelitis virus by LORING et al (1942; 1946). However, the question arises whether the observed particles were really the infectious unit or the smaller component which is currently thought to be associated with the complement-fixing property of poliomyelitis virus. Studies by QUIGLEY (1949) of a preparation purified by the use of methanol (POLLARD et al 1949) established the size of MM virus as between 11 and 16 mμ and GARD (1949) estimated its sedimentation constant as corresponding to that of LANSING or THEILER FA virus. The morphology of MM virus can therefore be regarded as substantially the same as that of Col SK virus.

The physical characteristics of EMC virus were studied in detail by WEIL, WARREN, BREESE, RUSS and JEFFRIES (1952). The virus passed through Gradocol membranes with an average pore diameter of 30 mμ; hence, its particle size was estimated to lie in the range of 8-15 mμ. It was further found that EMC virus, like WEE, Jap B, Polio, St. Louis and West Nile viruses, was not precipitated by protamine which carries down normal tissue components along with some other viruses, such as herpes, LCM, THEILER GD VII, rabies and vaccinia. Therefore, protamine precipitation provides a rapid, simple and effective means for partial purification of EMC virus-infected mouse brains. The protamine-clarified suspension can be further freed of normal components by enzymatic digestion (trypsin, chymotrypsin, ribonuclease, desoxyribosenuclease, papain) without damage to the virus, and ultracentrifugation of the digest yields preparations of considerable purity, as indicated by the ratio of infectivity to nitrogen content

(see Table 5). Preparations with $10^{15.2}$ to $10^{15.9}$ LD_{50} per gram of nitrogen obtained by this method contained particles with a sedimentation constant of 151 S. Chromium-shadowed electron micrograms showed round, almost spherical, highly uniform bodies, measuring about 38 mμ in diameter individually and 27 mμ in groups, which were considered to be the virus; some smaller particles of about 18 mμ were regarded as a component of normal mouse brain. (Fig. 3d.) These observations are in good agreement with the figures for Col SK virus as are the findings of the infectivity-nitrogen ratio for both purified virus preparations.

As far as Mengo virus is concerned, the lower limit of its filtrability through Gradocol membranes has been reported by DICK (1948) to be at 22 mμ porosity, placing its dimensions considerably below those of Col SK virus.

In summary, the viruses of the Col SK group have a particle size of between 10-15 mμ, as determined by ultrafiltration (based on the ELFORD correction formula) and of 25-30 mμ as determined by electronmicroscopy. The sedimentation constant as determined by ultracentrifugation is between 130 and 150 S. These measurements clearly separate these viruses from the group of arthropod-borne encephalitis viruses — which are larger-sized, with the possible exception of Semliki Forest virus — and place them in close proximity with human murine and porcine poliomyelitis viruses.

Physical-chemical Properties of Virus (Including Resistance to Disinfectants).

The viruses of the Col SK group will not pass through a dialysing membrane because of their particle size. Exposure of MM virus for 30 minutes to ultrasonic vibrations, generated by an oscillator operating at 1000 volts and putting out 500 watts, with a piezoelectric quartz crystal ground to a frequency of 600 kc, caused a progressive loss of potency of infected tissue culture fluids but did not completely destroy the virus (JUNGEBLUT 1945); however, exposure for 45 minutes at 750 kc and a power of 375 watts, brought about total destruction of MM virus with loss of immunogenic properties (NELIS and LAFONTAINE 1950). Treatment of Col SK virus with radioactive sodium (1-5 millicurie) injured the virus but did not kill it (JUNGEBLUT 1948); similarly, poliomyelitis virus survives massive exposure to X rays (LENZ and JUNGEBLUT 1932). On the other hand, exposure of Col SK and MM virus to ultraviolet irradiation (2800-3100 Ångstrom units) was followed by loss of potency which extended from partial inactivation (loss of peripheral infectivity) to complete destruction (MCKINSTRY and READING 1944; JUNGEBLUT 1945); similarly, poliomyelitis virus is readily destroyed by ultraviolet light of the same wavelength (JUNGEBLUT 1937). Col SK virus, like poliomyelitis virus, is very sensitive to desiccation (JUNGEBLUT 1945) and, although not completely destroyed by drying (KOPROWSKI 1953), cannot be safely preserved in the lyophilized state; however, both viruses retain their virulence for years in glycerine at 4° C. or frozen at —70° F. The viruses of the Col SK group have a thermal inactivation point near 55° C for 30 minutes; again, the figures are similar for poliomyelitis virus. BOURDILLON (1944) who determined the rate of thermal inactivation of partially purified Col SK virus at 49.5° C and at 56.5° C found the energy of activation of the reaction to be of the order of 100,000 calories per mole. The Col SK viruses are relatively stable in concentrated suspensions, within a p_H range of from p_H 4-10 (SCHATZ and PLAGER 1948), but deterioration of high dilutions sets in rapidly unless the medium is

buffered; the addition of 0.1% glycine prevents denaturation of the virus (Bourdillon 1944; McKinstry and Reading 1944).

The viruses of the Col SK group are resistant to 20% ethyl ether in the cold, a characteristic which again separates them from the viruses of the arthropod-borne encephalitis group. Like many other neurotropic viruses, they also resist the action of 5% phenol, but are inactivated by formalinization. With purified virus preparations, formalin concentrations of 0.04 to 0.08, held over short intervals, are sufficient to produce a killed, antigenic virus vaccine (Gard and Lindholm 1941; Jungeblut 1945; Frantzen 1948; Kauffman and Frantzen 1948). Also, treatment with ethylene oxide or nitrogen mustard-like drugs was used successfully by Powell and Jameson (1948) for the production of a totally inactivated vaccine with fair antigenic properties. Col SK virus is quickly inactivated by *in vitro* contact with oxidizing chemical agents, such as hydrogen peroxide, potassium permanganate, sodium hypochlorate, etc.; large quantities of the virus are also destroyed *in vitro* by milligrams of Vitamin C, probably through peroxide which is liberated upon reduction of ascorbic acid into dehydroascorbic acid. The virus is resistant to the action of many proteolytic enzymes, such as trypsin, etc., which makes possible its excretion in active form in the faeces.

Because of their uniform virulence, the viruses of the Col SK group have often been employed as test virus to study the virucidal effect of various chemicals under conditions applicable to the problem of disinfection of contaminated articles and of excreta from poliomyelitis patients, or the safeguarding of water supplies and food stuffs from viral contamination. Klarenbeek (1951) reported on the virucidal action against Col SK virus of various compounds (formaldehyde, chloramine, hexachlorophene, desogen, superol, sublimate). In later work the same author (Klarenbeek 1954) described inactivation of the virus by 90% propylene glycol after exposure for 24 hours at 4° C, but failed to inactivate the virus by alkyl sulphate, chloroxylenol, furacine or NaOH. Investigation of the virucidal effect on Col SK and vaccinia virus by a mixture of ethylene oxide and carbon dioxide yielded favorable results for both viruses, offering a simple and effective method of disinfection for certain materials, especially those of low heat-tolerance (Klarenbeek and van Tongeren 1954). In a series of experimental studies Bingel and Engelhardt (1953) found Col SK virus totally refractory to the action of alcohol, quaternary ammonia compounds and chlorated phenols, but obtained definite virucidal effects with alkaline lysol (4%), sublimate (4 ‰) and formalin (2%). The action of chlorine depends upon the amount of organic material present and the p_H of the medium. A disinfection of excreta with alkalinized cresols, mercury compounds or formaline has therefore chances of practical success. In further experiments the same authors studied the viability of Col SK virus in water, sewage, soil, and in air-dried preparations under the influence of UV light or sunlight. The virus remained alive in water and soil for several days and, in crude suspension, proved also quite resistant to UV light. Treatment of drinking water with oxon (0.15 mg/l), chlorine (0.25 mg/l) or chlorinedioxide (0.8 mg/l) was considered by Hettche and Schulz-Ehlbeck (1953) to be adequate for the destruction of Col SK virus; the figures are in general agreement with results of chlorination described for Lansing poliomyelitis virus and Coxsackie virus. Exceptionally effective as a virucidal agent for Col SK virus was parmetol, a disinfectant of unknown chemical composition, since a 10^{-2} virus suspension was inactivated by a 2% parmetol solution within 5 minutes and by a 1% solution within 1 hour (Schulz-Ehlbeck 1953). Comparative virucidal tests of various disinfectants against

Col SK virus and poliomyelitis virus (types I and II) were carried out by DREES (1956). Col SK virus was somewhat less resistant but the ratio of inactive/active virus was the same for all three viruses studied. Of the many preparations examined only formalin-soap solution, Tb-sputum-disinfectant, and two new phenolic compounds promised applicability to poliomyelitis prophylaxis.

The influence of pasteurization of milk on survival of Col SK virus was investigated by BINGEL and ENGELHARDT (1953) who obtained no reliable inactivation at 62° C for 30 minutes, or with the flash method of heating for 7 seconds at 85° C. The virus was definitely more resistant in milk than in water which corresponds with similar observations for poliomyelitis and Coxsackie virus. However, since temperatures as high as 90—100° C are sufficient for virus destruction, the authors feel that dipping fruit or vegetables in boiling water for seconds may provide enough safety margin for the consumption of raw foodstuffs during times of epidemics.

Hemagglutination.

Hemagglutination of sheep cells by brain suspensions of mice infected with Col SK or MM virus was first described in 1947 by HALLAUER. This discovery was subsequently confirmed and the basic characteristics of the phenomenon were studied in great detail by many investigators (HALLAUER 1947/48, 1950, 1951; BREMER and MUTSAARS 1948; OLITSKY and YAGER 1949; VERLINDE and DE BAAN 1949; DE BAAN 1950; DE BAAN et al. 1951; BREMER 1951; GARD and HELLER 1951; HORVATH and JUNGEBLUT 1952; VIVELL et al. 1952; HORVATH 1953). As the result of these studies it is now generally accepted that the various strains falling into the Col SK virus group, i. e. Col SK, MM, EMC, Mengo, F, Ortlieb, all possess hemagglutinating properties. There is universal agreement that the phenomenon is specific because suspensions of non-infected brains, under similar conditions, fail to hemagglutinate and hemagglutination can be inhibited specifically by antisera against the Col SK group of viruses. Among the neurotropic viruses, hemagglutination phenomena have since been described for the GDVII strain of THEILERS virus (LAHELLE and HORSEFALL 1949, FASTIER 1950/51, MORRIS 1952/53) and subsequently for most of the arthropod-borne encephalitis viruses (see CASALS, 1957), but so far no corresponding hemagglutinations have been observed with the three serological types of poliomyelitis virus (simian or murine strains) or with any of the Coxsackie viruses; early reports by HALLAUER, VERLINDE and DE BAAN and by BREMER and MUTSAARS on hemagglutination with MEF or Lansing mouse virus have not been confirmed by later investigators and hemagglutination reactions which occur with human stool suspensions (MILZER and ADELMAN, 1949) are too complex for critical interpretation (JUNGEBLUT, KODZA and BAUTISTA 1957). It is interesting, though, that poliomyelitis virus, under certain conditions, can be shown to become adsorbed *in vitro* on the surface of red cells (JUNGEBLUT, HOFMAN and VERLINDE 1953; JUNGEBLUT 1954; BAUTISTA, JUNGEBLUT and KODZA 1956), like lymphocytic choriomeningitis virus (SHWARTZMAN 1944), without causing clumping of the erythrocytes. The hemagglutination reaction with Col SK virus is of considerable theoretical and practical importance: 1) because it permits a more detailed investigation of the virus-cell relationship, opening, at the same time, the way for studies on inhibition of infection by chemical means, 2) because inhibition by specific antibody provides a simple method for serological tests with human and animal sera in epidemiological surveys.

Hemagglutinin.

The viral hemagglutinin is present in brain suspensions of infected animals and reaches its highest concentration in mice; it occurs in much smaller amounts in the brains of infected hamsters, guinea pigs, chick embryos and albino rats and it cannot be demonstrated at all in the brain or cord of infected cotton rats or cynomolgus monkeys. A low-titred non-specific hemagglutinin for sheep red cells, present in normal mouse brains and adsorbable by guinea pig kidney, can be eliminated through sharp centrifugation of the viral brain suspension. Although the viscera of infected mice contain large amounts of virus, no hemagglutination and no virus adsorption is demonstrable with organ extracts from liver, spleen, heart, skeletal muscle and lung, or with urine, nor with amniotic or allantoic fluid of infected chick embryos. The absence of hemagglutinin in tissue extracts and its variable concentration in the brains of different animal species does not seem to be due to the presence of inhibitors (DE BAAN et al. 1950; VERLINDE et al. 1951; VIVELL and MAUER 1952); nor is Col SK or MM virus hemagglutination inhibited by pseudomucinous ovarian cyst fluid which has high inhibitor activity against influenza virus hemagglutinin (GARD and

Table 6. *Hemagglutinin Titers of brains and cords from different animals infected with Col. SK Virus*
(B. HORVATH and C. W. JUNGEBLUT: J. Immunol. 68: 627, 1952)

Host	Infectivity (for Mice)	Hemagglutinin Titer (Sheep Cells)
Cynomolgus Monkey.........	10^{-1} — 10^{-3}	0
Guinea Pig................	10^{-3} — 10^{-5}	1:20
Rat.......................	10^{-5} — 10^{-7}	1:10 — 1:40
Hamster...................	10^{-6} — 10^{-7}	1:80
Mouse (During Incubation)...	10^{-6} — 10^{-7}	1:240
Mouse (Paralyzed)...........	10^{-9} — 10^{-10}	1:1280

HELLER 1951). As shown in Table 6, there is some correlation between hemagglutinin titer and infectivity of the CNS over the host range of Col SK virus, the HA reactions obtained with viral brain or cord suspensions increasing in strength from monkeys to rodents. The viral hemagglutinin is thermolabile, like the infectious virus particle, and is destroyed by heating for 1/2 hour at 56° C, but resists ether treatment; it also survives ultraviolet irradiation which destroys infectivity. The hemagglutinin is not dialyzable and passes through Seitz EK filters when the virus is suspended in broth. The hemagglutinating power of freshly prepared brain suspensions diminishes rapidly on standing, prior to the drop of infectivity. HALLAUER (1951) reported a gradual loss of hemagglutinating properties of EMC virus, as the result of successive brain-to-brain passages of the virus, which could be restored by egg passages; we have not observed this phenomenon with Col SK or MM virus. The hemagglutinin titers depend on the electrolyte balance of the medium, as will be discussed later. However, under the most favorable ionic environment, hemagglutinating endpoints do not exceed a range of 1 : 1000-1 : 2000 virus brain dilutions. Since the endpoint of infectivity is in the neighbourhood of dilutions approximating 10^{-8} or 10^{-9}, viruses with a potency less than 10^{-6} cannot be expected to yield hemagglutination under comparable conditions. It is also clear that the animal infection test is much more sensitive

than the hemagglutination reaction for the detection of active virus. Apparently, the difference is due to the fact that an inoculum of the order of 10 virus particles suffices to initiate progressive virus multiplication in the highly susceptible host whereas inordinately larger numbers of virus particles are evidently necessary to accumulate a layer on the surface of red cells which will result in bridge formation and eventual clumping of the erythrocytes. Since so much virus is used up for coating a single cell, it is obvious that satisfactory hemagglutination is only obtained by using very thin red cell suspensions, i. e. 0.1 or 0.25%. In fact, by varying the red blood cell concentration HORVATH and JUNGEBLUT (1952) were able to demonstrate a simple stoichiometric relationship between the amount of hemagglutinin and the number of red cells reacting in the end tube, as shown in Table 7. According to definition, one hemagglutinating unit

Table 7. *Stoichiometric relationship between Col. SK Virus hemagglutination and sheep red cells*
(B. HORVATH and C. W. JUNGEBLUT: J. Immunol. 68: 627, 1952)

Red Cell Concentration	Virus Dilutions									Cell Control
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560	
0.5%	4+	3+	3+	2+	2+	1+	—	—	—	—
0.25%	4+	4+	3+	3+	2+	2+	1+	—	—	—
0.125%	4+	4+	3+	3+	3+	2+	2+	1+	—	—

(HU) i. e. the least amount which agglutinates an equal volume of a 0.25% red blood cell suspension was present in this experiment in a 1 : 640 virus dilution. It will be seen that 2 HU were required to agglutinate an equal volume of a 0.5% red blood cell suspension whereas 1/2 HU was sufficient to give the same endpoint reaction with an equal volume of a 0.125% red blood cell suspension. Even under optimum environmental conditions, only red cells from certain animal species will hemagglutinate with viruses of the Col SK group. The phenomenon shows a characteristic spectrum of agglutinable and inagglutinable cells, like other viral hemagglutinations; red cells which fail to hemagglutinate also fail to adsorb the virus. We have found no significant differences in the hemagglutinating capacity of Col SK, MM, EMC, Mengo and F virus towards sheep or human red cells, provided potassium veronal buffer is used as diluent. As will be seen from Table 8, there is no correlation between any recognized red cell antigen, such as Forssmans antigen for instance, and agglutinability; nor is there any relationship with respect to susceptibility or insusceptibility of the host to infection with Col SK virus. In other words, the conditions are quite different from those underlying the adsorption of poliomyelitis virus on tissue cells of various animals which runs parallel with susceptibility of the species (KAPLAN 1955). Therefore, the precise nature of the red cell receptor cannot be further defined, except that it seems to be a trypsinresistant mucoprotein. That the reaction is dependent on a high degree of protein specificity is indicated by the fact that, in the experience of several authors, red cells from different sheep vary markedly in their hemagglutination reactions with Col SK virus (GASTAMBIDE-ODIER 1950; JUNGEBLUT and HORVATH 1952).

The important question whether hemagglutination is caused by the virus itself or by some other substances present in infected mouse brain can only be answered tentatively. The fact that the supernatant of a hemagglutinated virus-red cell combination loses most or all of its hemagglutinin with little or

no diminution of infectivity, has no bearing on the question because of the enormous quantitative difference in the amounts of virus required to bring about hemagglutination as compared with the amounts sufficient to infect mice. Evidence in favor of the identity of virus and hemagglutinin is suggested by the physical and chemical properties of both, as indicated by similarity in thermal inactivation point, inhability to pass through dialyzing membranes, ether resistance etc. Conclusive proof might be obtained if purification of the virus would run parallel with that of the hemagglutinin. Employing BOURDILLON's (1944) method, HORVATH (1953) could demonstrate such agreement at least during the first few steps of the purification process. Thus, following ether-extraction of the mouse brain suspension, full infectivity and full hemagglutinin titer were found in the bottom layer; on treatment of the ether-extracted suspension with acetate buffer of *p*H 4.6, both infectivity and hemagglutinin were completely precipitated, the supernatant not showing any activity at all. Another approach to the problem would be to investigate whether any correlation exists between the endpoints of neutralization and hemagglutination-inhibition with a given virus-immune serum combination. Data presented in Table 9 reveal that neutralization and hemagglutination-inhibition lie in entirely different ranges and, if one plots virus dilutions against serum dilutions for both infectivity and hemagglutination endpoints, two straight lines with different slopes and different intercepts are obtained. But if one plots the log

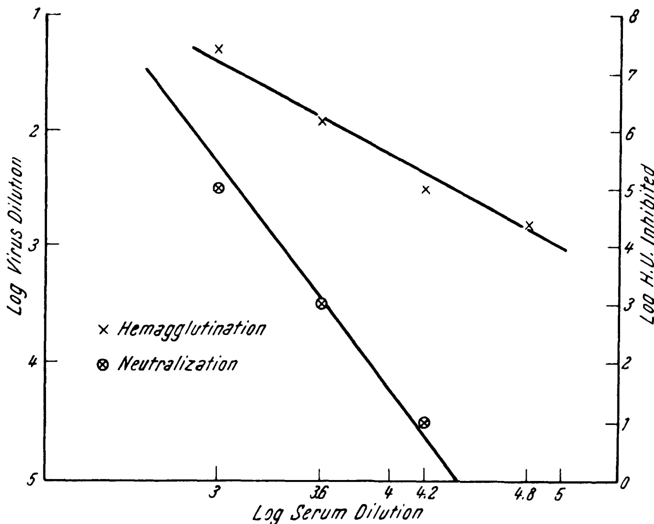


Fig. 5. Correlation between neutralization and hemagglutination-inhibition endpoints obtained with a hyperimmune monkey serum. (B. HORVATH and C. W. JUNGEBLUT; J. Immunol. 68, 627, 1952.)

Table 8. *Hemagglutination of red cells from different animals by viruses of the Col. SK group*

(Adapted from C. HALLAUER. Handbuch der Virusforschung. 2. Ergänzungsband, 1950)

Virus	Amphibia	Birds		Mammals										
	Frog	Chicken	Pigeon	Man	Monkey	Horse	Sheep	Pig	Hamster	Rabbit	G. Pig	Albino Rat	Cotton Rat	Mouse
COL SK, MM, EMC, Mengo	−	−	−	+	−	+	+	−	−	−	+	+	−	−

Note: Reactivity in optimum ionic environment.

of HU inhibited against the serum dilutions, the two lines overlap, as in figure 5. This means that there exists a relationship — although a somewhat complicated one — between neutralization and hemagglutination-inhibition, which can be expressed in numerical terms. The chances that such a relationship should

Table 9. *Correlation between Neutralization and Hemagglutination-Inhibition endpoints* (B. HORVATH and C. W. JUNGEBLUT: J. Immunol. 68: 627, 1952)

Dilution of Immune Serum	Dilution of Virus	Neutralization*	H. I. reciprocal of endpoint dil. of the Virus	H. U. inhibited	
				Number	Log_2
1/1000	320	2/4	10	32	5
	640	1/4			
	10^4	0/4			
	10^5	0/4			
	10^6	0/4			
1/4000	320	4/4			
	640	4/4			
	10^3	4/4			
	10^4	1/4	40	8	3
	10^5	1/4			
	10^6	0/4			
1/16,000	640	4/4			
	10^3	4/4			
	10^4	4/4			
	10^5	1/4	160	2	1
	10^6	0/4			
1/64,000	640	4/4			
	10^3	4/4			
	10^4	4/4			
	10^5	3/4	320	1	0
	10^6	2/4			
1/250,000	10^4	4/4	640	0	
	10^5	4/4			
	10^6	3/4			
Normal monkey serum, 1/4000 dilution	10^5	4/4			
	10^6	4/4			
	10^7	4/4			
	10^8	4/4			
	10^9	4/4			

* $\frac{\text{Numerator}}{\text{Denominator}} = \frac{\text{Number of Mice Paralyzed}}{\text{Number of Mice Injected}}$

exist if hemagglutinin and infectious agent were different substances appear rather remote. It seems to us that the weight of the total evidence available at present speaks definitely against the opinion voiced by GARD and HELLER (1951) that the hemagglutinin may be a nonspecific byproduct of viral infection of the CNS, liberated in some way from the injured tissue and causing autoantibody production, rather than the virus itself.

Hemagglutination Reaction.

The hemagglutination reaction goes through three successive stages: 1) adsorption of the hemagglutinin on the red cell surface, 2) aggregation of the cells, 3) elution of the hemagglutinin from the cells with dispersion of the erythrocytes. All three stages of the reaction are critically influenced by electrostatic forces, as was realized by HALLAUER at the time of his discovery, but chemical affinity also controls adsorption which lends specificity to the phenomenon. The importance of the electrolyte concentration for adsorption is reflected by HALLAUER's (1949, 1951) early studies which showed that maximum adsorption occurs only in an electrolyte-poor medium in which the NaCl content was reduced to one half of its physiological concentration, i. e. from 0.145 molar to 0.075 molar; for osmotic protection of the cells a 4.5% glucose solution was added in equal parts to the hypotonic salt solution. This basic phenomenon has since been confirmed by all later investigators. The effects of varying NaCl concentrations on hemagglutination, as reported by HALLAUER (1951) and by VIVELL and MAUER (1952), are shown in Table 10. It appears from this table that hemagglutination takes place at an optimum of about 0.075 molar concentration and that inhibition occurs below and above. The influence of various anions and cations on adsorption was subsequently studied by BREMER (1951), GARD and HELLER (1951), VIVELL and MAUER (1952) and HORVATH and JUNGEBLUT (1952). Inhibition by anions, depending on the number of negative electric charges, was described by GARD and HELLER who found the strongest inhibitory effects with hexametaphosphate; this suggests that blocking occurred at the cell surface, not with the virus. On the other hand, higher endpoints were observed by BREMER with KCl than with NaCl, while no hemagglutination occurred with $CaCl_2$. These findings were confirmed by VIVELL and MAUER (see Table 10), who found the optimum KCl concentration at isotonicity, i. e. 0.15 molar, as well as by HORVATH and JUNGEBLUT. Titrations of Col SK-MM virus hemagglutinin in different electrolytes, involving four cations of known biological importance, are presented in Table 11. Further studies by HORVATH and JUNGEBLUT (Table 12) showed that the cells adsorbed virus in NaCl and KCl, but failed to agglutinate in the bivalent electrolyte solutions. However, adsorption had apparently taken place in $CaCl_2$ and $MgCl_2$ because the cells agglutinated when resuspended in NaCl or KCl. This finding (contrary to BREMER's observation) could be verified by measuring the decrease in hemagglutinin content of supernatants removed from the cell-virus combination. Titration of these supernatants became possible after elimination of the $CaCl_2$ by dialysis, or by precipitation with sodium oxalate; with the latter method careful quantitation was necessary to avoid an excess of

Table 11. *Titration of Col SK Virus Hemagglutinin in different Electrolytes*
(B. HORVATH and C. W. JUNGEBLUT: J. Immunol. 68, 627, 1952)

Reciprocal of pipetted virus dilutions		10	20	40	80	160	320	640	1280	2560
M conc.	Electrolyte									
0.14	NaCl	3+	2+	1+	1+	—	—	—	—	—
0.14	KCl	4+	3+	3+	3+	3+	2+	1+	—	—
0.14	$CaCl_2$	—	—	—	—	—	—	—	—	—
0.08	$CaCl_2$	—	—	—	—	—	—	—	—	—
0.14	$MgCl_2$	—	—	—	—	—	—	—	—	—
0.08	$MgCl_2$	—	—	—	—	—	—	—	—	—
	Na veronal buffer	4+	3+	3+	3+	3+	3+	2+	1+	—

Table 10. *I. Effect of varying NaCl concentrations on Hemagglutination of sheep cells by MM Virus*
(C. HALLAUER. Arch. ges. Virusforschg. 4: 224, 1951)

Virus Dilutions	NaCl (Molar Concentrations)								
	0.025	0.05	0.075	0.1	0.15	0.2	0.25	0.3	0.35
1:20	3 +	3 +	3 +	3 +	3 +	3 +	3 +	(+)	—
1:40	3 +	3 +	3 +	3 +	3 +	+	(+)	—	—
1:80	3 +	3 +	3 +	3 +	3 +	(+)	—	—	—
1:160	2 +	3 +	3 +	3 +	3 +	—	—	—	—
1:320	+	3 +	3 +	3 +	2 +	—	—	—	—
1:640	±	2 +	3 +	3 +	+	—	—	—	—
1:1280	—	+	3 +	2 +	—	—	—	—	—
1:2560	—	(+)	3 +	+	—	—	—	—	—
1:5120	—	—	2 +	—	—	—	—	—	—
1:10240	—	—	+	—	—	—	—	—	—
Cell Control	—	—	—	—	—	—	—	—	—

II. Effect of varying NaCl and KCl concentrations on Hemagglutination of sheep cells by Leonhard Virus
(O. VIVELL and R. MAUER: Zeitschr. Immun. Forschg. 109: 246, 1952)

Virus Dilutions	NaCl (Molar Concentrations)						KCl (Molar Concentrations)							
	0.025	0.05	0.075	0.1	0.15	0.2	0.025	0.05	0.075	0.1	0.15	0.2	0.25	0.3
1:40	+	2 +	2 +	2 +	2 +	2 +	—	3 +	3 +	3 +	3 +	3 +	+	—
1:80	—	2 +	2 +	2 +	2 +	+	—	3 +	3 +	3 +	3 +	3 +	+	—
1:160	—	±	2 +	2 +	2 +	—	—	3 +	3 +	3 +	3 +	2 +	±	—
1:320	—	—	2 +	2 +	+	—	—	3 +	3 +	3 +	3 +	2 +	—	—
1:640	—	—	2 +	2 +	—	—	—	3 +	3 +	3 +	3 +	+	—	—
1:1280	—	—	+	+	—	—	—	2 +	3 +	3 +	3 +	±	—	—
1:2560	—	—	±	—	—	—	—	+	2 +	2 +	2 +	—	—	—
1:5120	—	—	—	—	—	—	—	±	+	+	2 +	—	—	—
1:10240	—	—	—	—	—	—	—	—	—	—	+	±	—	—
1:20480	—	—	—	—	—	—	—	—	—	—	±	—	—	—

Table 12. *Effect of Electrolytes on Adsorption and Agglutination of Col SK Virus*
(B. HORVATH and C. W. JUNGEBLUT: J. Immunol. 68, 627, 1952)

Virus adsorbed in					Cells resuspended in
NaCl	KCl	$CaCl_2$	$MgCl_2$	Na veronal buffer	
1+	3+	2+	1+	2+	NaCl
2+	2+	1+	2+	2+	KCl
—	—	—	—	—	$CaCl_2$
—	—	—	—	—	$MgCl_2$
3+	2+	2+	2+	2+	Na veronal buffer

oxalate, since oxalate ions will inhibit agglutination (GARD and HELLER). These observations indicate that hemagglutination is a phenomenon distinct from adsorption, suggesting that while adsorption is a necessary first step, completion of the reaction to visible clumping will occur only in a favorable ionic environment. Differential effects of Na and K ions on virus hemagglutination exist which are very definite and striking. Not only is the range of agglutinable cells widened by the presence of low concentrations of KCl (Table 13), but twice the physio-

Table 13. *Effect of Na and K Veronal Buffer on Hemagglutination by Col SK Virus at 20° C and 0° C of red cells from various species*
(B. HORVATH and C. W. JUNGEBLUT: J. Immunol. 68, 627, 1952)

Type of cells	20° C		0° C	
	Na	K	Na	K
Sheep I	320	1280	640	1280
Human group A, pool of 7	10	160	40	320
B, pool of 12	—	160	20	320
AB, pool of 2	20	320	20	320
O, pool of 8	—	160	20	640
Rabbit (one only)	—	—	—	—
Rat pooled	—	160	80	640
Mouse pooled	—	—	—	—
Guinea pig (one only)	—	160	20	320
Chicken (one only)	—	—	—	—

logical concentration of KCl may be used without even partial inhibition while NaCl completely inhibits in this range. (VIVELL and MAUER; HORVATH and JUNGEBLUT). Protein solutions do not differentiate between monovalent ions but the cell membrane is well known to be able to distinguish Na from K, keeping the concentration of the latter high inside and that of the former high outside. The observed differences, therefore, suggest that the cell membrane is involved in the process of virus adsorption.

Taking into consideration the effects of the variables discussed above, an improved method for carrying out hemagglutination tests was devised by HORVATH and JUNGEBLUT (1952) in which Mayer's veronal buffer is used as diluent, substituting K ions for Na ions. This buffer is made up as follows: 0.910 g of 5,5 diethylbarbituric acid in crystalline form is dissolved in 1000 cc of glass-distilled water, containing 0.147 M KCl, 1.82 cc 1 N KOH, with or without a final concentration of 0.000254 M $CaCl_2$ and 0.00083 M $MgCl_2$. (These low concentrations of Ca and Mg are not inhibitory).

Human O cells are usually collected in Alsever's solution and may be stored for two weeks. The cells are centrifuged, washed three times in veronal buffer, and the packed cells are then converted into a 0.25% suspension. Remarkably constant cell counts were obtained from the same donor, i. e. $78\text{-}80 \times 10^6$ red blood cells per cc. The test consists of combining equal volumes, usually 0.5 cc of red blood cell suspension in veronal buffer with serial twofold dilutions of a 10% virus suspension made up with veronal buffer. No difficulty is encountered if one adds another volume of veronal buffer to the test, a procedure which becomes necessary in studying inhibition by serum or the effects of other inhibitors. The results are usually read after overnight stay in the icebox; equally good reactions may be obtained by giving the tubes a short run at low speed in the cold-centrifuge and then shaking up the sediments, although the endpoint in titrations may be a little lower. The most striking feature of Col SK virus hemagglutination is that virus-agglutinated cells form rather heavy aggregates which are not easily separated by mechanical influences. Once the cells are broken up by continued agitation, they will agglutinate again in the cold to heavy clumps after a short time. A further check on endpoint agglutination may be made by microscopic observation; when kept at room temperature, the cells may disperse slightly after some time because of partial elution of the virus. The formation of a pattern seems to depend on a number of circumstances unrelated with real agglutination and pseudoreactions are occasionally seen with higher concentrations of normal mouse brain extracts. However, on the whole, endpoint determinations read by pattern or microscopically come to fairly close agreement. Standardization of the procedures involved in endpoint determinations is indispensable for quantitative interpretation of the reaction but is not as important for routine qualitative tests.

It had previously been shown by HALLAUER (1951) that adsorption of hemagglutinin is dependent on temperature, subject to the electrolyte content of the environment, in that adsorption in the electrolyte-poor medium occurred rapidly at 4° C. and 20° C. whereas it took place slowly and only in the cold in isotonic NaCl solution. Adsorption of Col SK virus hemagglutinin on sheep red cells was studied extensively by HORVATH and JUNGEBLUT (1952) as a function of time, initial hemagglutinin concentration and temperature. It is evident from Table 14 that adsorption at 0° C. is progressive with time and that 50% of the

Table 14. *Adsorption of Col SK Virus Hemagglutinin on sheep red cells as a function of time and initial Hemagglutinin concentration*

(B. HORVATH and C. W. JUNGEBLUT: J. Immunol. 68, 627, 1952)

Adsorption at 0° C. Amounts of hemagglutinin in H. U.

Initial Amount Added H. U.	Free in the Supernatant (Time in Minutes)								Adsorbed (Time in Minutes)							
	5	10	15	20	30	60	120	240	5	10	15	20	30	60	120	240
64	32	32	32	16	8	8	4	2	32	32	32	48	56	56	60	62
32	16	16	16	8	4	4	2	1	16	16	16	24	28	28	30	31
16	8	8	8	4	2	2	1	0	8	8	8	12	14	14	16	16
8	4	4	4	2	1	1	0	0	4	4	4	6	7	7	8	8
Adsorbed as % of the initial									50	50	50	75	88	88	94	97

initial amount, irrespective of its size, is always adsorbed in the first five minutes, 75% in twenty minutes, etc. When plotting the amount of free virus left in the supernatant against the initial amount added for certain time intervals, a number of straight lines are obtained, indicating that a fixed proportion of hemag-

glutinin is always adsorbed in a given time. (Fig. 6a.) One can also plot the data in such a fashion as to show the relation between time and amount of free or adsorbed virus more clearly, expressing adsorption as per cent of the initial

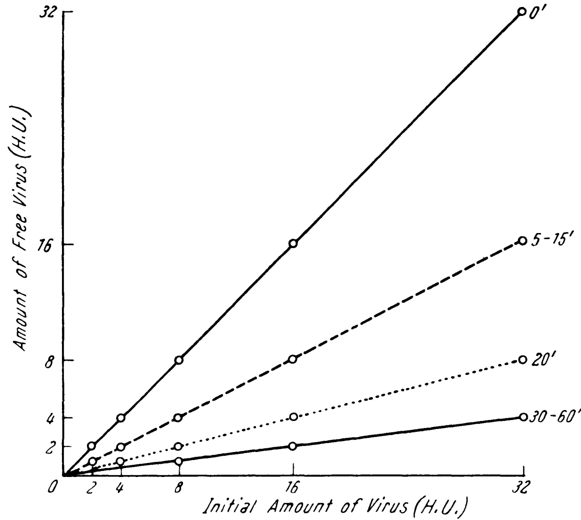


Fig. 6a. Adsorption of Col SK virus on sheep cells. Amount of free virus in the supernatant as a function of the amount initially added to RBC. 0° C. (B. HORVATH and C. W. JUNGEBLUT: J. Immunol. 68, 627, 1952.)

amount (Fig. 6b). To make intrapolation and extrapolation easier and to verify the equation of the curve in Fig. 6b as one of rectangular hyperbolae, the data

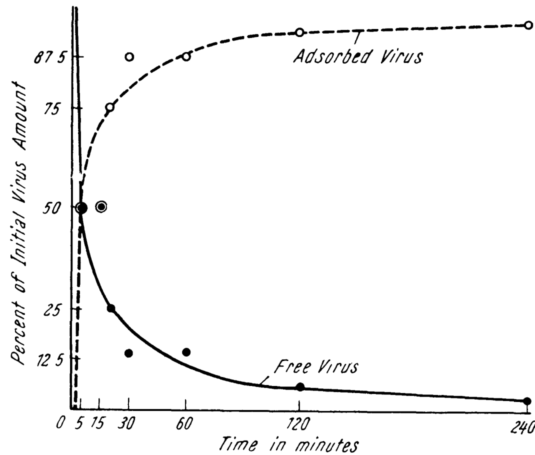


Fig. 6b. Adsorption of Col SK virus on sheep cells. Free and adsorbed virus in percentage of the initially added amount as a function of time. 0° C. (B. HORVATH and C. W. JUNGEBLUT: J. Immunol. 68, 627, 1952.)

may also be charted as per cent of virus adsorbed against the reciprocal of time. By doing so one arrives at a straight line, as shown in Fig. 6c. According to Fig. 6c, adsorption, then, is the following function of time: $y = b\text{-}1/t.\ k.$, where

y is the amount of virus adsorbed at time t, b is the amount adsorbed at infinite time, and k is a constant. It also appears that, when adsorption is studied at

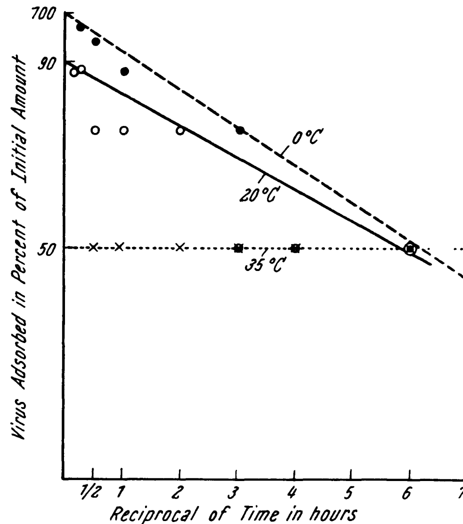


Fig. 6c. Adsorption of Col SK virus on sheep cells. Temperature dependence of adsorption. (B. HORVATH and C. W. JUNGEBLUT: J. Immunol. 68, 627, 1952.)

20° C. and at 35° C., adsorption goes slower and is less complete at higher temperatures than at 0° C.; nevertheless, significant adsorption occurs even at 35° C.

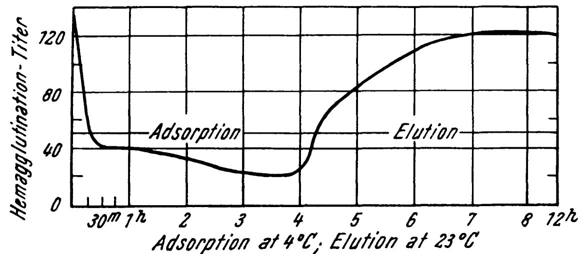


Fig. 6d. Adsorption and elution of MM virus in physiological NaCl solution. (W. KELLER and O. VIVELL, Ergeb. inn. Med. u. Kinderhk. 5, 1, 1954).

Elution.

The question of elution has been a matter of some controversy. According to HALLAUER's early experiments, elution occured quantitatively in hypertonic NaCl medium, at low and higher temperatures, but no elution was observed with isotonic NaCl medium. OLITZKY and YAGER (1944) described spontaneous elution with physiological NaCl solution upon elevation of temperature but their paper did not include titration results on supernatants. DE BAAN, who described such titrations, comes to the conclusion that there is no elution at all. VIVELL and MAUER (1952) who studied adsorption at 4° C. and elution at 23° C. in physiological salt solution, could show that about 80% of the bound hemagglutinin eluted (see fig. 6d); in physiological KCl solution no elution was obtained, presumably because of the rapidity of the adsorptive process. In our own ex-

perience (HORVATH and JUNGEBLUT 1952), with KVB as a medium, not more than 17% of the virus adsorbed in 0.147 M KCl (or NaCl) eluted on raising the temperature from 0° C. to 35° C. However, complete elution did occur when adsorption had taken place in 0.075 M KCl (or NaCl), supplemented with an equal volume of 4.5% glucose for isotcnicity (HORVATH 1953). In other words, HALLAUER's original results could be completely confirmed by observing exactly the same experimental conditions. This curious effect of low salt concentrations being just as critical during adsorption as high salt concentrations during elution, remains unexplained. The elution method may be used to prepare virus eluates of considerable purity, as shown by HORVATH in 1953 (see section on Morphology). Using such purified virus preparations, HORVATH also studied the electrophoretic mobility of red cells on which Col SK virus had been adsorbed, before and after elution. A definite decrease of electrophoretic mobility following virus adsorption could be recorded, but, after the virus had been eluted, the cells moved at the same velocity as normal red cells. Since only large amounts of adsorbed virus caused a decrease in mobility, it becomes virtually certain that hemagglutination, especially at or near the endpoint, is not due to the virus bringing about a decrease in the net negative charges of the red cells.

The sum total of the elution data indicate a fundamentally different mechanism of hemagglutination for Col SK virus as compared with that of influenza virus. In the first place, virus-treated cells are still capable, after elution, of reacting with the same virus or with fresh virus, and several hemagglutination cycles are possible as with THEILER GDVII virus hemagglutination. Secondly, red cells after contact with Col SK virus are not panagglutinable. An enzymatic activity of Col SK virus during hemagglutination is further unlikely because of the stoichiometric relationship between hemagglutinin and red cells and the strict dependence of adsorption and elution on varying electrolyte concentrations. Finally, the fact that red cells, after elution of the virus, recover their original electrophoretic mobility speaks for a mechanism at variance from that of influenza virus hemagglutination. Yet, while the virus does not seem to possess any receptor-destroying properties of its own, hemagglutination of Col SK virus can be inhibited by the action of certain enzymes which destroy viral cell receptors in a manner analogous to what is known for influenza virus [BURNET and STONE (1947)]. Thus, exposure of red cells to filtrates of *V. cholerae* (RDE) leaves such cells completely inagglutinable by Col SK virus (DE BAAN 1950, VERLINDE and DE BAAN 1949, JUNGEBLUT 1950). Interestingly enough, cholera vibrio RDE can also be shown to exert marked protective effects in mice against Col SK virus infection, provided both virus and enzyme are introduced at the same peripheral portal of entry (see section on chemotherapy); similar protection through *V. cholerae* RDE may be obtained in HeLa cell tissue cultures against infection with the cytopathogenic EMC virus (see section on Cultivation). Little is known about the elaboration of receptor-destroying enzymes by other enteric microorganisms, except *Clostridium Welchii*, which inhibits hemagglutination by influenza virus (BURNET, McCREA and STONE 1946) as well as by Col SK virus (KODZA and JUNGEBLUT 1957). HOWE et al. (1956, 1957) found among 30 strains of *Clostridium tertium* 19 which produced enzymes specifically splitting blood group A substance. Enzyme obtained from one strain (Iseki) also inactivated certain inhibitors of influenza virus hemagglutination and acted upon human erythrocytes to destroy the A, M and N antigen as well as the hemagglutinin receptors for influenza virus (strains PR 8 and PR 301) and Col SK virus, rendering the cells panagglutinable; chicken red cell receptors for several strains of influenza virus were likewise inactivated.

Inhibition of Col SK virus hemagglutination also occurs through the action on red cells of a receptor-destroying enzyme found in human saliva (and in human and animal stools) which appears to be closely related to bacterial RDE on the basis of cross agglutination tests with sera previously absorbed with enzyme modified red cells. (JUNGEBLUT, HORVATH and KNOX 1952; JUNGEBLUT and KNOX 1954; HOFMAN 1953/54.) This salivary receptor-destroying enzyme varies in amounts with different individuals and fluctuations in titer may occur from day to day in the same person. There is evidence that quantitative excretion patterns are inherited by a polygenic type of inheritance (JUNGEBLUT, KALLMAN, ROTH and GOODMAN 1956). (Table 15.) The enzyme seems to occur consistently with greater frequency in the saliva of poliomyelitis patients (acute and chronic cases) than in healthy individuals. (JUNGEBLUT and KNOX 1954; HOFMAN 1954.) (Table 16.) D'AMBROSIO and PIAZZA (1955) who examined a total of 133 saliva samples from 20 normal persons and 63 children with poliomyelitis detected the factor in question in the saliva of 10% of normal subjects and 52.4% of poliomyelitic children. Temporary elevations of titer in other infectious diseases have been described by MULÉ and SCAGLIONE (1956). Common to both

Table 15. *Similarity and Dissimilarity for low, medium and high Salivary RDE Secretion in monozygotic and dizygotic twin pairs*

(From C. W. JUNGEBLUT, F. KALLMAN, B. ROTH and H. GOODMAN: First Int. Congress Human Genetics, Copenhagen, 1956)

Twin Pairs	Totals	Similar	Dissimilar
Monozygotic	13	12 (92.3%)	1 (7.7%)
Dizygotic	12	5 (41.7%)	7 (58.3%)

Table 16. *Occurrence of Receptor-Destroying Enzyme in the saliva of healthy individuals and of poliomyelitis patients*

I. Data from: C. W. JUNGEBLUT and A. KNOX (J. Immunol. 73: 264, 1954)

Classification of donors according to type of reaction

Donors			Reactions of Donors		
Group	Age (years)	Number	Consistently negative	Intermittent	Consistently positive
Healthy persons	5 to 15	20	9 (45.0%)	11 (55.0%)	0 (0.0%)
	16 and over	73	41 (56.1%)	28 (38.3%)	4 (5.5%)
Total:	all ages	93	50 (53.7%)	39 (41.9%)	4 (4.3%)
Poliomyelitis patients	1 to 4	3	0	1	2
Acute	5 to 15	81	8 (9.9%)	53 (65.4%)	20 (24.7%)
	16 and over	89	7 (7.9%)	60 (67.4%)	22 (24.7%)
Total:	all ages	173	15 (8.7%)	114 (65.9%)	44 (25.4%)
Poliomyelitis patients	5 to 15	45	2 (4.4%)	27 (60.0%)	16 (35.6%)
Convalescent	16 and over	14	1 (7.2%)	8 (57.1%)	5 (35.7%)
Total:	all ages	59	3 (5.1%)	35 (59.3%)	21 (35.6%)

II. Data from: B. HOFMAN (J. Immunol. 73: 273, 1954)

Average percentages of positive, doubful and negative saliva samples for poliomyelitis patients and controls

Poliomyelitis Patients					Control Group				
Age	Number of donors	Average percentages			Age	Number of donors	Average percentages		
		Positive	Doubtful	Negative			Positive	Doubtful	Negative
years		%	%	%	*years*		%	%	%
0 - 6	19	51.1	17.6	31.3	0 - 6	2	58.7	28.8	12.5
6 - 12	21	59.3	19.0	21.7	6 - 12	20	34.8	30.2	35.0
12 - 18	12	51.9	27.2	20.9	12 - 18	2	11.1	33.4	55.5
18	12	37.3	21.0	41.7	18	23	18.2	14.8	67.0
Total	64	51.4	20.4	28.2	Total	47	26.6	22.8	50.6

salivary and bacterial enzymes is that, in addition to causing loss of virus receptors, a modification of the red cells occurs through the exposure of T antigen which renders them panagglutinable. It would therefore seem logical that the cellular virus receptor is part of the substrate which contains T antigen. On the other hand, mucinases present in ovarial cyst fluid, which are potent inhibitors of influenza virus hemagglutination, have been reported as being without any effect on Col SK virus hemagglutination. (KELLER and VIVELL 1954.)

In conclusion, the hemagglutination reactions of influenza virus and of Col SK share certain basic characteristics of their modus operandi, but they differ radically in others. The important question whether corresponding reacting groups occur on fixed susceptible tissue cells cannot be answered directly since HeLa cell suspensions do not clump in the presence of virus; yet such cells, previously treated with RDE, are fully protected against viral infection in tissue culture media (see section on Cultivation). It may briefly be mentioned that we could not demonstrate any agglutination of human spermatozoa by Col SK virus whereas spermagglutination has been described for viruses of the influenza-mumps-NDV group of viruses (CHU 1953).

Hemagglutination — Inhibition by Serum.

Since hemagglutination is inhibited by specific antibody, the hemagglutination test lends itself well to the *in vitro* demonstration of Col SK antibodies in human or animal sera. Earlier methods, which employed sheep cells and virus in NaCl solution, were handicapped by the fact that the presence of heterophile sheep agglutinins (FORSSMAN type) in many human sera necessitated a previous absorption with sheep cells or guinea pig kidney for their removal. The use of human O cells and of virus in K veronal buffer gives equally good hemagglutination and eliminates this difficulty, thereby greatly simplifying the procedure. (HORVATH and JUNGEBLUT 1952.) Since different mouse brains harvested from the same passage may differ appreciably in hemagglutinating potency, it is advisable to pool several brains in making up the virus suspension. Frozen brains preserve their hemagglutinating capacity better then glycerinated brains stored at 4^0 C and the thawing process seems actually to release more free virus. A non-specific inhibitor in the brain tissue may cause an occasional prezone of weaker reactions in the lower dilutions; its effects can be minimized by sharp centrifugation of the 10% mouse brain suspension. The endpoint of hemagglutination in this

system, i. e. 1 HU, lies usually between 1 : 160 and 1 : 640 dilution of virus suspension. The inhibition test currently used by us consists of mixing aliquots (0.2 cc) of a fixed dose of virus, containing 4 HU, with serum dilutions of 1 : 5, 1 : 10, 20, 40, 80 etc. and letting the mixture stand for one hour at room temperature. An amount of 0.2 cc of a 0.25% cell suspension is then added to each tube and the results are read after leaving the test in the icebox overnight; the reaction may be speeded up by cold centrifugation which permits an immediate provisional reading. The titer of the serum represents the highest dilution which completely inhibits clumping of the cells; it may be as high as 1 : 3000 with hyperimmune sera but convalescent sera rarely titer higher than 1 : 100 to, 1 : 1000 and are often lower. Controls are included: 1) to verify that the test dose of virus equals 4 HU and 2) to show that the serum alone does not cold-agglutinate the cells (many animal sera contain fairly strong hemagglutinins for human O cells which must be removed by previous absorption). In routine tests it is always advisable to run along a serum of known potency and a negative serum. The test appears to be specific since there is usually, but not invariably, agreement between hemagglutination inhibition and neutralization; moreover, both antibodies are destroyed at the same thermal inactivation point. As with all serological reactions, non-specific reactivity may occasionally occur in low serum dilutions; this may be related to PILLEMER's properdin system. For this reason, reactions are considered positive only when they exceed a serum dilution of 1 : 5. Results obtained by several authors with the hemagglutination — inhibition test and various human sera are given in another section (clinical observations).

Cultivation.

Attempts to cultivate Col SK virus *in vitro* on various cell substrates began with the early work of JUNGEBLUT and SANDERS (see also SANDERS and JUNGEBLUT) in 1940-1942. The type of tissue culture, originally described by SANDERS (1940) for other viruses, lent itself well to this purpose. It consists of growing the virus on minced embryonic tissue, suspended under controlled oxygen-carbon dioxyde tension in ox serum ultrafiltrate. When three different kinds of tissue were used, i. e. embryonic mouse brain, embryonic guinea pig brain, or whole chick embryo, high virus titers were obtained with mouse brain, intermediate titers with guinea pig brain, and low titers with whole chick material. The murine virus, therefore, represented an agent with decided predeliction for the tissues of its most susceptible host, i. e. the white mouse. It was further found that non-nervous embryonic mouse tissue is capable of maintaining a basic level of neurotropic virus growth, albeit definitely below that obtainable with tissue derived from central nervous system. These results were subsequently confirmed by SCHULTZ and IRWIN (1946). The optimum range of virus infectivity coincided with the optimum p_H for cell viability *in vitro*, having a maximum around p_H 7.3 to 7.6. In 1943, HUANG discovered that the degree of glycolysis and of acid production in tissue explants infected with Col SK virus was much lower than in uninfected controls. Hence, very little or no change occurred in the p_H and the color of phenol red in the infected medium remained the same, whereas it shifted quickly to the acid side, i. e. from red to yellow, in non-infected tissue controls. The principle of this simple visual method for detecting virus multiplication has subsequently found wide application in assaying the growth in tissue culture media of other viruses, including the poliomyelitis viruses. With the media employed in the early tests, the rate of growth for Col SK virus was extraordinarily fast in com-

parison with other methods in use at that time and maximum titers (10^{-6}) were reached within 10-72 hours, depending on the size of the inoculum. The virus titer was equally high in the fluid and in the cells, but the procedure did not permit the demonstration of cytopathogenic effects. Serial passage of Col SK virus in embryonic mouse brain tissue culture was easily achieved, provided transfers of infectious fluids were made with high and not with low dilutions; this phenomenon of self-interference was later recognized again by HENLE (1944) with allantoic fluids infected with influenza virus. With extended subcultivation *in vitro*, Col SK virus suffered a definite loss of infectivity for mice by peripheral injection, whereas intracerebral potency remained constant; interestingly enough, mice surviving inoculation of culture virus by peripheral routes acquired a relative resistance to reinfection with animal passage virus. A careful comparison of the mouse explant requirements for Col SK and MM virus, on the one hand, and of THEILER's GD VII and FA virus, on the other, was made by SHEAN and SCHULTZ (1950). Col SK and MM viruses grew freely on brain, lung, small intestine, heart muscle and tail tissue from embryonic mice, though not on tongue, skeletal muscle, kidney, liver or spleen; the THEILER viruses were more exacting in their growth requirements. All viruses studied grew on minced 5-day chick embryo tissue. Growth of MM virus in mouse testicular tissue was reported by CHAMBERS, SMITH and EVANS (1950; 1951) in flask cultures and by FABIYI (1955) in roller tube cultures; it also grows in various extraneural tissues of the hamster. (EVANS and CHAMBERS 1948; CHAMBERS, SMITH and EVANS 1950.) None of the poliomyelitis viruses, including the rodent-adapted strains (Lansing, MEF), have so far been cultivated succesfully in mouse or cotton rat embryonic tissue culture.

Efforts to propagate the early mouse passages of Col SK virus on the chorio-allantoic membrane of embryonated hen's eggs met with no success. In 1946, BRUTSAERT, JUNGEBLUT and KNOX showed that MM virus could be propagated in initial egg passages, but active virus disappeared after the fifth or sixth serial transfer. Later workers were more successful in growing both, Col SK and MM virus, in embryonated eggs. Thus, SCHULTZ and ENRIGHT (1946) could carry Col SK and MM virus through fourteen 9 day-old egg passages by chorio-allantoic, yolk sac, or intraembryonic inoculation, without evident diminution of virus; viral multiplication was associated with a high incidence of deaths in the embryos between 2-6 days, and the virus was found widely disseminated in the infected embryos. At 35° C incubation the average mortality of embryos was 75%, but at 37.5° C, only about 20%. With older eggs, the virus titer and the embryo mortality rate dropped, reaching zero with 17 day-old eggs. These observations were fully confirmed by POWELL and JAMIESON (1948) who encountered the major portion of the virus in the chick embryo proper and only small amounts in the peri-embryonic fluids. EMC and Mengo virus were propagated in embryonated eggs without difficulty by WARREN (1948) and by DICK (1950). Embryos infected with EMC virus died within 72-96 hours without pathognomonic lesions (WARREN), but those infected with Mengo virus showed extensive hemorrhages (no clumping of red cells was described) and were usually dead 48 to 96 hours after inoculation (DICK). That considerable differences exist in the adaptability to embryonated eggs among the various strains of viruses within the Col SK group is also shown by VERLINDE's observations (VERLINDE et al. 1951/52). Whereas Col SK and MM virus could be easily propagated in eggs, the European isolates behaved quite differently in that the F strain failed to grow at all and the AK as well as the Ortlieb strain could be carried only over a few egg passages (VERLINDE and HOFMAN 1952). THEILER's GDVII virus has also been reported as growing

in fertilized eggs, but of the poliomyelitis viruses only one Type II strain (MEF) has so far been adapted to growth in embryonated eggs with the production of neural and extraneural lesions in the chick embryo (ROCA GARCIA et al. 1952-55; CABASSO et al. 1952); egg cultivation has been described for the Type I Brazzaville strain of poliomyelitis virus by PELLISSIER (1954) but the data require confirmation. According to OKUNO et al. (1956) the Lansing strain of poliomyelitis virus can be carried over many passages in the chorio-allantoic cavity or yolk sac of 6-day old chick embryos; the evidence of virus presence is based neither on cytopathogenicity nor on mouse-infectivity but on the occurrence of interference with mumps virus multiplication in the Lansing-infected eggs.

Only sporadic attempts have been made until now to grow viruses of the Col SK group on tissue cultures with embryonic or adult cell substrates derived from monkey or man, utilizing modern methods of tissue cultivation. Col SK virus has been reported as growing on cynomolgus monkey testicular cell cultures (KRET 1955) but not on cynomolgus monkey kidney (WINSSER, personal communication). Attempts made by us to propagate Col SK or poliomyelitis virus on human spermatozoa gave negative results. Growth of MM virus has been obtained in cultures of testicular cells from rhesus monkeys (SMITH and EVANS 1954) and from man (CHAMBERS, SMITH and EVANS 1951), and also on human fetal fibroblasts (PINTER and ABRAHAM 1956). Mengo virus, according to BARSKI and LAMY (1955), multiplies abundantly in tissue cultures of kynocephalus monkey kidney cells, inducing cellular changes which appear after 2 days and cause complete destruction of the culture within 4 days. (Fig. 7.) This cytopathogenic effect could be transmitted with the culture supernatant and after 21 serial passages destruction of the entire cell population occurred within 48 hours following inoculation with 100 TC ID_{50} doses; the infectivity of the supernatant at this time was $10^{-6.25}$ if titrated in tissue culture and about one log less when titrated in mice. On stained preparations the cellular lesion produced by Mengo virus revealed some early changes in the nucleus but the development and type of this lesion differed clearly from that observed in similar cultures infected with poliomyelitis virus. The regularity and rapidity of cell destruction by Mengo virus seems to permit the use of this method for virus titration and for routine sero-neutralization tests as conveniently as with the poliomyelitis viruses. In fact, the first results obtained by BARSKI and LAMY with this method demonstrated the presence of antibodies against the Col SK group of viruses in about 8% of healthy inhabitants of Paris and showed an extraordinarily high percentage, i. e. over 50%, of positive sera among native pygmies of the African tropical forest. The authors also state that Mengo virus grows on embryonic tissues (kidney) of man and on adult or embryonic tissues (muscle, lung, kidney) of mice whereas no multiplication occurred with tissues from chickens, rabbits or dogs. Therefore, the uniform receptivity of tissue culture media prepared from man, monkey and mouse, all of which are susceptible to infection with Mengo virus, contrasts sharply with the selective growth *in vitro* of the rodent-adapted poliomyelitis viruses which, even though pathogenic for all three hosts, will multiply in tissue culture only on human and monkey cells, but not on normal mouse cells. The reasons for this discrepancy are not clear, except that it may serve as an indicator for the more advanced adaptation of human poliomyelitis virus in a possible transition from rodents to man.

As far as tumor cells are concerned, Col SK and MM virus cause no appreciable injury to HeLa cells (human uterine carcinoma) or KB cells (human mouth carcinoma) in tissue culture. Yet, Col SK virus appears to be adsorbed on HeLa cells and to maintain itself to some extent, without cytopathogenicity, as suggested by

high infectivity titers in mice of infected cells which had been thoroughly washed and by the fact that virus, pathogenic for mice, can be carried over into a second transplanted cell generation from infected, washed HeLa cells (JUNGEBLUT and KODZA 1957). Among malignant mouse cell cultures, L cells (Earl) are said

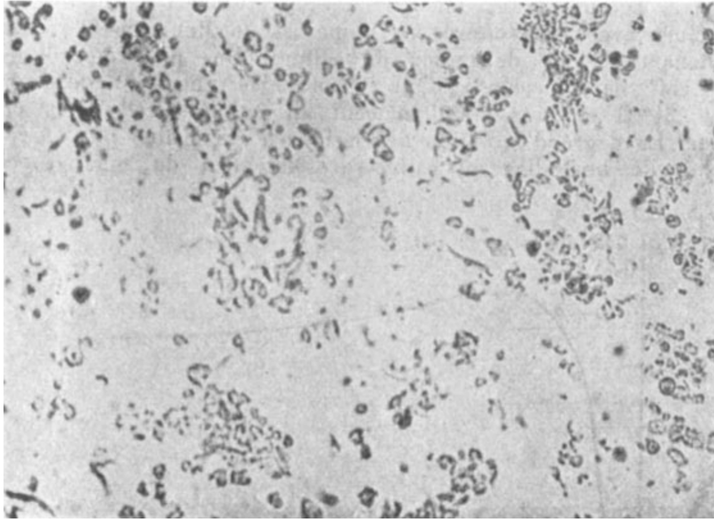


Fig. 7 a. Cultivation of Mengo Virus in Monkey kidney Tissue culture. Tissue culture 48 hours after inoculation with Mengo virus (19th TC passage). (G. BARSKI and M. LAMY: Ann. Inst. Past. 89. 318, 1955.)

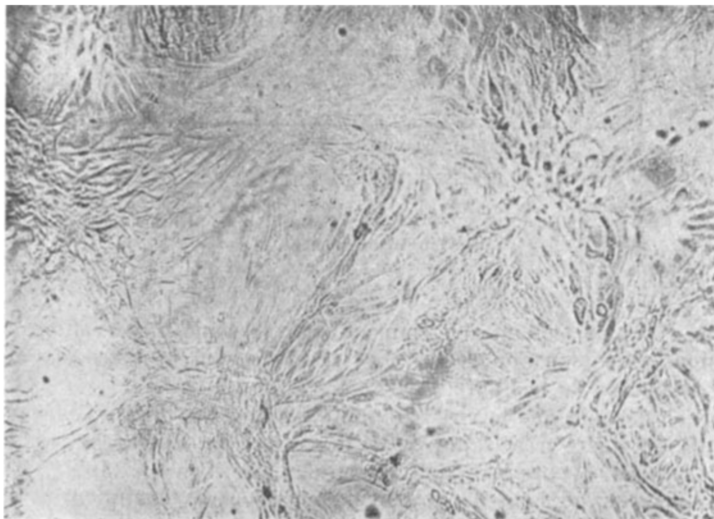


Fig. 7 b. Control tissue culture protected by specific Mengo virus immune serum.

to support non-cytopathogenic growth of EMC virus (SCHERER, 1953) but Col SK virus failed to multiply on two other transplantable mouse carcinoma or sarcoma cells studied by KRET (1955), both of which apparently permitted cytopathogenic growth of poliomyelitis virus. More striking results are obtained with EMC, F and Mengo virus on these and similar substrates. Thus, multiplication with

Table 17. *Multiplication of different Strains of the Col SK Virus Group in various tissue culture substrates*

Virus	Normal human Cells	Normal Monkey Cells Rhesus/Cyno	Normal chicken cells		Normal Hamster Cells	Normal Mouse Cells (Embryonic or Adult)	Tumor Cells						
							Human		Mouse				
			Embryonated Egg	Minced chick Tissue			Hela	KB	L	Ehrlich Ascites	Sarcoma (Kret)	Carcinoma (Kret)	Sarcoma 180
Col SK	?	− (Kidney)	+[2]	+	?	+	−	−	−	?	−	−	−
MM	+ (Testicle) (fetal fibroblasts[1])	+ (Testicle)	+[2]	+	+	+	−	−	−	?	?	?	?
EMC	?	?	+[2]	?	?	+	+[1]	+[1]	+[1]	+[3]	?	?	±[3]
Mengo	?	+[1] (Kidney)	+[1]	?	?	+	+[1]	+[1]	+[1]	+[3]	?	?	?
F	?	?	−	?	?	+	−	−	+[1]	?	?	?	?
Poliomyelitis ..	+[1]	+[1]	+[1]*	−	−	−	+[1]	+[1]	+[1]	?	+[1]	+[1]	−

\+ = Virus multiplication
± = Doubtful virus multiplication
− = No Virus multiplication
? = Unknown

[1] = Cytopathogenicity
[2] = Non-Cytopathogenic death
[3] Oncolysis
* Certain strains only

cytopathogenic effects were described for EMC virus by EAGLE et al. (1956) on KB cells and JUNGEBLUT and KODZA (1957) obtained cytopathogenic growth, yielding high titers of infectious and hemagglutinating virus, with EMC and with Mengo virus on HeLa cells as well as on L cells (see also SCHERER 1953). This cytopathogenic effect was specifically neutralized by immune serum. In harmony with these observations is an earlier report by KOPROWSKA and KOPROWSKI (1953) that mice bearing the EHRLICH ascites tumor are more susceptible to infection with Mengo virus than normal mice and that the virus multiplied at a faster rate in the tumor than in the brain; invasion of the tumor by the virus produced marked oncolytic effects, leading ultimately to destruction of the tumor cells. Growth of EMC virus in EHRLICH ascites tumor transplants, with marked oncolysis, was subsequently reported by LEVY and SNELLBAKER (1956). Mouse sarcoma 180 cells injected ip into mice also seem to favor multiplication of EMC virus, with definite oncolytic effects (JUNGEBLUT and KODZA 1957). It is of considerable interest that HeLa sheets infected with non-cytopathogenic Col SK virus resist subsequent challenge with cytopathogenic EMC virus and to some extent also with Type II poliomyelitis virus (MEF), undoubtedly due to interference. Of further interest is the fact that complete protection of HeLa cells, but not of L cells, against EMC virus infection can be obtained by previous adsorption of RDE derived either from *Vibrio cholerae*, or from human saliva, or from human stool (KODZA and JUNGEBLUT 1957). Protection against viral infection of isolated susceptible cells trough the action of receptor-destroying enzymes has heretofore not been demonstrated in such conclusive fashion as in these tissue culture experiments. The sum total of these observations seems to indicate quantitative rather than qualitative differences in virus-cell relationship between the Col SK and MM strains on the one hand, and the EMC and Mengo strains on the other. The cell responses produced by the various strains of the Col SK group of viruses in different tissue culture media are summarized in Table 17.

The factors which determine these selective growth phenomena on epithelial or fibroblastic cells of various hosts *in vitro* are not clear and require further detailed study in order to permit critical comparison of the respective growth requirements of the several virus strains in the Col SK group with those of the THEILER viruses, the Coxsackie viruses and the poliomyelitis viruses. Also, the possibilities of destroying the tumor agent by infection of tumor cells *in vitro* or *in vivo* with viruses of the Col SK group offer a promising field for systematic investigation of individual strain characteristics. The problem is of some clinical interest because of recent attempts which have been made to antagonize the neoplastic process in experimental animals or in patients by infection with certain neurotropic viruses. It is also a curious fact that cancer patients, as a group, give very rarely a history of previous paralytic infection with poliomyelitis virus.

Host Spectrum (Including Survival of Virus in Infected Insects).

The experimental host spectrum for the different strains of the Col SK virus group varies slightly, but all members of the group are highly pathogenic for most rodents, including albino mice, dormice *(Myoxus glis)* (SANZ-IBANEZ 1946), Orkney voles *(Microtus orcadensis)* FINDLAY and HOWARD 1951), golden hamsters *(Merocricetus aureatus)* and cotton rats *(Sigmodon)*, and to a lesser extent also for guinea pigs (JUNGEBLUT 1945). Guinea pigs are usually considered resistant to poliomyelitis infection. However in some early unpublished experiments (1947) in which we infected young guinea pigs ic with Y-SK virus, the animals apparently

developed a latent asymptomatic infection, as suggested by the appearance in the serum of specific neutralizing antibodies for this virus. Guinea pigs were later shown by GARD et al. (1956) to be good antibody producers following injection with poliomyelitis virus vaccine. SCHER (1953) found male mice more susceptible to EMC virus infection than female mice and described a curious effect of a factor present in female brains which enhances the mortality rate of both male and female mice infected with this virus. Generally, younger animals — including those of suckling age — are much more susceptible than older ones, especially when injected by peripheral routes (JUNGEBLUT 1947; SCHULTZ and ENRIGHT 1948). MACLAREN and SANDERS (1953) who studied the problem of age resistance with EMC virus and other neurotropic virus infections by examining the extent of virus multiplication in the tissues of old and young mice believe that the phenomenon can best be accounted for by assuming that the permeability of cells to virus decreases with age. The non-specific age resistance of adult mice to infection with Col SK virus is broken down through pregnancy, as was shown by KNOX (1950). Thus, the mortality of orally infected pregnant mice increased progressively, beginning with the 4th day of pregnancy, and reached at the last four days of gestation a rate almost twice (88%) that observed in a comparable non-pregnant control group (45%); immediately after parturition there was a full return of resistance to the non-pregnant level. The enhanced susceptibility of the pregnant mouse does not seem to depend wholly upon a greater permeability of the intestinal mucosa during gestation since pregnant mice, infected intravenously, also showed higher mortality rates than controls. A similar effect of pregnancy on the susceptibility of mice to infection with Coxsackie Group A and B viruses was described by BERGER and ROULET (1950) whereas DALLDORF and GIFFORD (1954) observed enhancement in gravid mice only with Group B Coxsackie virus infection. It is not known whether active or passive immunization would compensate for the temporary loss of resistance during pregnancy, a point which is of considerable importance for the corresponding problem in human poliomyelitis.

The symptomatology of the infection in mice and cotton rats is that of an overwhelming, invariably fatal encephalomyelitis, but encephalitic symptoms are much less marked in hamsters; peripheral infection of all three rodents with Col SK or MM virus often produces clear flaccid paralysis of front or hind legs while EMC, Mengo, Ortlieb and F virus tend to be more encephalitogenic. Even though the virus grows to top titers in hamsters, as in mice, about 50% of hamsters infected with ColSK or MM virus recover eventually from the disease; recovery may be complete or leave the animals with various degrees of residual paralysis and subsequent deformities. The appearence of such animals bears a striking resemblance to the clinical picture of recovery from paralytic poliomyelitis in man. In guinea pigs Col SK and

Fig. 8a–f. Symptomatology of Col SK virus infection in experimental animals.

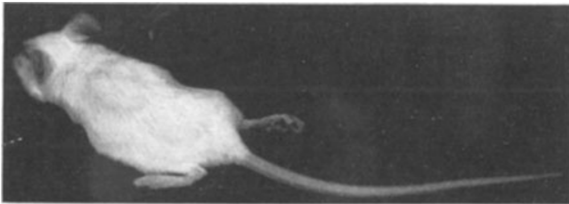


Fig. 8a. Albino mouse paralyzed by Col SK virus.

Fig. 8b. Albino mouse paralyzed by Col SK virus.

MM virus induce a typical poliomyelitic syndrome with no encephalitic signs and occasional involvement of isolated muscle groups; recovery with transient or permanent residual paralysis is frequently observed but death due to myocardial insufficiency may suddenly supervene. Virus titers in neural and extraneural tissues are much lower than in mice or hamsters and serial transmission of a cavian strain of virus does not always succeed (JUNGEBLUT et al. 1941/42; SCHULTZ and WHITE 1948). Approximate LD_{50} endpoints of mouse virus infectivity in these rodents are: mice and cotton rats: 10^{-9} ic, 10^{-8} ip, 10^{-2} orally; hamsters: 10^{-7} ic, 10^{-6} ip; guinea pigs: 10^{-3} ic, 10^{-1} ip (see Fig. 8). Albino rats, whether young or old, are quite resistant to Col SK or MM virus and usually develop an inapparent infection with high virus titers in the blood, viscera and brains for a period of about 2 weeks; no lesions are found in the symptomless rats despite extensive virus multiplication (JUNGEBLUT and SANDERS 1940; POWELL, JAMIESON and CULBERTSON 1948; FINDLAY and HOWARD 1951). On the other hand, EMC and Mengo virus were reported by KILHAM, MASON and DAVIES (1955) as causing paralytic and fatal illness, with brain and cord lesions, in albino rats of all ages by various routes of infection, though more frequently in younger rats. Other species of rats and mongooses when inoculated with these two viruses, ap-

Fig. 8c. Cotton Rat paralyzed by Col SK virus.

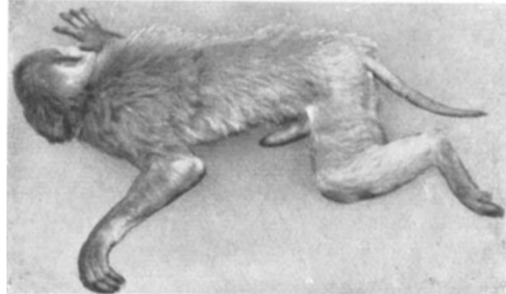


Fig. 8d. Rhesus Monkey paralyzed by F virus.

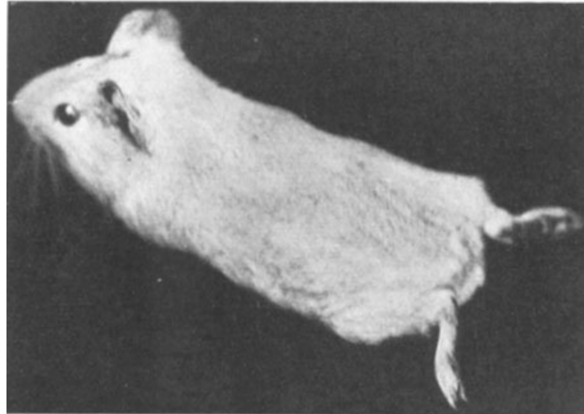


Fig. 8e. Paralyzed Guinea Pig following i. p. infection with MM virus (note flaccid paralysis with no encephalitic symptoms).

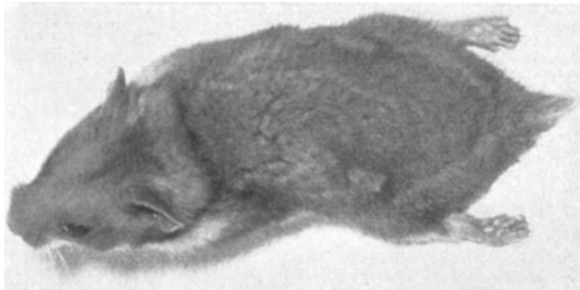


Fig. 8f. Paralyzed golden hamster following i. p. infection with Col SK virus (note flaccid paralysis with no encephalitic symptoms).

parently die principally from myocarditis. Black rats (*rattus rattus*), except in the suckling stage, were reported by the same authors (1956) as resistant to infection with EMC and Mengo virus and no continuous intestinal carrier state could be induced in them; however, multimammate rats (*Mastomys coucha*) and other field rats proved highly susceptible to both viruses and virus was recovered from faeces, intestinal wall, blood and various organs of infected animals. Field rats inoculated with EMC virus appeared to die chiefly from myocarditis. KILHAM et al. (1956) also found two species of mongooses (*Ichneumia albicanda* and *Mungos mungo*) to be highly susceptible to infection with Mengo virus by parenteral inoculation or by the oral route. Death occurred from acute cardiac failure and the principal damage was again to the heart, with little histopathological evidence of CNS involvement; also, the heart muscle contained greater amounts of virus than any other tissues tested and a phenomenal titer of 10^{-12} was obtained by inoculating mice with heart suspension from an infected mongoose (WEINBREN 1956). Two other types of wild carnivores belonging to the same family (VIVERRIDAE), i. e. genets (*Genetta tigrina stuhlmanni*) and palm civets (*Nandinia binotata arborica*), were resistant to infection with virus preparations fatal to mongooses. According to FINDLAY and HOWARD (1951), hedge hogs (*Erinaceus europaeus L.*), injected by central or peripheral routes, are also highly susceptible to Col SK virus infection, with the appearance of neural symptoms and the production of lesions in the midbrain, cord and heart. The same authors state that baby chicks may occasionally develop an inapparent infection; however, BRUTSAERT, JUNGEBLUT and KNOX (1946) found 1-2 day old chicks completely refractory, as did SCHULTZ and WHITE (1948). DEAN (1951) observed febrile illness in two sheep after subcutaneous injection of MM virus. We found young swine completely refractory to intracerebral injection of Col SK virus. Rabbits are highly resistant to all viruses of the group, but infected animals develop high-titered specific antibodies even though virus injected intracerebrally cannot be recovered from the site of inoculation at early or late intervals (JUNGEBLUT).

With respect to primates, rhesus monkeys infected ic with Col SK or MM virus develop paralytic symptoms only on rare occasions, yet the virus can be recovered from the brain and cord for as long as one week after inoculation. By contrast, Mengo, F and Ortlieb virus cause extensive flaccid paralysis in rhesus monkeys following ic inoculation (VERLINDE and HOFMAN 1952; JUNGEBLUT 1952); feeding infection in rhesus monkeys, with viremia and fecal virus excretion, has been described for F virus (VERLINDE) and for Mengo virus (DICK). It will also be remembered that the Mengo strain was originally isolated from the CNS of a naturally infected rhesus monkey and the EMC strain from the viscera of sick chimpanzees. All viruses of the Col SK group are variously pathogenic for Philippine or Java cynomolgus monkeys by intracerebral, intramuscular or intraperitoneal infection (10^{-2} ic; 10^{-1} ip or im). (JUNGEBLUT 1949; VERLINDE, DE BAAN and VERCRUYSSE 1950.) The symptoms are those of a flaccid lower-motor neuron paralysis, with little or no encephalitic syndrome; as in guinea pigs, the virus reaches low titers in cynomolgus monkeys and serial transmission is sometimes difficult to establish. Cercopithecus monkeys (African Green), when infected with Col SK virus, are somewhat less susceptile to paralytic involvement and their reaction begins to approach the subclinical response observed in rhesus (JUNGEBLUT 1950). Fatal infection with Mengo virus, fulminating in myocarditis, was reported by KILHAM et al. (1956) and by WEINBREN (1956) in African grivet monkeys (*Cercopithecus aethiops centralis*) and in African baboons (*Papio doguera*). Of New World monkeys, cebus capucinus,

and particularly spider monkeys (Ateles), are highly susceptible to infection with Col SK virus by the intracerebral route. Cebus monkeys have previously been reported as responding selectively to freshly isolated fecal strains but not to passage strains of poliomyelitis virus. Particularly interesting in this connection are spider monkeys (Ateles) because these animals possess a strictly selective susceptibility to Type I strains of poliomyelitis virus, being solidly refractory to intracerebral infection with Type II and Type III strains (JUNGEBLUT and RODANICHE 1954). Further analysis of this phenomenon showed that Ateles monkeys apparently lack cell-receptors for Typ II and Type III poliomyelitis virus (JUNGEBLUT and BAUTISTA 1956). As far as cold-blooded animals are concerned, CIACCIO (1955) could preserve Col SK virus by alternating passages from frog to mouse, but virus did not survive in serial frog passages. Finally, attempts by BRUTSAERT, JUNGEBLUT and KNOX (1946) to propagate Col SK virus in cultures of various intestinal bacteria or protozoa gave negative results.

Col SK virus has been carried in this Laboratory over 600 serial mouse passages. Transfers are made routinely by intracerebral injection of high dilutions (10^{-6} or 10^{-7}) of glycerinated or frozen brains in order to minimize the chances of picking up a low-titred latent THEILER virus in the mice and to facilitate the harvesting of brains from paralyzed mice before their death. This has resulted in the evolution of a virus (cerebral variant) with maximum potency and hemagglutinating power, yet with fully preserved peripheral infectivity.

The behaviour of Col SK virus, or of related viruses, in the infected insect is of special interest since Mengo virus could be isolated originally from various wild-caught mosquitoes *(Taeniorhynchus)*. However, DICK and GILLETT (see DICK 1953) found that *T. fuscopennatus*, *T. uniformis* and *T. africanus* were incapable of transmitting freshly isolated Mengo virus experimentally. In fact, while mosquitoes retained virus in their stomach for 8 days following an infected meal, there was no evidence of infection of the insect or of viral multiplication. Similarly, VANELLA, KISSLING and CHAMBERLAIN (1956), working with EMC virus, failed to transmit infection with two other species of mosquitoes, i. e. *Aedes aegypti* and *Mansonia perturbans*, after feeding on infected mice. Finally, KILHAM, MASON and DAVIES (1956) found no multiplication of Mengo virus in *T. fuscopennatus* or *Aedes aegypti* after hemocele inoculation and infected mosquitoes did not transmit infection to mice or rats. The conditions are therefore analogous to those under which mosquitoes, through contamination with faeces, may become temporary mechanical carriers of poliomyelitis virus but quite different from those prevailing in true insect-borne virus diseases, like yellow fever or the arthropod-borne encephalomyelitis viruses, in which transmission depends upon actual infection, with viral increase, in the vector. FINDLAY and HOWARD (1951) reported that, although Col SK virus is taken up from the blood of infected hedgehogs by fleas *(Archeopsylla erinacea)*, it is not transmitted by the bite of such fleas to other animals. The same authors, working with two species of cockroaches, *Periplaneta americanus* and *Blatella germanica*, described survival for 3-4 days of Col SK virus after injection into the haemotocele sac, or after feeding, in the American cockroach, but not in the other species. Lansing virus, under comparable conditions, survived in the American cockroach for 15 days (HURLBUT 1950) and THEILERS virus for 1-6 days (SYVERTON and FISCHER 1950). Essentially similar observations were made by SCHULZ-EHLBECK (1952) with ticks *(Ornithodorus moubata)* infected with Col SK virus by feeding on infected mice. The virus survived in the insect for short periods of time, but transmission did not occur through the bite of infected ticks. An endoparasite (*Porocephalus armillatus*), commonly present in mongooses, was found by KIL-

HAM et al. (1956) to contain Mengo virus when recovered from infected animals, but serial passage was not attempted. Finally, CIACCIO (1955) reported that Col SK virus remains alive for 10 days in the adult form of *Tenebra molitor* and in the larval form of *Bombyx mori*, but serial passage in these arthropods proved impossible.

Pathology of Experimental Infection with Col SK Group of Viruses.

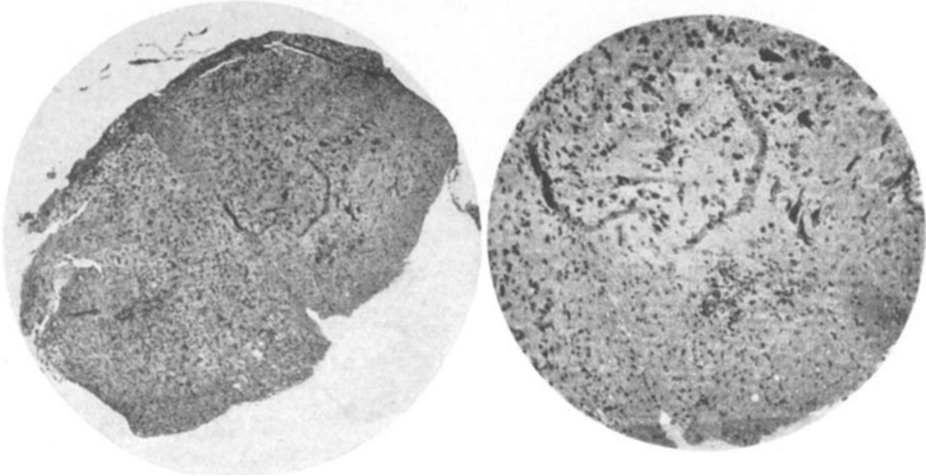
Except for a single fatal human case with extensive myocardial involvement at the autopsy, which has been ascribed to infection with F virus, all data on the pathogenic properties of the Col SK group of viruses are based on experimentally infected animals. The role of these viruses as human pathogens is, therefore, still obscure and comparisons with other well-defined human disease entities with similar clinical symptomatology, such as poliomyelitis, are necessarily limited to an interpretation of pathological findings in monkeys and rodents following infection with these agents. From a strictly pragmatic viewpoint, this state of affairs imposes certain restrictions on the ability to formulate valid conclusions regarding the relationship of these viruses to those of the poliomyelitis group. Yet, since the pathology of poliomyelitic infection in laboratory animals imitates so closely that of the human disease, an evaluation of pathological changes on purely experimental grounds offers a sound basis for a critical analysis of the entire range of the morbid process induced by these viruses.

The basic characteristic common to all viruses of the Col SK group is that they are capable of setting up lesions along the entire axis of the central nervous system — irrespective of whether infection is induced by central or peripheral inoculation — as well as in striate muscle, in heart muscle, and in lymphatic tissues. The systemic distribution of pathology is doubtlessly dependent, to some extent, on a massive viremia which appears early in the infectious process; lesions in other viscera are of minor importance in comparison with these primary localizations. With respect to tissue tropism the virus is, therefore, a neurotropic, myotropic, and lymphotropic agent and evidence can be found that these tropisms are interchangeable, through a selection of variants, by adapting the virus to either neural or extraneural growth (JUNGEBLUT and STEENBERG 1950; WARREN 1952; KUWATA et al. 1954). The pathological process, as seen under different conditions of infection, also manifests a wide range of intensity, extending from a rapidly fatal polioencephalomyelitis to a peripheral myositis and/or myocarditis with eventual repair, or to a sub-clinical intestinal carrier state. Thus, it becomes clear that the dynamics of the infection initiated by parapoliomyelitis virus (Col SK virus) are directly superimposable on the complex and varied pattern of infection with Coxsackie and poliomyelitis viruses in man or in animals (GAEDECKE 1952).

The individual pathological responses of the CNS to Col SK and to poliomyelitis virus vary in different animals, probably because of peculiarities of anatomic structure and metabolic function. In mice, paralyzed by ic injection of either virus, the changes in the CNS are alike in that lesions of the anterior horn are fairly constant and of similar type (ganglion cell necrosis with incipient neuronophagia), but the myelitis is overshadowed by an intensive involvement of the brain stem, the rhinencephalon, the cerebellum, and the entire cerebral cortex (JUNGEBLUT and SANDERS 1940; LILLIE and ARMSTRONG 1940; WOLF 1942; EHRICH and FOSTER 1944; FOSTER and EHRICH 1944; VIEUCHANGE 1945). GORSUNOVA (1955) who observed paralysis in mice or cotton rats after oral

infection with Lansing virus reported degeneration especially in cells of the hippocampus; in some instances focal myocarditis or pericarditis was also present. This panencephalitic picture (see Fig. 9a-c) is not present in mice injected intraspinally with mouse-adapted Type I poliomyelitis virus (LI and SCHAEFFER 1953)

Fig. 9a–d. Central nervous system lesions in rodents infected with Col SK virus.



Sections through spinal cords of mice paralyzed by Col SK virus.

Fig. 9a. Neuronophagia, perivascular infiltration, microglial proliferation in anterior horn. (C. W. JUNGEBLUT and M. SANDERS: J. exper. Med. 72, 407, 1940.)

Fig. 9b. Higher magnification of Fig. 9a.

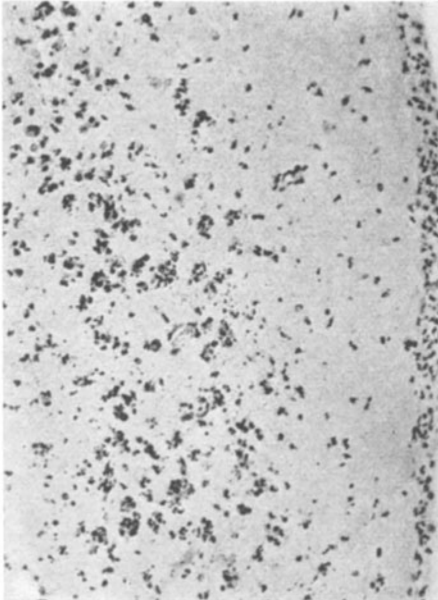


Fig. 9c. Mouse paralyzed by Col SK virus. Cerebrum (anterior third) focus of necrosis of cortical ganglion cells with neuronophagia. (H. E. strain.) (A. WOLF: J. exper. Med. 76, 53, 1942.)

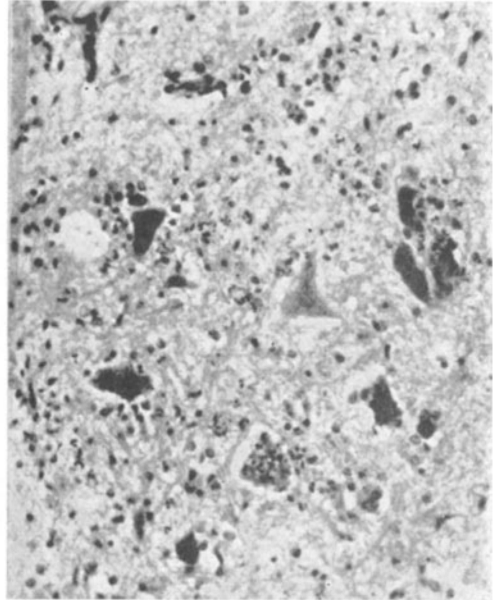


Fig. 9d. Spinal cord lesion of guinea pig infected with Col SK virus. Anterior horn showing nerve cells in various stages of cytolysis. (A. WOLF: J. exper. Med. 76, 53, 1942.)

and ESCHENBRENNER (1956) found no brain lesions at all in mice infected with a spinal variant of this strain. The pathology of Type III poliomyelitis virus infection in mice produced by intraspinal injection of the mouse-adapted Leon strain (LI and HABEL 1951) has not been examined in detail. The histopathological changes induced by Col SK-MM virus in hamsters (DALLDORF and WHITNEY 1945) or more particularly in guinea pigs (WOLF 1942) show the anterior horns as the site of the severest damage (see Fig. 9d); lesions diminish progressively, both in intensity and frequency, as one ascends the central nervous system. Eosinophilic intranuclear inclusion bodies in nerve cells, resembling those occurring in experimental poliomyelitis, were described by WOLF in guinea pigs infected with Col SK virus. Cortical lesions, when present, are minor and, as a rule, associated with the inoculation tract; the olfactory bulbs present only isolated lesions and the cerebellum is not involved at all. Moreover, guinea pigs injected intramuscularly with MM virus show a high rate of concordance (76.7 %) between the localization of initial paralysis and the chosen portal of entry for the virus (JUNGEBLUT 1947). In other words, in the larger rodents, which have more resistance to the virus than the mouse, distribution and histological detail of the lesions approach the pathology of simian and human poliomyelitis very nearly.

In monkeys, the histopathology of infection with various strains of the Col SK group has been studied by JUNGEBLUT (1949), by VERLINDE et al. (1950, 1952), and more recently in great detail by KALM (1955). All three authors are in agreement that the cord lesions produced in rhesus (F, Ortlieb) or cynomolgus and cercopithecus monkeys (Col, SK, MM) are essentially the same as those following infection with the poliomyelitis viruses. The pathological changes are located principally in the anterior horns, especially at the cervical and lumbar levels of the spinal cord; occasionally they are unilateral and correspond with the paralytic involvement of regional muscle groups. In the acute stage they consist of edema, multiple hemorrhages, perivascular infiltration with leucocytes and lymphocytes and partial or complete necrosis of groups of motor ganglion cells, often with well-developed neuronophagia. Cytolysis of the individual nerve cell goes through the same phases as in poliomyelitis and longitudinal cord sections show clearly the distribution of neuronal lesions along the course of the blood vessels (LARUELLE, personal communication); in recovered monkeys, typical gliosis with scar formation may be seen as in the chronic stage of poliomyelitis. As far

Fig. 10a–f. *Central nervous system lesions in monkeys infected with Col SK virus.*

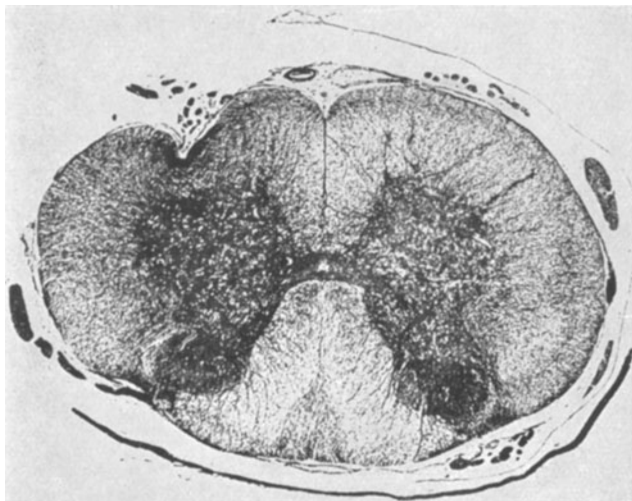


Fig. 10a. Cynomolgus monkey infected i. c. with Col SK mouse virus. Section through cervical cord showing lesion in right anterior horn.

as brain lesions are concerned, VERLINDE as well as JUNGEBLUT found in Col SK virus-infected cynomolgus monkeys neuronal damage and leucocytic infiltration in the medulla, the brain stem and in the motor area of the cerebral

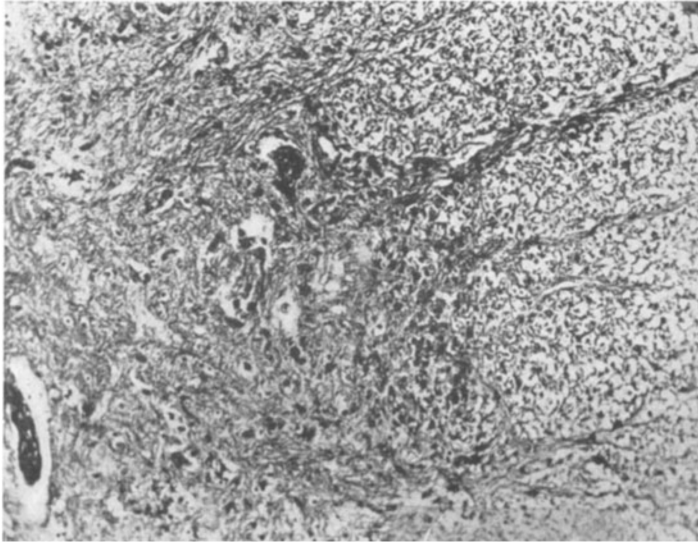


Fig. 10b. Cynomolgus monkey infected i. c. with Col SK mouse virus. Higher magnification of lesion shown in Fig. 10a. Perivascular infiltration and extensive destruction of ganglion cells in anterior horn.

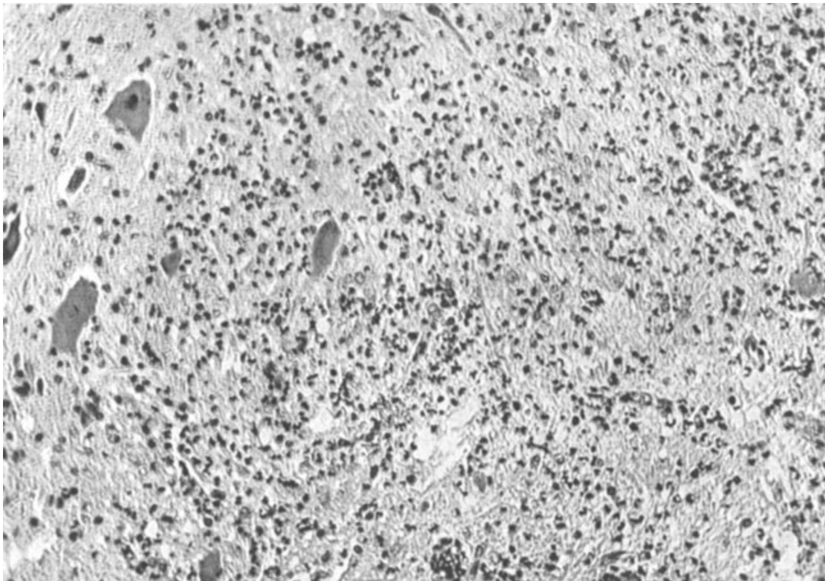


Fig. 10c. Spider monkey (Ateles niger) infected i. c. and i. m. with Col SK virus. Section through anterior horn of lumbar cord. Extensive necrosis of nerve cells.

cortex. The cerebellum showed lesions in the roof, but the cortical zone remained free and the Purkinje cells were well preserved; meningeal involvement was usually slight. The pathological picture, therefore, appeared to be essentially the

same as that produced by poliomyelitis virus (see Fig. 10 a-f). Different conclusions were reached by KALM, however, who examined serial brain sections in order to compare the distribution of cerebral lesions in monkeys following

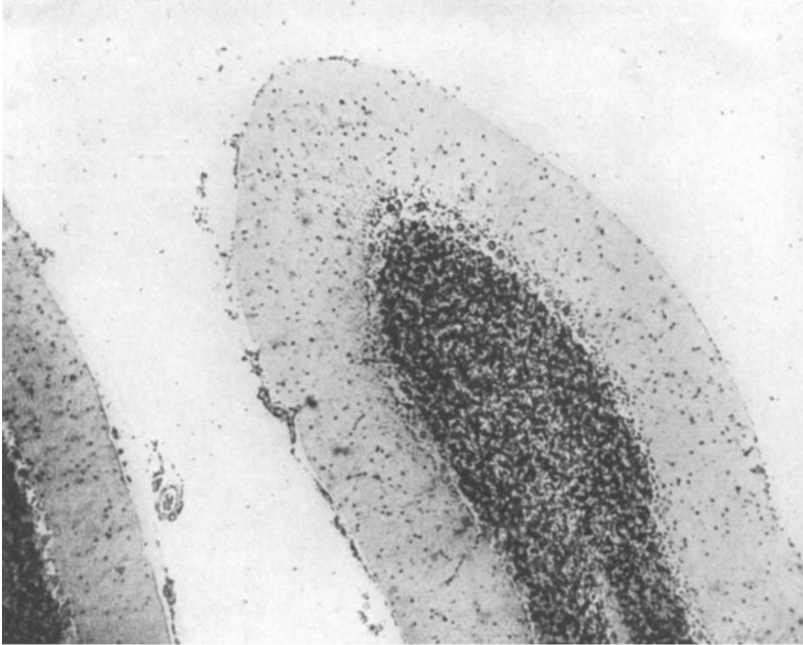


Fig. 10 d. Cynomolgus monkey paralyzed by i. c. injection of Col. SK virus. Minute vascular leptomeningeal lesion. Full preservation of Purkinje cells.

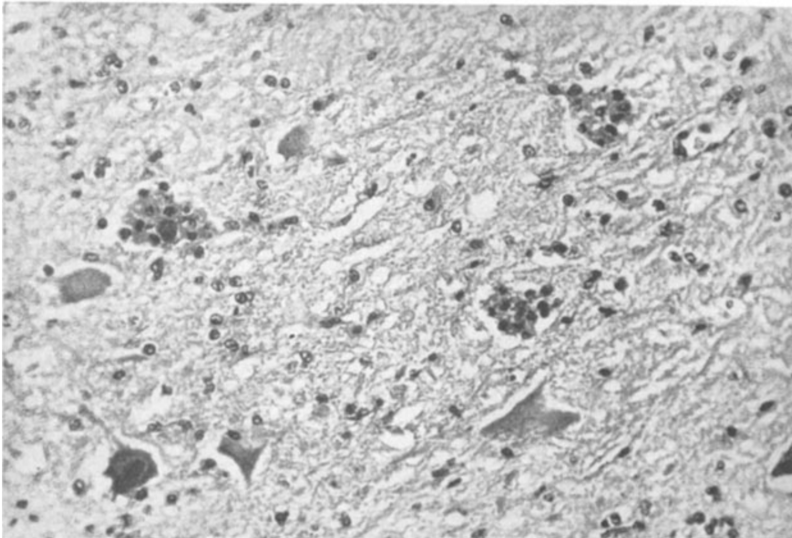


Fig. 10 e. Rhesus AT 59. Cavian F strain. V. Monkey passage. 900 ×. Anterior horn, lumbar enlargement, showing nerve cell necrosis and neuronophagia. (C. W. JUNGEBLUT: Arch. ges. Virusforschung IV, 568, 1952.)

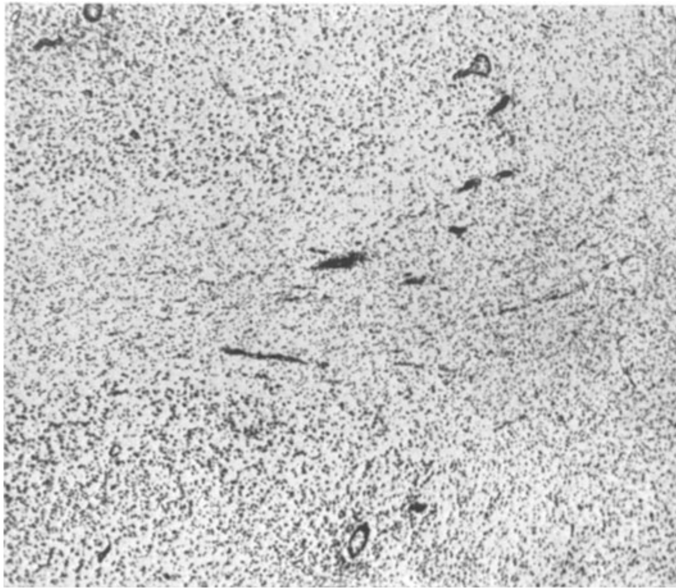


Fig. 10f. Mac. rhesus infected i. c. with F virus. Perivascular cell infiltration in subcortical layer of cerebrum. (H. KALM: Arch. ges. Virusforschung 6, 183, 1955.)

infection with parapoliomyelitis (Col SK, F, Ortlieb) or with poliomyelitis (Brunhilde, Leon) virus. (Fig. 11a-c.) These studies demonstrated the occurrence in parapoliomyelitic infection of perivascular focal lesions in certain subcortical zones which are normally not involved in poliomyelitic infection, and the lesions increased in intensity and frequency from Col SK virus to F and Ortlieb virus. In particular, Col SK virus infection showed an extension of the pathological process to the temporal cortex and the hippocampus which are usually, but not always, spared by poliomyelitic infection (see pages 278/279 of Poliomyelitis Monograph); on the other hand, F and Ortlieb virus infection led to generalized inflammatory changes over the entire cerebral cortex, comparable to infection with Jap B encephalitis virus. In KALM's experience, the cerebellar cortex showed lesions with all three strains of parapoliomyelitis virus after intracerebral infection, but such lesions were absent in peripherally infected animals. Other pathognomonic localizations, such as the cerebellar nuclei, the pons and the formatio reticularis in the medulla oblongata were identical for parapoliomyelitic and poliomyelitic infection.

We are inclined to agree with RUSTIGIAN and PAPPENHEIMER (1949) that differences between "encephalitic" and "paralytic" strains, provided the viruses share the same morphological and physical-chemical properties, should not be over-emphasized, or else one is forced into the absurd position of having to separate viral strains (Brazzaville — Type I poliomyelitis; THEILER GDVII — THEILER TO) that are antigenically alike. In as much as all viruses in this large family are probably mixed populations of "cerebral" and "spinal" variants, the selection of one or the other may depend on factors connected with the host rather than on divergent properties of the virus. This viewpoint is also supported by clinical observations which indicate a wide diffusion of spinal paralytic forms, bulbar forms, encephalitic forms, and mixed forms of the disease, often in the same family (see JUNGEBLUT 1951, p. 15). However, experimental evidence cannot be ignored which tends to show that classical

poliomyelitis virus has more affinity for the spinal cord than for the brain, both with regard to its pathological effects and the amounts of virus generated, in comparison with Col SK virus. Therefore, a clear separation of Col SK virus, under the name of parapoliomyelitis virus, seems to be in order.

Fig. 11a–c. *Localization of pathological process in the central nervous system of monkeys following infection with Col SK group of viruses.* (H. KALM: Arch. ges. Virusforschung **6**, 183, 1955.)

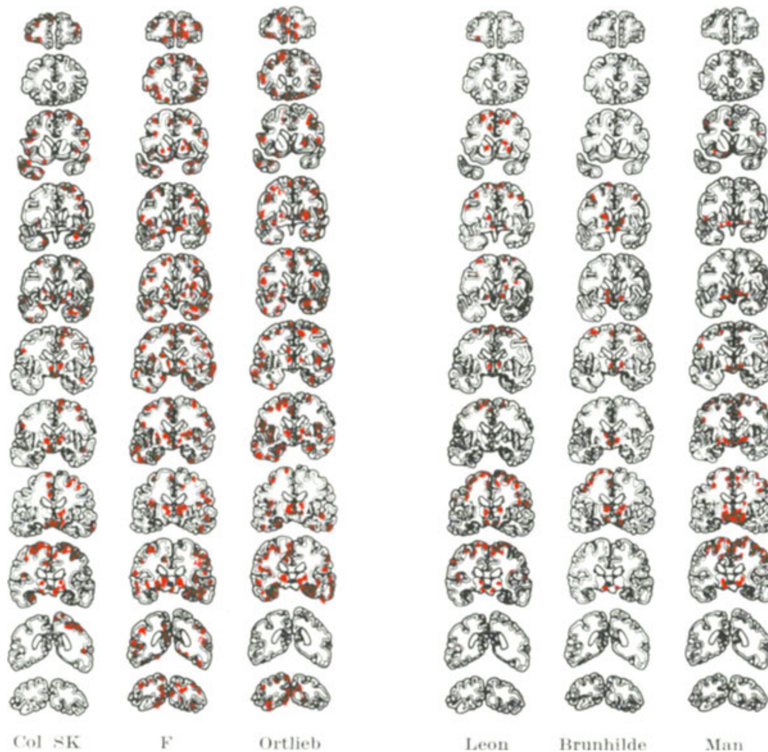


Fig. 11a. Localization after intracerebral infection.

Lesions produced by Col SK virus in the peripheral nervous system, i. e. in the peripheral motor nerve and its terminal organs, are described in the comprehensive studies of SANZ-IBANEZ (1944). This author was also the first to demonstrate the actual presence of the virus in the paralyzed muscle of guinea pigs following infection by the intraperitoneal route. That specific lesions also occur in the muscle fiber itself was shown by RUSTIGIAN and PAPPENHEIMER in 1949, who injected Col SK virus intramuscularly into mice. Within a few hours, even before the appearance of paralytic symptoms, the local injection of virus was followed by the development of a massive myositis involving most of the muscle groups in the injected area; homologous immune serum prevented these lesions. The pathological process consisted of a widespread necrosis of individual muscle fibers, accompanied by edema and a marked inflammatory cell response; regeneration of new fibers took place *pari passu* with the destruction of original fibers, a conspicuous feature of the lesions after the 4th day (Fig. 12a-c). A similar myositis could be produced with a high-titred strain of THEILER's virus (GDVII) and to some extent also with a TO strain, but not with the Lansing virus nor with a number of other unrelated neurotropic viruses.

These muscle lesions do not occur in intracerebrally infected mice, even though the paralyzed muscles contain considerable amounts of virus. The process can be enhanced by the administration of hyalouronidase which accentuates the invasiveness of the virus (VERLINDE 1952; GAEDEKE et al. 1954). The important question as to the relative importance of central nervous system or peripheral muscle lesions for the production of paralysis in the mouse cannot be answered unequivocally. Electromyographic studies by GAEDEKE et al. (1954) of MM virus-infected mice showed that paralysis following intracerebral virus injection was due to centrally-conditioned spasms whereas flaccid pareses resulted from peripheral injection of the virus. Yet, careful observations by these authors failed to indicate any clear-cut parallelism between the histological picture of muscle pathology and the development of paralysis. In view of our imperfect knowledge on the mutual functional relationships between central nervous system and skeletal musculature, it was concluded that both neurogenic and myogenic disturbances are probably participating in the production of paralysis.

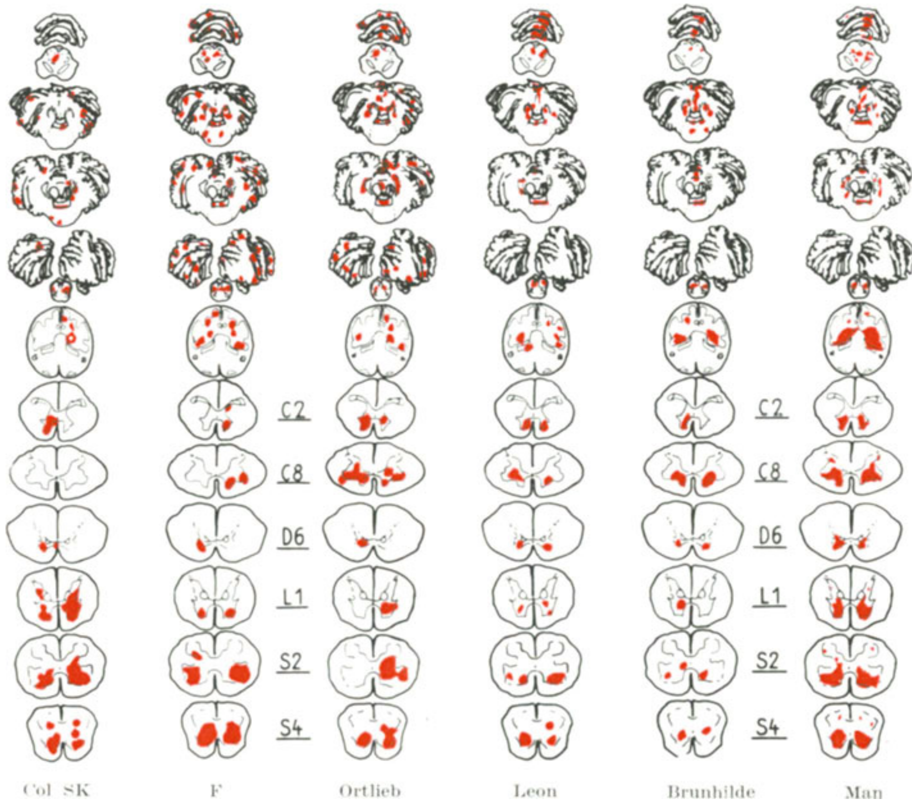


Fig. 11 b. Localization after intracerebral infection.

Similar muscle lesions may be obtained in guinea pigs following intramuscular injection of Col SK virus, except that evolution of the lesion requires more time in consonance with the lower virulence of the virus and the slower course of the experimental disease in guinea pigs as compared with mice. We found the lesion to be strictly local in the sense that it occurred only in the initially paralyzed leg and not in subsequently paralyzed extremities; nor could it be produced when paralysis was initiated by intraperitoneal injection of the virus,

except when a "visceral" variant of the virus was employed. Corresponding muscle lesions may be demonstrated in cynomolgus monkeys following intramuscular injection of Col SK virus and free virus can be recovered from the muscle tissue (JUNGEBLUT; VERLINDE). As in guinea pigs, there occurs a localized

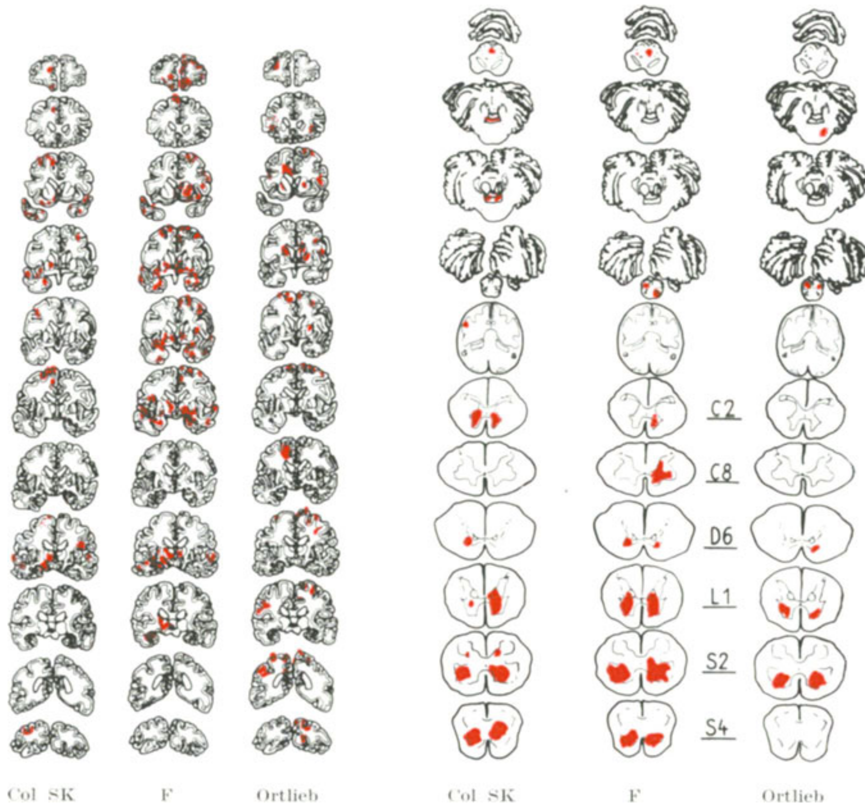


Fig. 11c. Localization after intramuscular infection.

myositis in the paralyzed limb, accompanied by segmental, unilateral destruction of the anterior horn. These facts would suggest a continous spread of virus from the point of first contact with the peripheral muscle fiber to the innervating ganglion cell, followed by the appearance of more or less synchronized serial lesions along the path of viral travel.

Reports on the occurrence of lesions at the myoneural junction in human poliomyelitis, located at the motor end plate or within the adjacent muscle fiber, are few and contradictory [(see JUNGEBLUT (1950) page 4; also SANZ IBANEZ (1945), DENST and NEUBURGER (1950), WOHLFART (1952) and BOWDEN (1952)]. It is difficult, of course, to decide whether such changes are primary or secondary and whether they truly represent the point of initial attack of the virus. In monkeys experimentally infected with poliomyelitis virus TOOMEY and WEAVER (1937) found peripheral nerve lesions and CAREY (1943) described disappearance of motor end plates with degeneration proceeding in a centripetal direction along many motor nerves. Myositis in monkey muscles at the site of poliomyelitis virus inoculation, but not in the contralateral limb injected with normal tissue, was reported by VERLINDE; however, these lesions were less

Fig. 12a–c. *Muscle Lesions in rodents and monkeys infected with Col SK virus.*

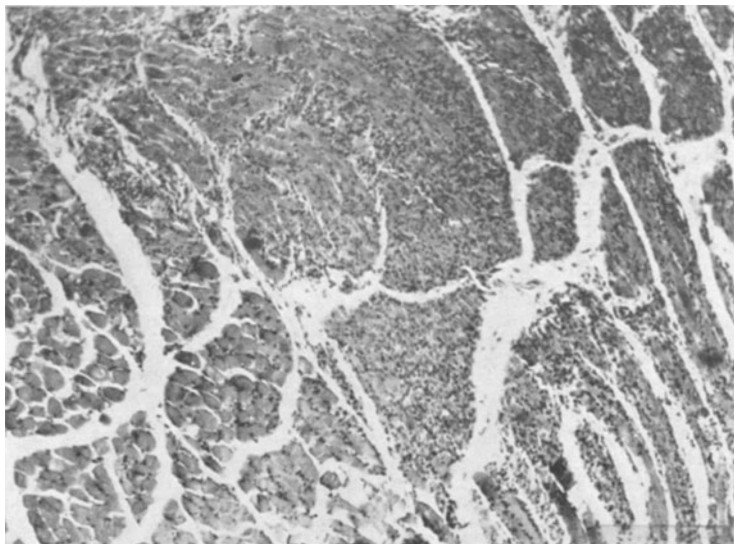


Fig. 12a. Paralyzed leg muscle of guinea pig infected intramuscularly with the brain strain of Columbia SK virus, showing myositis with extensive destruction of muscle fibers. (C. W. JUNGEBLUT and E. STEENBERG, Arch. Path. **49**, 574, 1950.)

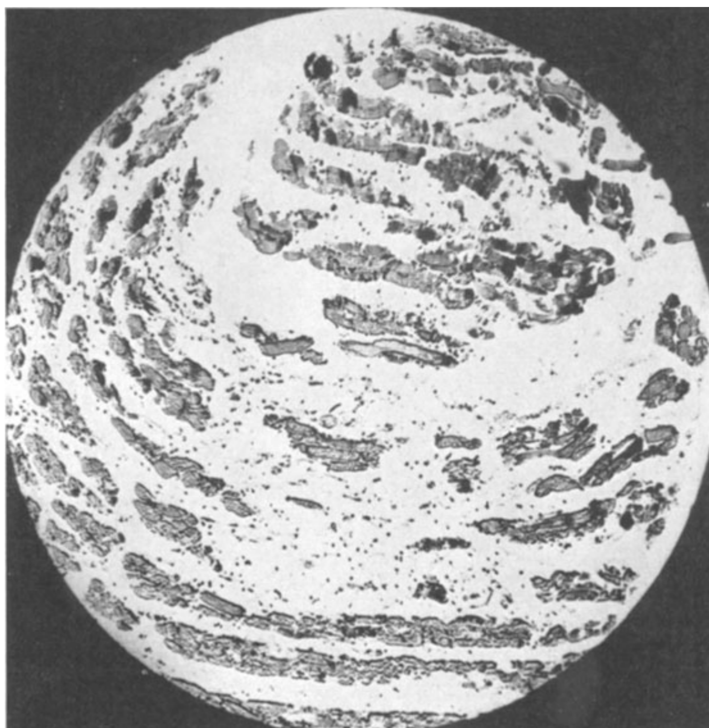


Fig. 12b. Paralyzed leg of mouse infected intraperitoneally with splenic strain of Col SK virus, showing diffuse myositis with muscle cell necrosis. (C. W. JUNGEBLUT and E. STEENBERG, Arch. Path. **49**, 574, 1950.)

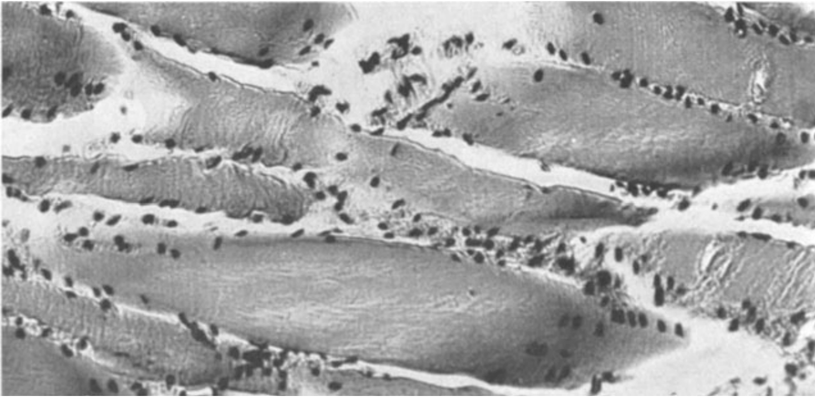


Fig. 12c. Myositis in spider monkey following i. m. injection of Col. SK virus. Paralyzed leg (high magnification) (C. W. JUNGEBLUT).

Fig. 13a–c. *Pathological changes in lymphatic tissues produced by infection of mice with MM, Ortlieb and Tietze virus.* (R. GAEDEKE and K. BETKE: Zeitschr. f. Naturforsch. 7, 401, 1952).

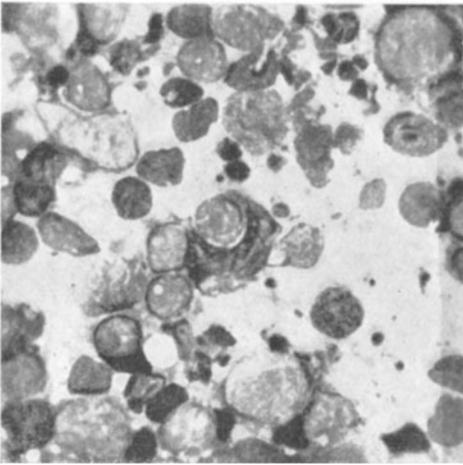


Fig. 13a. Lymphnode: pyknosis of nuclei and cellular debris.

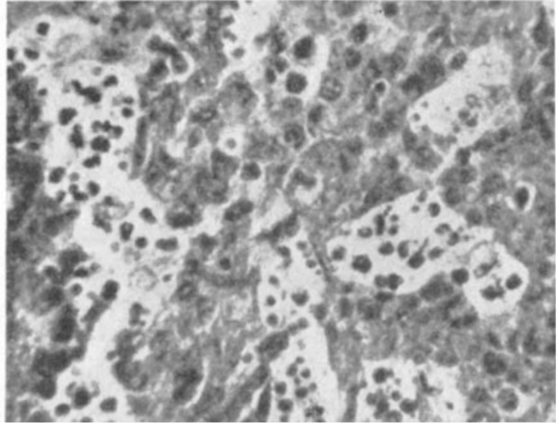


Fig. 13b. Spleen: Hyperplasia of reticulum, marked destruction of lymphocytes.

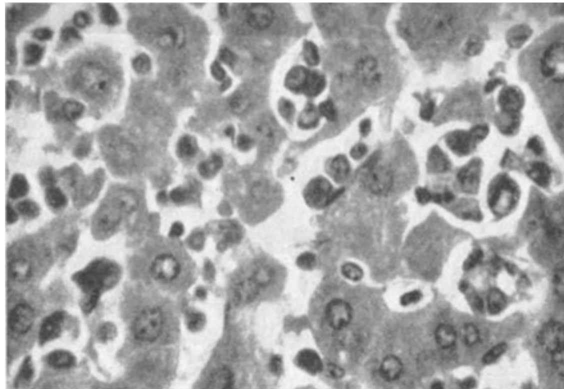


Fig. 13c. Liver: marked proliferation of interstitial cells.

extensive than those induced by Col SK virus and the muscle tissue contained no free virus. Poliomyelitis virus produces no significant local lesions in intramuscularly injected rodents, i. e. mice or hamsters (GAEDEKE et al. 1955). Yet, the virus will set up a severe myositic process when the animals receive cortisone, as was clearly shown by SHWARTZMAN (1954). The myositis observed in cortisone-treated hamsters after injection with MEF virus is essentially the same as that following Col SK virus infection, but ARONSON and SHWARTZMAN (1956) draw attention to a marked difference with respect to the lipotropism of the two viruses. Thus, whereas poliomyelitis virus — like Coxsackie virus — showed a singular affinity for brown fat, resulting in fulminant necrosis of the lipid-bearing cytoplasm with concomitant viral multiplication, no undisputable alteration of brown fat could be appreciated with Col SK virus. GAEDEKE and BETKE (1952), however, demonstrated a more or less marked loss of lipid storage in the adrenal cortex of Col SK virus-infected guinea pigs. The same authors also emphasize the marked affinity of Col SK virus for lymphatic tissue, as shown by edema, destruction of lymphocytes and hyperplastic proliferation of the reticular elements in lymph nodes, spleen and liver [see also SANZ-IBANEZ (1944)]. A similar lymphotropism of poliomyelitis virus has been described by many pathologists from the early observations of BURROWS (1931) to the recent work of WENNER (1951) (see Fig. 13a-c).

The myotropic properties of the viruses of the Col SK group are reflected not only by their ability to produce a myositic process in skeletal striated muscle but also by their capacity to invade the heart muscle, the most vulnerable muscular organ, and to cause severe myocardial damage. The earliest reference to cardiac pathology is SANZ-IBANEZ's description, in 1944, of interstitial myocarditis in guinea pigs infected with Col SK virus. These findings were subsequently confirmed by all investigators in the field working with different strains of the Col SK group and with different species of rodents or monkeys, or even in the chick embryo infected with MEF virus (Fig. 14a-c). The myocardial lesions usually occur as discrete foci consisting of edema, hemorrhage, interstitial collection of round cells, and necrosis of individual muscle fibers but occasionally the involvement is patchy and covers a wider area. The authenticity of myocarditis, endocarditis and pericarditis in the larger rodents or in monkeys seems well enough established but corresponding observations in mice should be interpreted with caution because of the frequent occurrence of "spontaneous" myocardial lesions, possibly due to latent THEILER virus in-

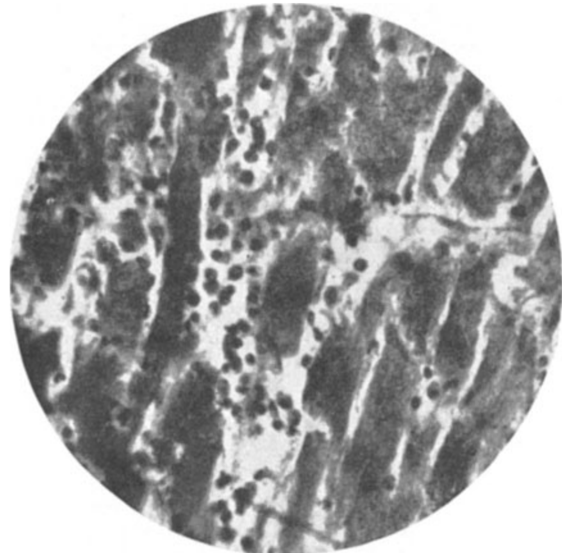


Fig. 14a. Myocarditis in 5 year old chimpanzee, naturally infected with EMC virus. (E. C. H. SCHMIDT,: Am. J. Path. 24, 97, 1948.)

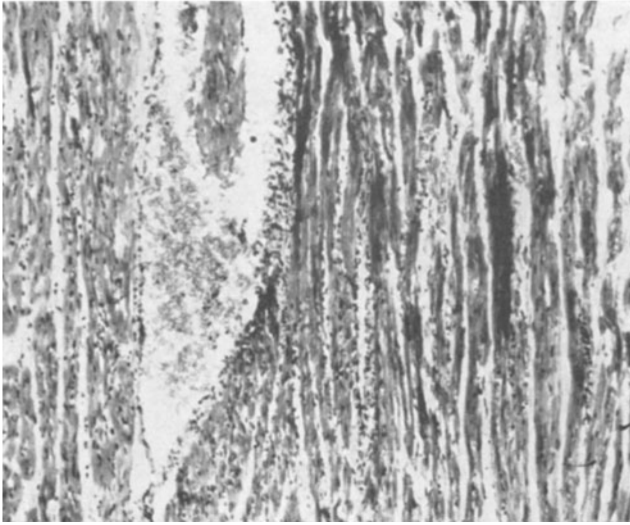


Fig. 14b. Cercopithecus 4: Col. SK virus 10^{-2} i. p. Massive myocarditis with muscle fiber fragmentation and interstitial cell collections. (C. W. JUNGEBLUT: Bull. N. Y. Acad. Med. **26**, 591, 1950.)

fection, in healthy mice [LENKE and LOEWE (1941); ANGEVINE and FUERTH (1943); GREY (1949); LARUELLE and REUMONT (1952)]. The classical example of myocardial involvement in infection with these viruses — which prompted the choice of the designation "encephalomyocarditis virus" for this group of agents at large — is, of course, EMC virus. (SCHMIDT 1948.) In the original host, i. e. the chimpanzee, the myocarditis-producing property of this strain even overshadowed its ability to produce paralysis which became manifest only upon transfer to Laboratory animals. During early rodent passages the virus combined marked neurotropism and viscerotropism, inducing both myocarditis and encephalomyelitis in mice, hamsters and (to some extent) in guinea pigs, but rapid serial passages by the intracerebral route soon led to fixation so that the strain produced only paralysis and no longer myocarditis. However, when virus harvested from infected spleens was carried as a spleen-strain peripherally in mice, lesions in

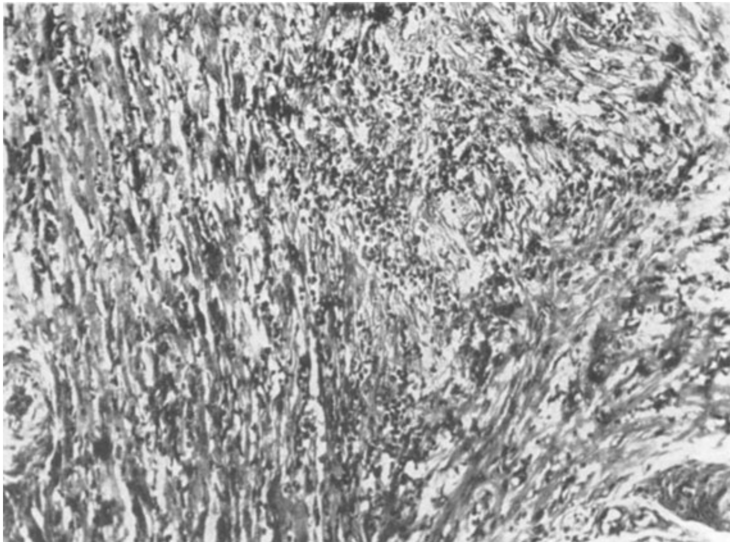


Fig. 14c. Rhesus AR 79. The monkey died with generalized weakness, following intracerebral injection with Y-SK mouse virus (I suckling mouse passage). This is a section through the heart showing focal myocarditis with interstitial cellular infiltration and necrosis of muscle fibers. (C. W. JUNGEBLUT: J. Ped. **37**, 109, 1950.)

the heart and skeletal muscle reappeared once more in the paralyzed animals; identical results are obtained when Col SK virus is subjected to similar experimental procedures. Of the various primates infected with Col SK or MM virus cercopithecus monkeys (African Greens) contract myocardial lesions with a frequency and severity not observed in other experimental hosts (JUNGEBLUT 1950). There seems to be no simple relationship between the development of cardiac or neural lesions because myocarditis occurs equally often in paralyzed as in non-paralyzed monkeys; in monkeys infected with Mengo virus myocardial involvement may be the only pathological feature. (KILHAM et al. 1956.) An interesting comparison was made by KILHAM (1955) for several species of rodents with respect to the frequency of cardiac or neural lesions in these animals and corresponding changes in the clinical picture of the disease after infection with Mengo virus. As a general rule, albino or black rats suffered encephalitis while mongooses and multimammate rats died of myocarditis. In the wild rat (*Arvicanthis abyssinicus*) relatively unadapted low-level strains of the agent, which cause myocarditis, rapidly become neurotropic and produce only an encephalitis in these animals when they are infected with high-level passage material (WEINBREN, 1956). This evolution from myotropism to neurotropism which is so characteristic of the Col SK group of viruses bears a striking analogy to similar phenomena with the Coxsackie and poliomyelitis viruses.

It may be inferred from the sum total of these observations: 1) that viruses of the Col SK group are complex agents possessing both neurotropic and viscerotropic qualities, 2) that the viscerotropic quality may be dormant during passages from brain to brain, and 3) that the lost viscerotropic quality can be restored through propagation on extraneural tissues. There is at least a possibility that a viscerotropic phase may exist without any neurotropic activity at all. It is clear that the latter form, with further modifications, would be the ideal starting point for a living vaccine.

References to heart lesions in experimental poliomyelitis are few and, as a rule, are merely part of a general discussion of the visceral pathology in the experimental disease. VERLINDE has described focal interstitial myocarditis in cynomolgus monkeys infected with poliomyelitis passage virus. Y-SK virus has once been isolated from the heart of an infected infant rhesus monkey (HORSTMANN et al., 1947), and a presumable variant of Y-SK virus, obtained through passage in infant mice, produces typical flaccid paralysis in rhesus monkeys with extensive myocardial involvement (JUNGEBLUT 1950, see page 13). Myocarditis has also been described in the chick embryo inoculated with the egg-adapted MEF strain (LOVE and ROCA-GARCIA, 1955). So far not many freshly isolated human strains have been examined for their ability to produce myocarditis in experimental infection. However, two strains of poliomyelitis virus isolated by us (JUNGEBLUT and EDWARDS, 1951) from the cord and from the heart in fatal cases and another strain recovered from the paralyzed muscle during the acute stage of the disease (JUNGEBLUT and STEVENS, 1950) have produced paralysis and myocarditis in several of the cynomolgus sub-passages. In all these instances the type of lesions corresponds with those induced by Col SK virus infection (see Fig. 15a-d). It seems that poliomyelitis virus — like Col SK virus — may undergo a similar shift from viscerotropism to neurotropism as the result of neurotropic fixation. For our difficulties in establishing serial transmission of heart isolates contrast sharply with the ease with which neural isolates of the same strain could be carried through successive monkey passages. Additional evidence in favor of this viewpoint may be found in the results of reinfection experiments which indicate a different response of animals,

previously infected with human material, towards challenge with a strictly neurotropic strain (Aycock) than with a viscerotropic-neurotropic strain (Col SK)

Fig. 15a—d. *Myocarditis in man and in monkeys infected with human poliomyelitis virus.*

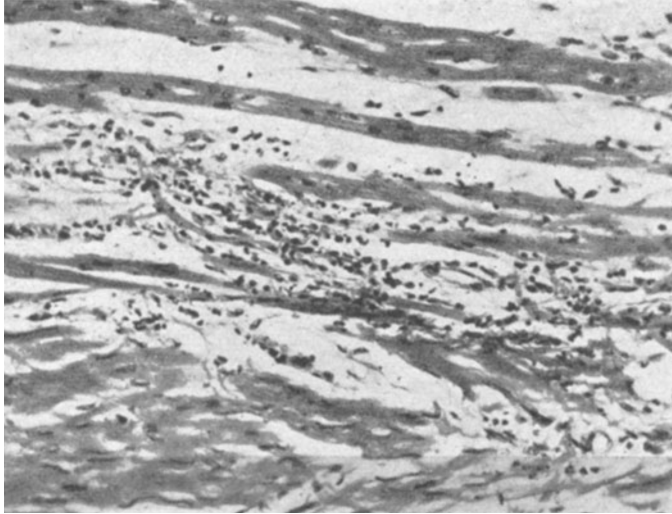
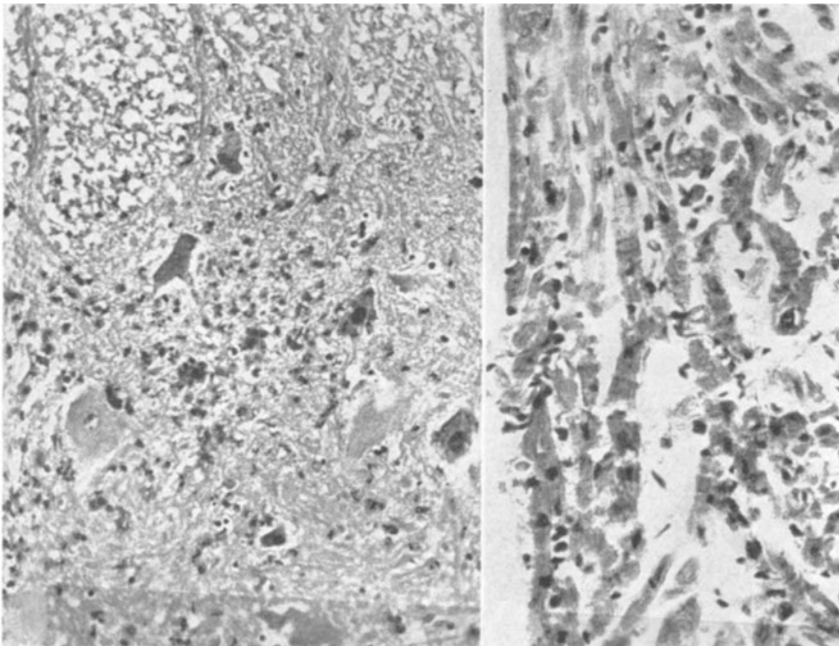


Fig. 15a. Severe acute myocarditis in human poliomyelitis. Focal necrosis of myocardial fibers and infiltration of neutrophils.



Spinal cord and heart lesions in rhesus monkey with 2nd passage poliomyelitis virus isolated from human heart of fatal case.

Fig. 15b. Anterior horn of monkey with typical poliomyelitic lesion.

Fig. 15c. Myocardium of monkey showing focal clumping of myocardial fibers and early fragmentation.

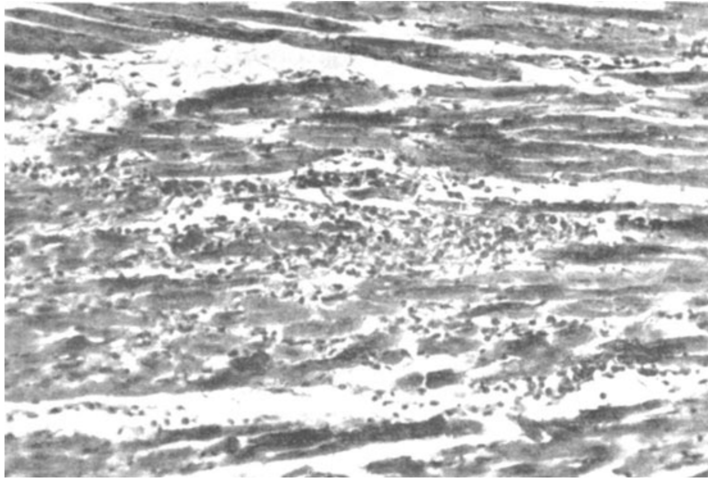


Fig. 15d. Cynomolgus 111: Poliomyelitis virus (isolated from human paralyzed muscle) 10^{-1} i. c. Focal myocarditis with muscle fiber necrosis and interstitial cellular reaction.

of virus (JUNGEBLUT and EDWARDS). To wit, whereas cynomolgus monkeys which had survived paralytic or non-paralytic infection with human poliomyelitis virus (cord or heart) escaped paralysis when reinfected with Col SK virus, no protection was demonstrable in monkeys with a similar history upon reinfection with Type II poliomyelitis passage virus (AYCOCK strain). These probabilities can scarcely be accepted as conclusions but are offered as suggestions for a point of departure for further inquiry into this important problem.

An impressive body of data has accumulated in recent years which points to frequent and severe involvement of the heart muscle in the human disease. The characteristic lesion, first described by SAPHIR and WILE in 1942 (see also SAPHIR, 1945) and subsequently confirmed by many authors, consists of a focal necrosis of individual muscle fibers, with varying degrees of inflammatory cell response in the affected areas (BONCEK et al., 1949). Since the extent of pathological alteration shows considerable variation and virus can be recovered from hearts with or without obvious myocardial involvement (JUNGEBLUT and EDWARDS), the activity of the infectious agent in the heart muscle may not always culminate in the production of substantial local damage, and may possibly

Table 18. *Myocarditis in Human Poliomyelitis.*

I. Myocarditis in Fatal Human Poliomyelitis.

Author	Year	Total Cases	Myocarditis	Per Cent
SAPHIR and WILE	1942	7	6	85
PEALE and LUCCHESI ..	1943	9	7	77
GEFTER et AL.	1947	11	6	54
DOLGOPOL and CRAGAN	1948	87	16	18
LUDDEN and EDWARDS	1948	35	14	40
SPAIN et AL.	1950	14	12	85
Total	1942 — 1950	163	61	34

II. Electrocardiographic Abnormalities in Non-Fatal Human Poliomyelitis.

Author	Year	Total Cases	EKG Abnormalities	Per Cent
BATTRO et AL*	1943	20 (Acute)	4	20
GEFTER et AL	1947	226 (Acute)	32	14
FIRPI et AL	1949	44 (Acute)	10	23
SCHMIDT-KESSEN	1949	43 (Acute)	9	21
BRADFORD and ANDERSON**	1950	155 (Acute)	20	12
JOOS and YU	1950	23 (Acute)	5	21
FRISCHKNECHT and ZELLWEGER***	1950	52 (Acute)	21	40
LAAKE****	1951	265 (Acute)	84	32
BENGTSSON and JOHNSSON*****	1952	200 (Acute)	23	12
Total	1943—1952	1028 (Acute)	208	20

* Normal EKG with all of 18 Chronic Cases.
** Of 5 Fatal Cases 4 had abnormal EKG.
*** 17 Transient; 4 Persistent.
**** Spinal Paral.: 53.5%; Bulbar: 40.5%; Non-Paral.: 3.2%.
***** 25% Sera Giving Positive HA-Inh. Tests against EMC Virus; 15% Positive HA-Inh. Reactions in Poliomyelitis, regardless of Complications.

be reversible in some cases. Nevertheless, the high incidence of histologically demonstrable acute myocarditis, characterized by muscle fiber necrosis and associated cellular infiltration, in fatal cases of acute poliomyelitis is amply illustrated by the statistics given in Table 18; it is likewise reflected in electrocardiographic studies during the lifetime of poliomyelitis patients. Myocarditis may be found, of course, in many other infectious diseases, including some of viral origin, and may represent a toxic manifestation of the infectious process. However, the faithful reproduction of experimental myocarditis in the monkey with poliomyelitis virus isolated from the human heart, mentioned above, supports the same conclusion which LUDDEN and EDWARDS (1949) had previously reached, namely "that the lesions in the heart muscle cannot be satisfactorily explained without ascribing them to the virus of poliomyelitis".

The ultimate significance of poliomyelitic or poliomyelitis-like myocarditis in man remains to be determined, especially in view of the sporadic occurrence of cases of "cryptic or idiopathic" myocarditis of unknown etiology in children and in adults (KOCH 1950; SAPHIR 1952; BLATTNER 1953). Between 1937 and 1944 so many cases of acute myocarditis occurred among infants in certain districts of Munich that STOEBER, who in 1952 reviewed the findings in 140 autopsies, referred to the disease as "epidemic myocarditis of infants". In the absence of any bacterial etiology, STOEBER believed that a virus may be responsible and suggested that either agents of the Col SK or Coxsackie groups might be involved. No definite evidence, however, was obtained to support the hypothesis. KELLER and VIVELL (1954) studied 12 cases of "encephalomyocarditis", described by BETKE and HARMS in 1953 in Freiburg, without being able either to isolate virus or to demonstrate antibody formation against Col SK virus. A similar outbreak, closely resembling that reported by STOEBER, occurred in 1953 among new-born children in Victoria, Australia, but, again, no virus was isolated. Reports have lately come from South Africa in 1955 and 1956 describing meningo-encephalitis with myocarditis in a nursery at Johannesburg (MONTGOMERY et al. 1955). In this instance Coxsackie Group B, Type 3 virus was obtained from the faeces of one of the surviving children and from the brain of two fatal cases. In 1955 VAN CREVELD and DE JAGER published the clinical data relating to a group of 5 cases of myocarditis in newborn infants in Holland. One virus strain, isolated by DEKKING from the heart muscle, was identified by DALLDORF as Group B type 4 Coxsackie virus; the same virus was isolated by VERLINDE et al. (1956) from the heart muscle

of all 5 infants and from the brain of one. The latter authors also succeeded in producing a fatal infection in a baby cynomolgus monkey with this virus with local myositis, myocarditis and recovery of the same virus from the brain and heart of the animal. ENDERS (personal communication) had a similar experience in Boston when he could isolate a virus from the cord of a fatal case, using human kidney cell tissue culture as a medium, which was identified as Coxsackie Group B, Type 3 virus. These observations represent the first examples of injury to meninges, spinal cord and myocardium in man by viruses belonging to the Coxsackie group.

Pathogenesis of Col SK Virus Infection.

It was clear from the early studies of JUNGEBLUT and SANDERS (1940) that infection with Col SK virus produces a systemic disease with subsequent localization in the central nervous system and excretion of the virus in the alimentary tract. This conclusion was based on the fact that virus could be found in neural as well as extraneural tissues, including the blood stream, and that viral multiplication *in vitro* occurred on non-nervous cell substrates. Subsequent experiments by other authors, working with Col SK virus or with other strains of the group, have confirmed and expanded these findings. Most important, perhaps, is the demonstration of specific lesions in the skeletal or cardiac muscle which suggest that the presence of the virus in such tissues is correlated with virus multiplication *in vivo*. In 1948 EVANS and CHAMBERS carried out a careful analysis of the kinetics involved in the distribution of MM virus between extraneural and neural sites by plotting virus concentrations in different tissues against time intervals. (Fig. 16.) Their data brought conclusive proof that, following injection in the foot

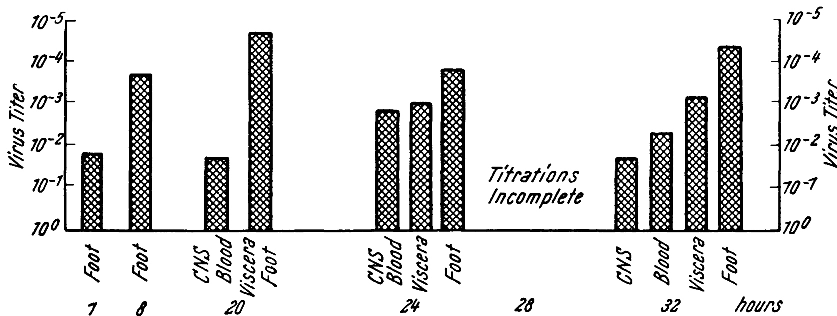


Fig. 16. Concentration of MM virus in hamster tissues at intervals after inoculation into foot. (C. A. EVANS and V. C. CHAMBERS. Proc. Soc. Exp. Biol. and Med. 68, 439, 1948.)

pad of hamsters, the virus increased quantitatively in local tissues, followed in turn by viral increases in the blood and viscera, and that virus appeared in the CNS only after a critical threshhold concentration had been reached extraneurally. In agreement with these findings SANZ-IBANEZ (1948) could isolate Col SK virus from the muscle of infected mice during the pre-paralytic period. The chronological sequence of events leaves little doubt that invasion of the CNS, under the conditions of these tests, was terminal. A similar mode of virus multiplication and dissemination presumably also controls the mechanism of infection with poliomyelitis virus since ARONSON and SHWARTZMAN (1953) observed a definite correlation between foci of myositis and proliferation of virus in MEF-infected hamsters. With Coxsackie virus infection, larger amounts of virus may actually be found in the paralyzed muscles than in the brain. The sum total of these

observations adds up to one conclusion, namely, that Col SK virus, as well as Coxsackie and poliomyelitis virus, possess not only neurotropic properties which are demonstrable by direct introduction of these viruses into the CNS, but are also endowed with well-developed viscerotropic qualities which enable them to propagate actively in non-nervous tissues (especially muscle) prior to their invasion of the brain or spinal cord. In this respect, these viruses are not distinct from certain other neurotropic viruses, such as B virus, vesicular stomatitis, or equine encephalomyelitis virus, which share the same properties. There is this difference, however, between the poliomyelitis and the encephalitis group of viruses that the former, in their neurotropism, exhibit marked preference for the lower motor neuron, particularly the anterior horn, whereas the latter have a tendency to spread indiscriminately across blood vessels throughout the axis of the entire central nervous system. Absence of enterotropic qualities with the encephalitis viruses, which is so highly characteristic for both, the Col SK viruses and the poliomyelitis viruses, further emphasizes the divergent properties among these groups of viral agents.

The question still remains open as to the actual mechanism of progression in the infectious process, i. e. how the virus reaches the CNS from a peripheral portal of entry, whether by nerve transport, or via the bloodstream, or by both routes. Our own observations in MM or Col SK virus-infected guinea pigs and monkeys, in which the localization of paralysis as well as of spinal cord lesion was definitely correlated with the choice of a predetermined muscular site of injection, demonstrate the ability of these viruses to travel along peripheral nerves. However, in mice injected intramuscularly with Col SK virus, severance of the sciatic nerve inhibited neither the development of local myositis nor the occurrence of generalized CNS involvement (RUSTIGIAN and PAPPENHEIMER 1949). Similarly, experiments by SANDERS (1953), who attempted to trace the appearance of EMC virus in the hypoglossal nucleus of mice following injection of the virus into the tongue, showed that the virus was recoverable from the hypoglossal nuclei at an interval which almost coincided with its first appearance in the blood stream (Fig. 17). The different results are probably explained by the greater susceptibility of the mouse to infection with these viruses. From what has been said, it would appear that viruses of the Col SK group may choose either neuronal pathways or the hematogenous route, or a combination of both avenues of infection, depending on sets of conditions which are probably determined by a balance between the virulence of the virus and the susceptibility of the host. A selection of viscerotropic and

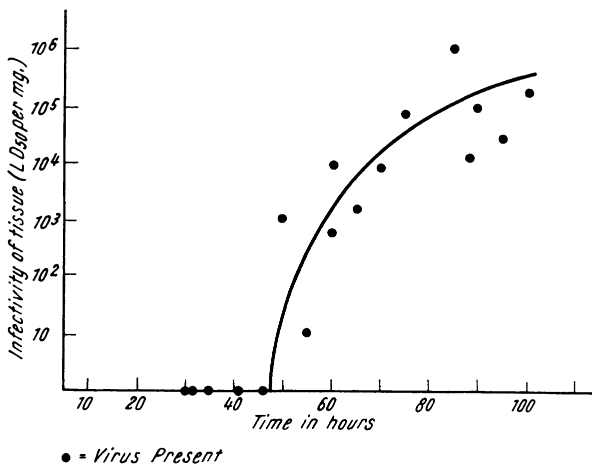


Fig. 17. EMC virus. Titration of virus infectivity in the region of the hypoglossal nucleus at intervals after injection of virus into the tongue. (F. K. SANDERS: Multiplication cycles in neurotropic viruses. The nature of virus multiplication. Camb. Univ. Press. 1953.

neurotropic variants may also take place as suggested by experiments of KUWATA et al. (1954), who found that Col SK virus, after serial passages from muscle to muscle, had increased its myotropism at the expense of its neurotropism, as shown by comparison of the muscle strain with the original brain-passage strain.

As pointed out by us as early as 1950, it seems likely that the pathogenesis of human infection with poliomyelitis virus, which is characterized by a succession of various stages that advance the infectious process from the asymptomatic to the symptomatic phase, with characteristic halting points at various levels, follows a fundamentally similar pattern. In subsequent years essentially the same opinion has been expressed by three different workers in three different countries. Thus, VERLINDE, in 1953, writes the following resumé on the subject: "Dans l'étiologie de la poliomyélite, l'attention a été retenu, non seulement par l'éxistence des divers types immunologiques de virus poliomyélitiques proprement dits, mais aussi par les groupes des virus Coxsackie et Columbia SK, qui semblent bien intervenir dans l'étiologie de certaines maladies dont la symptomatologie peut rappeler, à s'y méprendre, la poliomyélite abortive ou extraneurale, la poliomyélite à forme méningée et même, parfois, la poliomyélite paralytique. L'étude des formes extraneurales a fait concevoir d'une façon nouvelle la pathogénie de la poliomyélite. Les nouvelles recherches expérimentales indiquent que le virus dispose de deux routes d'invasion. La première, la voie hématogène, le conduit à certains tissus non-nerveux; cette dispersion se manifestera généralement par le développement d'une forme extraneurale. La seconde, la voie neurogène, n'est qu'occasionallement suivie par le virus; celui-ci se rend le long des nerfs aux cellules nerveuses du système nerveux central et détermine, de ce fait, l'apparition de paralysie. Sur la longue route, hématogène et neurogène, de la porte d'entrée naturelle jusqu'au système nerveux central, le virus peut être arrêté à differents niveaux qui déterminent la forme clinique de la maladie." In the same year, ARONSON and SHWARTZMAN (1953) draw the following conclusions from their studies: "The virus of poliomyelitis has been separated in the past from other neurotropic viruses as endowed with a highly selective neurotropism. Within recent years the lack of viscerotropism was considered characteristic of the poliomyelitis virus. The findings recorded above would indicate a broader tissue affinity for the virus of poliomyelitis and hence a closer relationship to other viruses exhibiting the binary potentiality of myo-neurotropism in the appropriate host." Finally, KELLER and VIVELL (1954), in their discussion of the same subject, write as follows: „Es ist kaum anzunehmen, daß ohne die Erforschung der Parapoliomyelitisviren die Kenntnis von der Viremie, dem Viscerotropismus, der Gewebs- und Eikultur usw., der echten Poliomyelitisviren solche Fortschritte in relativ kurzer Zeit, und entgegen mancher vorgefaßten Meinung, gemacht hätte.“ ... „Es darf daher heute gesagt werden, daß das Studium pathogenetischer Probleme der Poliomyelitis durch die Untersuchungen an den Parapoliomyelitisviren entscheidend gefördert wurde. Gerade im Zusammenhang mit den pathogenetischen und anatomischen Untersuchungen muß nochmals betont werden, daß die neueste Entwicklung der Poliomyelitisforschung offensichtlich zeigt, daß die anfangs so sehr betonten Unterschiede zwischen den für Mäuse hochvirulenten Parapoliomyelitisviren und den klassischen Poliomyelitisstämmen doch nicht mehr bestehen, oder wenigstens nicht mehr so grundsätzlichen Charakter tragen.“

Influence of Chemical Agents on Course of Experimental Infection with Col SK Virus.

Influence of Hormones and Vitamins.

Because of the uniformity of the peripheral infection with Col SK virus in rodents, many attempts have been made to influence its course by the administration of chemical agents to the infected animal. Beginning with hormone or vitamin treatment, which may initiate antiviral effects by altering the physio-

logical defense responses of the host, work by FOLEY and AYCOCK (1945) showed that the mortality from MM virus infection in estrogen-treated mice (castrate or uncastrate) is significantly lower than in untreated controls. These effects could only be produced in nasally infected mice, not when the virus was given intracerebrally, or even intraperitoneally. In similar experiments with mice, infected orally with a dose of MM virus sufficient to kill 68% of the controls, ANDERSON and BOLIN (1946) observed complete protection with progesterone treatment and a significant reduction of mortality with stilbestrol or testosterone-proprionate treatment; on the other hand, the administration of desoxycorticosterone acetate failed to induce any protective effects. ANDERSON 1946 concluded "that the protectiveness is associated with the administration of those hormones which modify the carbohydrate metabolism of the body". The problem may, therefore, be comparable to the involvement of carbohydrate metabolism in the mechanism of abnormal cell proliferation in malignancy. The results obtained with these hormones parallel earlier ones obtained by JUNGEBLUT and ENGLE (1934) and also those reported by AYCOCK (1937; 1940) in monkeys infected with poliomyelitis virus. It seems possible that these hormones modify cell permeability in such a way as to prevent entry of the virus. On the other hand, cortisone treatment enhances the progress of infection with Col SK virus as it does with poliomyelitis virus (FINDLAY and HOWARD 1952; ARONSON and SHWARTZMAN 1953). The susceptibility of mice to peripheral infection with MM virus could not be altered by the administration of crystalline thyroxin (GOLLAN 1948) whereas HOLTMAN (1946) reported a prolongation of the incubation period in mice infected with Lansing virus under the influence of thyroactive substances, excepting thiouracil.

Attempts to modify the course of experimental infection in mice, hamsters or guinea pigs by the administration of Vitamin C led to no conclusive results in our hands even though as little as 1 mg. of ascorbic acid will inactivate *in vitro* as much as 5000 fatal doses of the virus. These negative results are, therefore, in contrast with earlier successful Vitamin C experiments in monkeys infected with poliomyelitis virus (JUNGEBLUT 1937-1939). Because of the suspected interrelationship between ascorbic acid and the function of adrenal corticosteroids it seemed possible that Vitamin C may antagonize the exalting effects of cortisone on the infection. However, experiments in which cortisone-treated hamsters, which had been infected ip with either Col SK or with MEF virus, received large doses of ascorbic acid, did not bear out this hypothesis. Likewise, it proved impossible to reduce the greatly increased susceptibility of pregnant mice to oral infection with Col SK virus, previously described by KNOX (1950), through the administration of Vitamin C.

Another approach to the preventive problem consists of studying the effect of various protein diets on the course of MM virus infection in mice. The diets used by BIETER and WRIGHT (1949), and later by O'DELL et al. (1953), included mainly nucleic acids (desoxyribosenucleic acid, ribose nucleic acid, pentonucleotide) and nucleic acid components (adenylic acid, adenine sulfate, guanylic acid, guanine, uracil, etc.). These compounds were usually incorporated in the diet and the virus was given by inoculation of the foot pad, as earlier suggested by EVANS and CHAMBERS (1948). Generally, a high protein diet seemed effective in reducing the mortality when it was composed of a completely synthetic preparation with 60% casein and 2% ribose nucleic acid. The rationale of this approach, however, which consists in attempting to minimize viral damage by replacing lost nucleoproteins, seems open to question in view of the fact that the static patterns of nucleic acids in tissues infected with another virus, i. e. herpes, remain

fairly well fixed, despite the morphological cell change. It is therefore not surprising that the administration of large doses of various amino acids, with the exception of phenylalanine, failed to inhibit MEF virus infection in mice (SCHIOTT 1954). More important yet, when mice are fed diets deficient in one or several amino acids, especially tryptophane and isoleucine, multiplication of poliomyelitis or of THEILERS virus in the CNS of the infected animals is actually retarded as compared with non-deficient controls (DAVIES et al. 1949; 1952). In addition, substantial evidence exists from studies with Lansing virus-infected mice that the animals derived some protection from being fed a diet deficient in the Vitamin B complex. The sum total of these results is difficult to reconcile with the data reported by O'DELL.

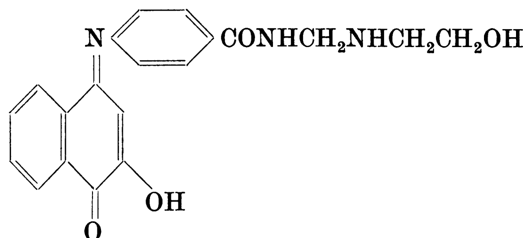
Chemotherapy.

The viruses of the Col SK group have offered an attractive model to many investigators concerned with the chemotherapy of viral infection, and the approaches to the problem are manifold. Even though viruses, unlike higher microorganismal forms, are agents which multiply at the expense of the living cell, it seems *a priori* not impossible that antimicrobial chemicals, particularly certain dyes, or even antibiotics, may have unsuspected antiviral activity; such effects could be produced either by altering the electrical charge of the virus particle itself, or by blocking certain functional protein groups of the cell protoplasma. For instance, inhibition of tobacco mosaic virus has been described by KAUSCHE (1950) for the triphenylmethane series of dyes; in the case of victoriablau and nachtblau, this inhibition may be referable to an actual attachment of the virus particle to the colloidal dye particle. Of the many dyes and chemotherapeutic substances employed in the treatment or prophylaxis of Col SK or MM virus infection in mice most have given negative results but a few offer what seems to be at least a chance of success.

The first of these substances tried is trypan red which WOOD and RUSSOFF (1945) found moderately effective against MM virus infection when virus and dye were given by the intraperitoneal route. A certain specificity of this antiviral action seems undeniable because trypan blue, also an acid dye with similar structure, was ineffective under the same experimental conditions. The effect of trypan red against MM virus was confirmed by BIETER and WRIGHT (1949) as well as by MURRAY et al. (1949), and HURST et al. (1952) found trypan red protective against EEE virus infection. Even greater chemotherapeutic action was noted by the same author (HURST et al. 1952) with the basic acridine dye mecaprine (atabrine) against certain small neurotropic viruses including EEE, WEE and Rift Valley Fever, but not Theiler virus or mouse-adapted poliomyelitis virus; THOMPSON and LAVENDER (1953) found atabrine useless against Semliki Forest virus. The common property between trypan red, mecaprine and other macromolecular dyes is that they all tend to be concentrated in the reticulo-endothelial system (HURST 1956). HAMMON, AIRD and SATHER (1948) who repeated WOOD and RUSOFF's experiments with trypan red and MM virus infection obtained only negative results when dye and virus were given by separate routes. HAMMON's conclusion, however, that the apparent protective effect is due to nonspecific protection afforded by certain inert substances when injected prior to virus inoculation in the same area, does not seem entirely valid since BIETER and WRIGHT's and MURRAY's results were obtained by feeding the dye to ip-infected animals. An even greater protection, according to MURRAY et al., can be obtained by orally administered Congo red. BIETER and WRIGHT have also presented extensive data on the screening of a large number of dyes and chemical substances given by drug-diet to mice infected ip with very small doses of MM virus, producing a mortality rate in the controls of about 90% after a 28 day interval. Basing their

conclusions on ratios of survival and the amount of virus recoverable from the brains of treated and untreated mice, they found among a total of 393 compounds investigated some activity in groups of benzine dyes, azo dyes, nucleic acid derivatives, pteroylglutamic acid derivatives and in various miscellaneous organic chemicals, including simple phenolic compounds, antimalarials, etc. The criteria of infection chosen by these authors are not in agreement with our own experience on the infectivity of MM virus. Therefore, we suspect that it might be difficult or impossible to obtain confirmation of these results on repetition. Attempting to make use of the selective localization of sudan red in the fat cells as a possible blocking agent for virus multiplication, we ran several experiments in which mice fed a 1% sudan diet were tested for their resistance to intraperitoneal infection with Col SK virus. The results were entirely negative, which is in line with ARONSON and SHWARTZMAN's (1956) finding that Col SK virus spares the fat cells whereas the brown fat apparently is a specific locus of viral multiplication for poliomyelitis and Coxsackie viruses. A study of certain organic arsenicals by MCKINSTRY and READING (1945) was prompted by the fact that arsenic produces its chemotherapeutic effect by inactivating sulfhydril groups in certain of the protozoa. In view of the essential role played by these groups in the reproductive process, it was hoped that arsenicals might sufficiently interfere with their action to prevent multiplication of the virus in the infected host. Of 22 organic arsenicals studied, three (neoarsphenamine, stovarsol, tryparsamide) exerted a slight antiviral effect against Col SK infection in mice. The virus was given ip and the drugs by other routes, starting on the day of infection. The antiviral action of neoarsphenamine was completely nullified by the administration of cysteine or glutathione, suggesting that their effect may have been by interfering in some manner with the sulfhydryl system in the host-virus interaction. Previously, we had obtained essentially negative results with the administration of arsenicals to monkeys infected with poliomyelitis virus (JUNGEBLUT 1930). SANDERS and SUBARROW (1948) reported that darvisul, a sulfonamide which competes with another bacterial metabolite, i. e. paraminobenzoic acid, had a marked antiviral effect against Col SK virus infection in mice. However these results could not be confirmed by five independent investigators (JUNGEBLUT 1949, LO GRIPPO et al. 1949, COX et al. 1949, WEIL and WARREN 1949, FRANCIS and BROWN 1949).

In 1951, SCHNITZER, BUCK and STEIGER, after testing unsuccessfully 700 chemical substances, discovered that compounds of a series of 2-hydroxyl, 4-naphtoquinonimines possessed more or less marked activity when administered ip or sc to mice, prior to intraperitoneal infection with Col SK or MM virus. Among a group of 160 compounds of this type 67 were active. One of these, designated as Ro-3532/1,



after a single ip injection of 500 mg/kg, given shortly after an ip infection with 10-20 LD_{50} of virus, prevented the death of approximately 80% of the animals; a single sc dose, under similar experimental conditions, protected about 50% of the mice. Surviving mice were fully susceptible to reinfection with Col SK virus. The type of antiviral activity suggested some form of direct interaction between virus and drug. The experiments also showed that, once virus had entered the cells of the host, it could no longer be influenced by the

chemical. The above results were fully confirmed by JUNGEBLUT (1951) who also reported a similar chemotherapeutic effect in infection with F virus, whereas the drug exerted only a minimum degree of protection against EMC virus infection (Fig. 18). These studies were not continued because the margin between

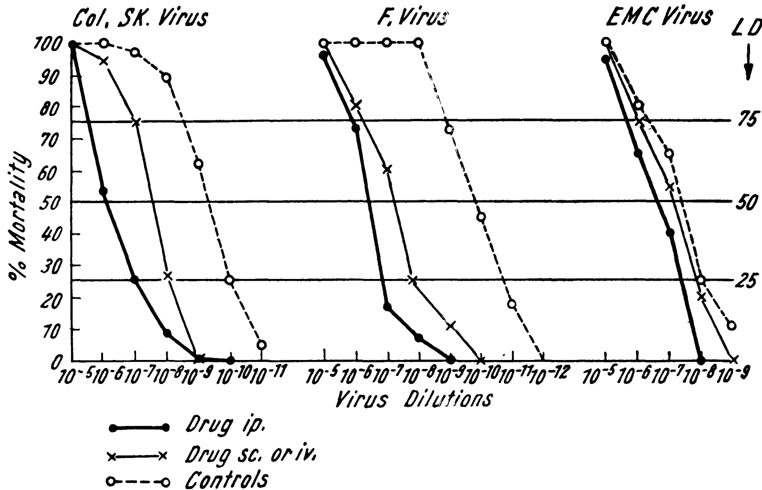


Fig. 18. Effect of compound Ro 2-3532/1 on the intraperitoneal infection of mice with Col SK, F and EMC virus. (C. W. JUNGEBLUT: Proc. Soc. Exp. Biol. and Med. 77, 176, 1951.)

the effective and tolerated dose was too close to entertain any hopes for a human application of this drug.

The possible usefulness against Col SK virus and poliomyelitis virus infection of a certain antibiotic principle found in mold filtrates of *Penicillium stoloniferum* and *Penicillium funiculosum* is suggested by reports of POWELL and his coworkers in 1952 as well as of SHOPE in 1953. POWELL et al. (1952), working with a mold filtrate obtained from *P. stoloniferum* cultures and designated by them as M 5-8450, found considerable chemoprophylactic action, coupled with slight chemotherapeutic action, against MM and Semliki Forest virus in mice. Preventive action against 100 LD_{50} of virus given intraperitoneally could be shown by injecting mice parenterally with a single dose of 0.05 to 0.25 cc of the crude filtrate, provided its administration anteceeded infection by 24 hours; negative results were obtained with ic infected mice and with oral administration of the drug. These results could be duplicated in mice infected peripherally with a hamster-passaged line of MEF (Type II) poliomyelitis virus (POWELL and CULBERTSON 1953). Subsequent experiments by HULL and LAVELLE (1953) made it clear that pre-treatment of tissue-cultured monkey testicular cells with relatively small amounts of M 5-8450 inhibited the cytopathogenic effects of all three immunological types of poliomyelitis virus. That the antibiotic exerts its effect on the cells rather than on the virus is indicated by the fact that pre-treatment of the cells is necessary to demonstrate protection and that viable virus could be recovered from the undamaged treated tissue culture as late as 5 days after inoculation. The evidence points to the probability that the mode of action may be one of interrupting or preventing some stage in the reproductive cycle of the virus, although an effect on virus attachment to the cell is not precluded. The point could presumably be settled by hemagglutination-inhibition experiments with Col SK virus. In view of these promising results COCHRAN, BROWN

and FRANCIS (1954) undertook to investigate whether any antiviral effects of M 5-8450 could be elicited in cynomolgus monkeys infected subcutaneously with Type I poliomyelitis virus (Mahoney strain). It was concluded that pretreatment with at least 100 cc of the crude filtrate reduced the morbidity and lengthened the incubation period of the infected animals. SHOPE, working independently with a mold filtrate of *P. funiculosum*, reported in 1953 the discovery of an active principle in the filtrate, designated by him as helenine, which was effective against Col SK and Semliki Forest virus infection in mice. The substance exerted its maximum effect when given within the first 10 hours after peripheral infection and showed the best therapeutic results with doses of virus ranging from 10-1000 MFD. SHOPE concluded that the antibiotic delays the entrance of the virus into the CNS and retards its build-up to lethal levels. Apparently, the substance acted essentially as a viro-static agent, causing an inhibition or interruption of viral multiplication, or modification of the neuroinvasiveness of the virus. Since maximum therapeutic effect was obtained with relatively small doses, the substance may produce a "triggering" action on some antiviral function of the host (SHOPE); as HURST (1956) suggests, the phenomenon seems to be similar to that observed by BAUER (1955) with isatin thiosemicarbazone in infection with vaccinia virus. While the chemical nature of the principle is unknown, the presence of a large proportion of polysaccharides in crude preparations suggests the possible importance of this class of substances for the antiviral effect. SHOPE believes that helenine differs from the antiviral polysaccharide reported by HORSFALL and MACCARTY (1947) and from the antiviral penicillin impurity reported by GROUPÉ and RAKE (1947) but that it may be related to the preparation employed by POWELL and CULBERTSON. Helenine was examined, in 1956, by COCHRAN and FRANCIS for antiviral effects in mice and monkeys against peripheral infection with poliomyelitis virus (MEF; Mahoney). The substance was capable of prolonging the incubation period of the disease in mice and had marked prophylactic activity in monkeys, as indicated by a reduction of the incidence of paralysis from 100% in 11 controls to 18% in 11 treated animals. In the present state of their development, the preparations of POWELL and of SHOPE do not appear to have direct therapeutic application. However, their proven prophylactic effectiveness under various experimental conditions should encourage further work towards purification of the active principle and more detailed investigation of its properties and mechanism of action. There are no further reports on tests with other antibiotics against Col SK virus infection, except that a crystalline antibiotic isolated from cultures of *Nocardia formica* which has activity against the influenza-mumps-New Castle group of viruses is inactive against Col SK virus in mice (MCCLELLAND 1953).

Another approach to the chemotherapy of viral infection is directed toward inhibition of the intracellular reproduction process. It is believed that viruses, after gaining access to the susceptible cells, exert a directive influence on the synthetic mechanisms of the cell, diverting it from normal function to that of synthesis of virus molecules. Since these synthetic processes are catalyzed by certain enzyme systems, it appears probable that virus reproduction may be inhibited either directly by compounds which inactivate these catalysts, or indirectly by the phenomenon of drug-enzyme-substrate competition. The latter would involve the use of analogues, or of substances similar enough in chemical configuration to a virus component to react with the enzyme system concerned, yet sufficiently dissimilar to serve as a normal functional unit of the parasite. Since many viruses appear to be essentially nucleoprotein macromolecules, such compounds might possibly inhibit their proliferation by competing with the

normal virus pyrimidine components (cytosine, thymine, uracil) for the synthetic enzyme systems involved. It is also known that ribosenuclease arrests protein synthesis in plant cells and that analogues (such as thiouracil) which inhibit RNA production also inhibit the synthesis of tobacco mosaic virus. Inasmuch as poliomyelitis virus consists entirely of RNA, ribosenuclease may specifically interfere with intra-cellular multiplication of this virus (see BARSKI and CORNEFERT 1956).

Studies by McKINSTRY and READING (1944), which follow this line of approach, showed that five of 42 pyrimidines tested exerted a slightly favorable influence against Col SK virus infection in mice, as indicated by prolongation of life or survival of the treated animals, as compared with the controls. Four of the five effective compounds were ethyl esters of certain 2-oxo-4-R-6-methyl-1,-2,-3,-4-tetrahydro-5-pyrimidinecarboxylic acids. These may be considered as derivatives of uracil in which the functional group in position 4 and the hydrogen atoms in position 5 and 6 have been replaced. Antiviral action seemed to be definitely associated with the nature of the substituent in position 4. Introduction of the phenyl, 4-hydroxyphenyl, n-hexyl, styryl and 3-methoxy-4-hydroxyphenyl groups at this point yielded active compounds whereas a number of other related or unrelated substituents were inactive. A complex pyrimidine, i. e. 5-(2: 4-dichlorophenoxy)-4-hydroxy-2-mercaptopyrimidine, which produced some favorable effects against vaccinia infection, had no action on Col SK virus infection in mice (THOMPSON et al. 1951). A similar relation between chemical structure of purine antagonists and antiviral properties against poliomyelitis virus has been described by BROWN (1952), BROWN and ACKERMAN (1951) and by AINSLIE (1952).

In this connection it is of interest to refer to other experiments designed to interfere with viral propagation by placing the virus in contact with chemical compounds of a configuration similar to that of cell receptors which are known to adsorb or bind virus prior to the process of intracellular multiplication. Since these cell receptors are generally of mucoprotein character, it seemed logical that polysaccharides, under favorable conditions of contact, might be able to combine firmly with virus to form a non-infectious complex. In keeping with this hypothesis, we could demonstrate that *in vitro* prepared mixtures of Col SK virus and various bacterial capsular polysaccharides (pneumococcus, streptococcus, Klebsiella) had apparently lost the greater part of their virulence for mice as well as their hemagglutinating power. Therapeutic results *in vivo* were reported by HUMMEL and REIMOLD (1954) who treated MM virus-infected mice by intravenous injection with highmolecular polymers of varying chemical derivation. Most likely, the observed effects were due to an adsorption of free virus in the bloodstream during the viremic phase of the infection by these colloidal substances. It seems possible that chemicals of this kind, such as blood group substance, tissue mucoproteins, bacterial and synthetic polysaccharides, might be useful not only for intercepting circulating virus, but also for reducing the content of free virus in the intestinal canal. In fact, the seasonal cycle in the excretion of fecal virus in man may conceivably depend upon a suppression of free virus by the greater presence in the winter months of capsular polysaccharides released from upper respiratory microorganisms; inversely, a splitting of this complex may occur through the action of certain receptor-destroying enzymes liberated by the enteric bacterial flora of the summer months. It is interesting that MANDEL and RACKER (1953) have already offered experimental evidence, using THEILERS GD VII virus infection in mice as a model, to show that the intestinal tract of the mouse contains a polysaccharide which binds virus and also an enzyme which splits the polysaccharide receptor-virus complex.

Finally, another approach to the chemotherapy of virus infection is possible

through the administration of suitable receptor-destroying enzymes. The group of Col SK viruses, like the influenza group, lend themselves well to this purpose since the activity of such enzymes can be demonstrated and quantitatively measured by *in vitro* hemagglutination-inhibition tests. That the receptor-destroying enzyme of *V. cholerae* (RDE), for instance, is capable of producing antiviral effects against Col SK virus *in vitro* as well as *in vivo* was shown by VERLINDE and DE BAAN (1949) and later again by JUNGEBLUT (1950). The magnitude

Table 19. *I. Effect of Cholera Vibrio Filtrate (RDE) on Col. SK Virus Infection in Mice.*
(Data from J. D. VERLINDE and P. DE BAAN: Ann. Inst. Past. 77: 1, 1949.)

Treatment RDE 1:100 0.5 cc. IP	Infection* Col. SK Virus 10^{-6} 0.1 cc. IP		Average Survival Time Days
1 Injection	34/56	(60%)	8
2—7 Injections	6/24	(25%)	12
Control (No Injection)	28/30	(93%)	5

*) Numerator = Number of Mice Paralyzed.
Denominator = Number of Mice Injected.

II. Prophylactic Effect of R.D.E. on Col. SK Virus Infection in Mice.
(Data from C. W. JUNGEBLUT: Bull. N. Y. Acad. Med. 26: 571, 1950.)

Mice (10-12 gms.)	Col. SK Infection	Number of Mice	Cumulative Mortality Days									Mortality Rate	
			1-4	5	6	7	8	9-11	12	13	14		
RDE*	10^{-6} i.p.	20	0	0	4	5	7	7	8	8	8	8/20	40%
Controls**	10^{-6} i.p.	20	0	16	18	18	19	19	19	20		20/20	100%
Controls ..	10^{-7} i.p.	5	0	2	4	5						5/5	100%
Controls ..	10^{-8} i.p.	5	0	2	2	3	3	3	4	4	4	4/5	80%

* 2 cc. RDE (P&S cholera strain) given 5 hours before infection.
** 2 cc. NaCl solution given 5 hours before infection.

III. Reinfection (16 days later) of mice surviving from Experiment II.

Mice (20 gms.)	Col. SK Infection	Number of Mice	Cumulative Mortality Days							Mortality Rate	
			1-3	4	5	6	7-9	10	11-14		
RDE-treated survivors	10^{-4} i.p.	12	0	2	4	4	4	6	6	6/12	50%
Controls..........	10^{-4} i.p.	5	0	4	5					5/5	100%

of these effects is illustrated in Table 19 which gives the results obtained by these investigators. The fact that surviving mice, unlike those treated with a true chemotherapeutic substance, are partly refractory to reinfection is evidence that the virus was not destroyed but that the animals, in the absence of functioning cell receptors, were free to develop neutralizing antibodies and immunity during the asymptomatic interval of the infection. Similar receptor-destroying enzymes elaborated by CL. WELCHII, which inhibit hemagglutination by influenza virus

(BURNET, McCREA and STONE 1946), have not yet been examined for their ability to prevent infection with Col SK virus. But the administration of rattle snake venom to mice, prior to infection with Col SK virus, has in our experience produced some definite protective effects; similar protection against poliomyelitic infection has been described by LÉPINE (1948) and by SANDERS et al. (1953). It would seem that some combination between enzymatic substances of this type, which prevent virus anchorage on the cellular surface, and some other drug or analogue, which inhibits directly or indirectly actual multiplication of the virus, may offer the best chances for a successful chemotherapy of viral infection.

From what has been said it is clear that the Col SK group of viruses provide a practical model for the study of chemotherapeutic problems in virus diseases, especially those that are closely related to poliomyelitic infection. To summarize, the several experimental approaches consist of: 1) use of certain dyes, especially those belonging to the triphenylmethane series which have been shown to fix tobacco mosaic virus (KAUSCHE 1950) and which inhibit multiplication of the influenza group (HOYLE 1949; FLEISHER 1949) and of foot and mouth disease virus (CIACCIO et al. 1954), possibly by affecting the metabolism of ribonucleic acid in the infected cell; 2) use of certain substances, such as sodium fluoroacetate, which inhibits influenza virus and suppresses Lansing and Mahoney virus, probably by interrupting the Krebs cycle so as to interfere with the oxidation and utilization of citric acid, thus leading to a depletion of certain amino acids which are diverted to citrate formation; 3) studies on the effect of amino acid imbalance, produced by feeding excess methionine, which lowers the susceptibility of mice to Lansing infection; 4) use of certain pyrimidines which may slow up enzymatic processes necessary for virus synthesis; 5) use of polysaccharides or mucoproteins, which combine with virus-like cell receptors, or of synthetic organic polymers which are capable of adsorbing free virus in blood and possibly also in faeces; 6) use of receptor-destroying enzymes which may block temporarily the attachment of virus on the susceptible cell; 7) use of chemicals or antibiotics which counteract virus multiplication in an as yet unknown manner. Since viruses of the Col SK group grow well on certain tissue substrates *in vitro*, the experimental possibilities are further expanded by employing tissue culture techniques which permit a more precise determination of viral growth curves under the influence of any of the various substances mentioned above than can be obtained by infectivity tests in animals (see section on Cultivation).

Advancement in the chemotherapy of viral diseases would be considerably aided if the precise biochemical changes were known that occur in infected tissues through the derangement of cellular metabolism. Unfortunately, there is but little information on this problem. FISHGOLD (1951) reported that the capacity of normal mouse brain to reduce triphenyltetrazolium chloride is much impaired in brains harvested from Col SK virus-infected mice when tested in pyruvate and glutamate substrates, but not in succinate; this did not occur with ectromelia virus-infected mouse brains. Observations on changes in enzyme activities by infection were reported by BAUER (1953) who found that cholinesterase levels of MM virus-infected mouse brains were significantly below the normal value; the same was true for infection with neurotropic yellow fever, neurovaccinia and FA THEILER viruses (LITTLE and LAWSON 1951), but not for LCM or Lansing polio viruses. BAUER also found the Mg content of mouse brains depressed below normal values as the result of infection with neurotropic yellow fever virus or MM virus and suggests that this Mg deficiency may explain the nervous hyperactivity characteristic of this type of encephalitis. QUIGLEY (1954) who studied the activity of pyruvic and xanthine oxidase in the brains of mice infected with different neurotropic viruses found an increase in both enzymatic activities for MM virus whereas Coxsackie virus-infected brains showed an increase of pyruvic oxidase only and GDVII virus-infected brains had less xanthine oxidase than com-

parable normal tissue. Studies by GOLLAN and VISSCHER (1951) on the water and electrolyte level of brain tissue of mice infected with MM virus revealed a marked increase in the water and sodium content as compared with normal controls but the potassium content was not significantly changed. The results indicate that the paralytic infection produced an extracellular edema of the brain. Edema of the central nervous system is a well recognized feature of poliomyelitic infection and has been thought to contribute to the extent of neuronal damage whereas disturbance of the sodium-potassium balance may have a bearing on the degree of myocardial involvement (HALL and SHERMAN 1953). As one might expect, the appearance of paralysis in MM virus-infected mice is accompanied by a drop of K values in the skeletal muscle (GAEDEKE et al. 1954). Determinations by GOLLAN et al. (1948) of phosphorus-containing compounds of the whole brain of MM virus-infected mice did not reveal any changes in the inorganic, acid-soluble lipids, nucleic acid, "protein residue", or total phosphorus fractions, Nor did they show any significant differences from normal brain in the desoxyribosenucleic acid content whereas the content of ribosenucleic acid was reduced in infected brains. The effect of EMC virus infection on similar metabolic functions of Ehrlich ascites tumor cells was investigated by LEVY and SNELLBAKER (1956). In partial agreement with GOLLAN et al no change was found in the cellular concentration of acid-soluble P, phospholipid, RNA or DNA as a result of infection, nor in the purine and pyrimidine composition of RNA or DNA of the cell. However, by using radioactive tracers (P^{32}), an increase in the turn-over rate of several phosphorus-containing metabolites could be demonstrated.

Interference Phenomena.

The term interference is applied to the suppression of virus activity by a process which is not concerned with antibody function but is mediated through cellular mechanisms that either prevent fixation of the virus on the surface of the cell or interrupt the intracellular cycle of viral multiplication. Because little is known about the precise forces that come into play, i. e. whether such sparing effects are brought about by loss of extracellular virus receptors or by blockade of intracellular enzyme systems (or perhaps by direct interaction between active and inactive virus), the phenomenon was aptly named "viral interference"; the entire process is not unlike the antagonistic action between certain chemotherapeutic substances, first designated as "chemotherapeutic interference" by BROWNING and GULBRANSON in 1920. Interference may be observed in systems which combine a given living virus with the same virus in inactive or non-pathogenic form, or in combinations consisting of two different related, or even unrelated viruses. The process may, therefore, appear to be non-specific in one sense, but in another it must obviously depend upon some overlapping degree of basic biochemical specificity. In two-virus systems, one of the two agents is usually suppressed, whereas the other survives; if exclusion of either agents becomes possible through proper adjustment of viral quantity or of time relationships, one speaks of mutual or reciprocal interference. Interference can be demonstrated by mixing the two components *in vitro* as well as by their separate introduction *in vivo*. Many examples are found in the literature on auto-interference as well as of two-virus interference systems with plant viruses, bacterial viruses, and animal viruses (VIVELL 1951); the latter include several neurotropic viruses in addition to viruses of the influenza-mumps-Newcastle group. Interference phenomena with Col SK virus were reported by JUNGEBLUT and SANDERS at the time of discovery of the strain.

Auto-interference with Col SK virus was first noted in embryonic mouse brain tissue culture experiments in which transfer of infectious fluids from one generation to another succeeded better with high than with low dilutions. The

phenomenon was not further investigated but presumably was due to the fact that inactive virus which accumulated in low concentrations in the medium following the phase of logarithmic viral multiplication effectively inhibited growth of the highly potent active virus, unless the two components were separated by suitable dilution (see also HENLE and HENLE 1944). Auto-interference was again encountered by VIVELL (1951) in mice which had been subjected to double infection with MM virus. The sparing effect occurred only when the virus was re-introduced at the same portal of entry and depended definitely on critical limitations of virus dosage and time intervals between the two infection cycles

Table 20. *Auto-Interference with MM Virus*
(From O. VIVELL: Zeitschr. Kinderhlk. 70: 113, 1951)

Number of Mice	First Infection	Second Infection (24 Hrs. later)	Average Survival Time (Days)	Number of Surviving Animals %
10	10^{-2}	10^{-2}	4.4	0
17	10^{-3}	10^{-2}	5.4	0
21	10^{-4}	10^{-2}	6.1	28.5
20	10^{-5}	10^{-2}	6.7	30.0
6	10^{-6}	10^{-2}	4.2	0

(Table 20). Auto-interference is probably also responsible for the rhythmic distribution of deaths in the mortality curve of MM virus-infected mice, as described by GAEDEKE and KANZLER (1953), and may explain comparable fluctuations in the mortality rates of mice infected with MEF poliomyelitis virus (STANLEY 1952) or with Coxsackie virus (HOWES 1952). The process may be related to LEVADITI's concept of "auto-sterilization". An analogous phenomenon, causing a reduction in virus titer by means of an inhibitor present in infected monkey cords, has previously been described for poliomyelitis virus by JUNGEBLUT in 1934. Similar virus inhibitors were found by GARD (1944) in crude extracts, prepared from the brains of mice infected with THEILER virus, and again by SANDERS (1950/1953) in mouse brains infected with EMC virus. Experiments by MELNICK (1952) also demonstrated the presence of a virus-binding accessory substance in the cords of mice infected with Lansing poliomyelitis virus and FRANCIS and CHU (1953) correlated the adsorption of poliomyelitis virus by grey matter of monkey or human brain with a specific tissue protein. None of these virus inhibitors have been obtained in any state of purity and it seems more likely that they represent inactive forms of the virus itself, which cause self-interference with active virus, than tissue-bound substances with antibody character, which inactivate the virus by neutralization.

In their first publication on Col SK virus, JUNGEBLUT and SANDERS (1940) reported the existence of a powerful antagonism between this virus and several strains of poliomyelitis virus as evidenced by the fact that Col SK mouse virus, when introduced simultaneously or at short intervals before or after infection with Y-SK or Aycock simian virus, prevented the occurrence of paralysis in the injected monkeys; similar protection could be obtained with tissue culture preparations of Col SK virus. It was also determined in subsequent experiments (JUNGEBLUT and SANDERS 1942; JUNGEBLUT 1945, 1948) that heated Col SK virus lost its interfering ability whereas ultraviolet-irradiated preparations had retained some of this power. Attempts to separate by physical or chemical processes from live Col SK virus a non-pathogenic principle capable of inter-

Table 21. *Interference between Col SK or MM Virus and Poliomyelitis Type II Virus in Rhesus Monkeys*

(C. W. JUNGEBLUT and M. SANDERS: J. Exp. Med. 76 : 127, 1942; C. W. JUNGEBLUT: J. Exp. Med. 81 : 275, 1945)

Prophylactic Experiments

Parapoliomyelitis Virus	Mode of Prophylaxis	Infection with Poliomyelitis Type II Virus	Number of Monkeys	Result	
				Paralysis	No Paralysis
Col. SK	3-5 Injections before Infection (In some cases also after Infection)	RMV or Aycock	26	13	13
Controls	—	RMV or Aycock	19	19	0
MM	3 Injections before*) Infection (In some cases also after Infection)	RMV or Aycock	7	5	2
Controls	—	RMV or Aycock	6	6	0

*) 2 Monkeys received Tissue Culture Preparations of MM Virus.

Therapeutic Experiments

Parapoliomyelitis Virus	Mode of Therapy	Infection with Poliomyelitis Type II Virus	Number of Monkeys	Result	
				Paralysis	No Paralysis
Col SK	No Interval or 3-5 days after Infection	SK (Simian)	23	3	20
Controls	—	SK (Simian)	11	9	2
Col SK	No Interval or 3-5 days after Infection	RMV	20	13	7
Controls	—	RMV	9	9	0
Col SK	No Interval or 3-5 days after Infection	Aycock	45	21	24
Controls	—	Aycock	30	30	0
MM	No Interval or 3-5*) days after Infection	Aycock or RMV	17	9	8
Controls	—	Aycock or RMV	5	5	0

*) 9 Monkeys received Tissue Culture Preparations of MM Virus.

fering with poliomyelitis virus were unsuccessful. Interference could be demonstrated irrespective of whether monkey and murine virus (Col SK or MM) were injected in form of *in vitro* prepared mixtures or whether the two viruses were introduced *in vivo* by separate channels of infection. With mouse virus injected intravenously, distinct protective effects were obtainable, in some instances up to 48 hours after intracerebral infection with poliomyelitis virus (Table 21). It became also clear that the protection resulted from domination of the more potent mouse virus over poliomyelitis virus, as shown by isolation of the former and not the latter virus from the protected rhesus monkey. The fact that interference is quantitative, in that definite proportions obtain between the opposing agents, is illustrated by the results of experiments in which mouse and monkey virus were combined *in vitro* in graded doses and the combinations were injected intracerebrally into rhesus monkeys (Table 22); it is also indicated by the necessity

Table 22. *Quantitative Interference between Col. SK Virus and Type II Poliomyelitis Virus in Mixture Tests.*

Poliomyelitis Virus (Aycock or RMV)	Col. SK Virus	Number of Monkeys	Result	
			Paralysis	No Paralysis
1:10	1:10	2	1	1
1:100	1:10	2	0	2
1:500	1:10	2	1	1
1:1000	1:10	2	0	2
1:5000	1:10	2	0	2
1:10 000	1:10	2	0	2
1:10	1:10	2	0	2
1:10	1:100	3	1	2
1:10	1:1000	3	2	1
1:10	1:10 000	3	3	0

Controls: All of 9 Monkeys injected with Poliomyelitis Virus 1:10—1:10 000 (alone, or in combination with heated Col. SK Virus, normal or herpetic mouse brain) developed paralysis.

for maintaining a proper time interval between injections when the two viruses are given separately. The phenomenon was specific, in so far as it was examined, because only very slight interference against Type II poliomyelitis virus was obtained when THEILER virus-infected mouse brain was substituted for Col SK virus; herpetic mouse brain, or normal mouse brain gave entirely negative results. Similarly, LÉPINE and ATANASIU (1950) found no interference between Lansing poliomyelitis virus and Teschen disease virus.

New information on interference between viruses of the Col SK group and the several immunological types of poliomyelitis virus became available in 1954 through VERLINDE and MOLRON's studies of the murine AK strain. These authors had no difficulty in demonstrating solid protection of rhesus monkeys against intracerebral infection with two Type II strains of poliomyelitis virus, i. e. the simian AK strain and the Aycock strain, by injecting either Col SK virus or the murine AK strain as late as 3-5 days after infection with poliomyelitis virus. However, no such interference could be obtained against Type I (Mahoney) or Type III (Leon) poliomyelitis virus. Recent experiments of JUNGEBLUT and BAUTISTA (1956) in spider monkeys, which are naturally resistant to Type II and Type III poliomyelitis virus but are susceptible to Type I poliomyelitis

virus and to Col SK virus, showed no suppression of Col SK virus by either Type II or Type III poliomyelitis virus; effective interference, however, could be demonstrated in rhesus monkeys between Col SK and Type I poliomyelitis virus (Brunhilde) but not between Col SK virus and Type III poliomyelitis virus (Leon). It remains for further systematic studies, possibly in suitable tissue culture media (see section on Cultivation), to resolve the conflicting data with respect to the interference system between Col SK virus and Type I poliomyelitis virus. The problem is of particular interest in view of the fact that freshly isolated Type II strains of poliomyelitis virus, as a group, appear to differ from the other two serological type strains by being more easily adaptable to rodents (SILVERBERG, HABEL and SHELOKOV 1955).

Reverse interference in rodents cannot be easily demonstrated in the mouse which is much more susceptible to Col SK virus than to any of the rodent-adapted strains of poliomyelitis virus (see also VERLINDE). Yet, when mice are injected with balanced mixtures of Col SK (10^{-6}) and Y-SK (10^{-1}) viruses, which had previously been held in the icebox for 24 hours, the animals may survive with no symptoms and neither virus is recoverable from the mice, suggesting that a point of total extinction of the two viral agents had been reached (JUNGEBLUT). The fact that, under certain conditions, two potent viruses may annihilate each other seems to us of considerable theoretical and practical importance. By choosing a less susceptible rodent host, interference between poliomyelitis virus and MM virus, with suppression of MM virus, was readily achieved in the hamster by DALLDORF and WHITNEY (1943), and in the guinea pig by JUNGEBLUT (1948). In both instances, a considerable percentage of animals prepared with simian or human poliomyelitis virus resisted subsequent challenge with small doses of MM virus, the morbidity rate in the prepared groups being approximately one fourth to one fifth that observed in the control groups. RHODES and CHAPMAN (1944), who repeated the interference experiments in hamsters with MM virus and poliomyelitis virus, failed to observe any suppression of MM virus; the discrepant results are probably due to the larger dose of challenge virus used by these authors. That cross protection between Col SK and MEF virus can be obtained in the hamster under carefully controlled conditions was again shown in recent experiments by BAUTISTA, JUNGEBLUT and KODZA 1957 (see Table 23).

Table 23. *Cross Protection in Hamsters between Col. SK and MEF Virus Infection.* (BAUTISTA, JUNGEBLUT and KODZA: J. Inf. Dis. 1957.)

First Infection				Interval	Second Infection			
No of animals	Virus	Dose	Result		No of animals	Virus	Dose	Result
34	Col. SK	10^{-4}—10^{-6} ip	$^{26}/_{34}$	3 weeks	24		10^{-2} ic	$^{7}/_{24}$
					Controls:10	MEF	10^{-2} ic	$^{10}/_{10}$
30	Col. SK	10^{-4} 10^{-5} ip	$^{24}/_{30}$	3 weeks	22	MEF	10^{-2} ic	$^{3}/_{22}$
30	Col. SK	10^{-6} 10^{-7} ip	$^{22}/_{30}$	3 weeks	15	MEF	10^{-2} ic	$^{6}/_{15}$
38	MEF	10^{-1} ip	$^{1}/_{38}$	3 weeks	38	MEF	10^{-2} ic	$^{6}/_{38}$
					Controls:39		10^{-2} ic	$^{25}/_{39}$
32	Col. SK	10^{-4} 10^{-5} ip	$^{28}/_{32}$	4 weeks	19	MEF	10^{-2} ic	$^{6}/_{19}$
24	Col. SK	10^{-6} 10^{-7} ip	$^{21}/_{24}$	4 weeks	2	MEF	10^{-2} ic	$^{2}/_{2}$
25	MEF	10^{-1} ip	$^{0}/_{25}$	4 weeks	23	MEF	10^{-2} ic	$^{6}/_{23}$
					Controls:20		10^{-2} ic	$^{19}/_{29}$

Numerator: number of hamsters paralyzed.
Denominator: number of hamsters injected.

The protective effects were unrelated with specific antibody action and occurred at time intervals at which no living residual virus was recoverable from the animal, suggesting that resistance was brought about by some metabolic cellular reaction or deficiency induced by the primary infection. These results point up the difficulty of clearly separating non-immunological cross protection from interference, in the strict sense of the word, which presumably depends on the actual presence of the interfering virus in the tissues at the time of reinfection. It also remains unexplained why these experiments furnished no evidence for the occurrence of increased virulence, or "virus exaltation", which FINDLAY and HOWARD (1948) had previously described with mixed infections in mice with Col SK and MEF virus. The chances of using successfully the reverse interference system for diagnostic purposes in the detection of poliomyelitis virus in faeces, blood, et. seem remote, partly because of the comparatively low concentration of the virus in pathological human material, and partly because the limits of specificity of the reaction are not known. Attempts to demonstrate reverse interference between poliomyelitis virus and Col SK virus in embryonated eggs led to inconclusive results in our experience.

The occurrence of interference between the viruses of the Col SK group and clearly unrelated viruses has engaged the attention of several authors. Interference effects between EMC virus and Theiler GD VII virus, described by SANDERS (1953), are open to question because survival of either virus was determined by hemagglutination titration with human 0 cells and sheep erythrocytes which do not satisfactorily differentiate between the two viral agents (see section on hemagglutination). On the other hand, DALLDORF and WHITNEY (1943) and RHODES and CHAPMAN (1949) found that interference occurs when hamsters are inoculated ic with lymphocytic choriomeningitis virus and several days later ip with MM virus. A significant number of such animals failed to develop paralysis due to MM virus infection, but not when the sequence was reversed; occasionally, the animals also remained free from symptoms of LCM virus infection. Further work by RHODES and CHAPMAN (1950) showed that the survivors of such interference experiments had in their tissues (neural and extraneural) the same amount of LCM virus as controls inoculated with this virus alone whereas the amount of MM virus was significantly less than that recoverable from controls inoculated with MM virus alone. In other words, previous involvement of the cells with LCM virus prevented MM virus from multiplying in the same cells. These results are analogous to early observations by DALLDORF (1939) who discovered that LCM virus infection in monkeys exercised a sparing effect on subsequent infection with poliomyelitis virus (RMV strain) and that this sparing effect was associated with a suppression of poliomyelitis virus in the CNS of the monkey. Similar interference between Coxsackie and poliomyelitis viruses was later described by the same author (DALLDORF 1951). Another example of this type of interference is the effect of ectromelia infection in mice on subsequent infection with Col SK virus, as reported by SCHULZ-EHLBECK (1954); the author observed a milder course of Col SK virus infection in such mice and noted that the protective effect coincided with the time of maximum spread of the mouse pox virus. In both instances we are dealing with systemic infections by viruses with viscerotropic and neurotropic properties which inhibit the development of an infection with a pantropic viral agent and it would be interesting to know what the common denominator is of such interference.

A marked antagonism or sparing effect of influenza, mumps and vaccinia viruses [but not of poliomyelitis (Lansing), GD VII Theiler, MM, or herpes viruses] against pertussis infection in mice was described by COHEN (1953). The phenomenon resembles interference between viral agents and the virus-bacterium-host system opens up new possibilities for studying the mechanisms of cellular response to infectious agents.

In conclusion, the most striking manifestation of interference by Col SK virus is its ability to induce a high-grade protection in rhesus monkeys against anteceding simultaneous, or subsequent infection with poliomyelitis virus; the reverse

phenomenon in rodents, while well enough established, is perhaps of lesser immediate importance. There is evidence that this interference is not due to a metabolic viral product but depends upon some direct interaction between the living virus particle and the susceptible cell, rendering the latter temporarily refractory to infection with poliomyelitis virus which presumably attacks the same cell territories. One can only speculate on the nature of this cell change but the protection, as a rule, is only transient since monkeys surviving Col SK-poliomyelitis virus interference without paralytic symptoms remain fully susceptible to poliomyelitic reinfection. The sparing effect of previous infection with unrelated viruses on subsequent infection with Col SK virus may depend on basically similar mechanisms, even though the specific forces that come into play are unknown. All observations are in agreement that the protective effects originate under conditions which preclude the intervention of conventional processes of immunity and if there is any specificity, it would have to be on the level of virus-cell reactivity. While it is generally believed that exclusion of one virus through interference with another is mediated by pre-occupation of cell receptors or blocking of intracellular multiplication sites, we have also pointed to evidence which suggests a possibility for some direct interaction between Col SK and poliomyelitis virus upon prolonged *in vitro* contact with each other, resulting in the loss of both agents. The dynamics of this process, which represents the opposite of recombination, are currently not well understood and deserve further inquiry. Practical applications of interference principles to the prevention or therapy of the human disease are, at present at least, not in sight.

Immunology.

The several strains of the Col SK group form one compact immunological family because of the similarity of their antigenic properties, as determined by neutralization, hemagglutination-inhibition, or challenge resistance. The same immune reactions separate these viruses sharply from all other neurotropic viruses, especially the THEILER group and the poliomyelitis group [WARREN, SMADEL and DICK (1949); DICK, (1949)]. While the various strains of the Col SK group are practically indistinguishable by immunological tests, biological differences between the individual viruses are sufficiently marked to ensure the authenticity of the isolates. These differences are principally concerned with the degree of pathogenicity for certain Laboratory animals (rhesus monkeys, cynomolgus monkeys, guinea pigs, albino rats), the ability to multiply in the embryonated hen's egg, and adaptability to cytopathogenic growth in various tissue culture media.

Overlapping serological reactivity between Col SK-MM virus and Type II poliomyelitis virus, described in earlier publications (JUNGEBLUT 1944; DALLDORF and WHITNEY 1945), as occurring in unilateral direction only, has not been confirmed by subsequent investigation. In fact, recent work by VERLINDE and his associates (1953/54) suggests that when a virus with Col SK properties is neutralized by Lansing serum the virus represents a mixture of Col SK and poliomyelitis Type II virus or else an agent of complex nature (recombinant ?) possessing pathogenic characteristics and antigenic components of both viruses. It is also now recognized that whatever cross protection may be obtained *in vivo* between Col SK and poliomyelitis virus is probably due to non-specific tissue immunity or interference phenomena. Yet, it is difficult to explain satisfactorily the divergence between earlier results and later observations. For Type II poliomyelitis antisera (Y-SK, Aycock) prepared in monkeys or in rabbits

Table 24. *Neutralization of Col SK Virus and of Y-SK Virus by Type II Anti-Poliomyelitis Sera.*

(C. W. JUNGEBLUT, Unpublished Data 1947—1948.)

Immunizing Virus	Immune Sera (Undiluted)	Col SK Virus Dilutions (ip Test) 10^{-3} T	10^{-3} C	10^{-5} T	10^{-5} C	10^{-6} T	10^{-6} C	10^{-7} T	10^{-7} C	10^{-8} T	10^{-8} C	Y-SK (IC Test) 10^{-1} T	10^{-1} C
Y-SK *) (Type II)	3 Guinea pigs ***) (pooled)					6/6	9/6	6/6	6/6	5/6	5/6	0/7	13/16
	3 Rabbits (pooled) Bleeding: 1	3/3	3/3	2/3	3/3	2/3	3/3	0/3	3/3	0/3	6/6	6/8	8/8
	2	3/3	3/3	3/3	3/3	3/3	3/3	2/3	3/3	0/3	6/6	5/8	8/8
	3	0/18	9/9	0/18	9/9	0/18	9/9	0/18	9/9			12/26	26/32
	Rhesus Monkey (AR 3) Bleeding: 1	2/3	3/3	2/3	3/3	2/3	3/3	0/3	3/3			7/8	8/8
	2	3/3	3/3	3/3	3/3	1/3	3/3	0/3	3/3	0/3	3/3	0/8	13/16
	3	3/3	3/3	3/3	3/3	1/7	6/6	0/7	6/6	0/7	6/6	1/8	13/16
	4	2/6	6/6	2/6	6/6	0/6	6/6	0/6	6/6			1/8	13/16
	Rhesus Monkey (AR 8) Bleeding: 1	3/3	3/3	5/6	6/6	3/3	3/3	6/9	6/6	1/6	6/6	5/6	8/8
	2	3/4	3/3	4/6	6/6	0/6	6/6	0/6	6/6	0/6	6/6	1/8	13/16
	3	0/6	3/3	0/6	6/6	0/6	6/6	0/6	6/6	0/6	6/6	0/8	13/16
Aycock (Type II)	Rhesus Monkey (AQ 34) Bleeding: 1	3/3	3/3	3/3	3/3	3/3	3/3						
	2	3/3	3/3	2/3	3/3	0/3	3/3						
	3	0/3	3/3	0/3	3/3	0/3	3/3					1/7	8/11
	4	1/3	3/3	0/3	3/3	0/3	3/3					0/8	8/8
Brunhilde (Type I)	5 Rhesus Monkeys (pooled) Bleeding: 3	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	5/6	7/8	7/7
	4	6/6	6/6	6/6	6/6	6/6	6/6	6/6	6/6	5/6	6/6	8/8	8/8
Y-SK **) (Type II)	2 Guinea Pigs (pooled) Bleeding: 1					3/3	3/3	3/3	3/3	2/3	4/4	0/8	7/8
	2					4/4	4/4	2/4	4/4	0/4	4/4	0/8	7/8
	3					4/4	4/4	3/4	4/4	0/4	3/4	0/8	8/8
	3 Rabbits (pooled) Bleeding: 1					11/12	12/12	8/12	12/12	0/12	12/12	7/8	6/8
	2					8/12	12/12	1/12	12/12	0/12	12/12	6/8	6/8
	3					8/12	11/12	1/12	10/12	2/12	6/12	6/8	7/8
	2 Rhesus Monkeys (pooled) Bleeding: 1			8/8	8/8			5/8	8/8	1/8	8/8	0/16	14/16
	2			8/8	8/8	7/8	8/8	2/8	8/8	0/8	8/8		
	3			7/8	8/8	6/8	8/8	0/8	8/8	0/8	8/8		
	4			8/8	8/8	5/8	8/8	3/8	8/8	0/8	8/8		
Y-SK (Type II)	Chimpanzee 3062 ****)	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3				
	Rhesus Monkey 3/25 ****)					4/4	4/4	4/4	4/4	3/4	4/4	1/8	7/8
	Rhesus Monkey 45 5/6 ****)			4/4	4/4	0/4	4/4	0/4	4/4	0/4	2/4	1/8	5/8
	Cynomolgus Monkey 3627 ****)			4/4	4/4	3/4	4/4	1/3	4/4	1/1	4/4	0/8	7/8

Legend: T = Test (Immune Serum.)
C = Control (Normal Serum).
Numerator = Number of Mice Paralyzed.
Denominator = Number of Mice Injected.

*) The Y-Sk Virus used for these Injections was propagated at Columbia University Laboratory.
**) The Y-SK Virus used for these Injections was prepared at Yale University Laboratory.
***) These Guinea Pigs were injected i. c. with 0,1 cc of 10% Y-SK Mouse Virus and the symptomless animals were bled 2 weeks later.
****) Sera prepared at Yale University. All other sera were prepared at Columbia University.

in this Laboratory showed neutralizing effects on Col SK virus not only in our hands but also when tested elsewhere (MELNICK 1950; DICK 1949); conversely, Type II poliomyelitis antisera, prepared in other Laboratories, failed to neutralize Col SK virus under comparable conditions. Since the non-neutralizing poliomyelitis antisera had lower homologous antibody titers, a possibility exists that such cross reactions occurred under special conditions which prevailed at that time, namely when Type II poliomyelitis antisera of unusual potency were tested against early mouse passages of Col SK virus. The point in question is illustrated by the original protocol shown in Table 24. Be this as it may, there is presently no longer any disagreement that antisera against the three serological prototypes of poliomyelitis virus, sufficiently potent to neutralize completely 10^5 or 10^6 infectious doses of the homologous viruses in tissue culture medium, have no neutralizing effect on viruses of the Col SK group, irrespective of whether such tests are run in rodents or in monkeys with the intracerebral or intraperitoneal method of testing, or are performed in tissue culture.

Further uncertainty arose from the description of so-called "high" strains of Y-SK, Lansing and MEF virus, or even THEILER virus, which seemed to originate as variants from the "low" parent strains during rapid serial passage in adult or suckling mice, or in cotton rats (see JUNGEBLUT 1951, p. 19). Strains thus produced possessed greatly increased powers of invasiveness from peripheral portals of entry, approaching the pathogenicity of Col SK virus. When examined serologically, it appeared that these "high" strains, in some instances, had preserved their antigenic integrity whereas at other times they seemed to undergo certain changes in their antigenic structure, placing them again close to Col SK virus. Thus, with the high-low MEF combinations antigenic identity could be established since antisera against the two viruses gave complete neutralisation in homologous and heterologous cross tests. Neither high nor low MEF virus showed any overlapping reactivity with Col SK or MM virus, but both MEF strains crossed with low Lansing virus. In the case of the high-low Lansing strains, neutralization occurred between low Lansing antiserum and high Lansing virus, but not in the reverse direction. High Lansing antiserum, however, completely neutralized high MEF virus and, in some instances, also Col SK virus (ENRIGHT and SCHULTZ 1947; JUNGEBLUT 1949). Finally, with the high strain of THEILERS GD VII virus, the serological evidence indicated again that the invasive strain was identical with the non-invasive strain and no crossing was observed with Col SK virus (JUNGEBLUT 1944). Observations referring to changes in pathogenicity with the MEF strain were subsequently confirmed by SELZER, SACKS and VAN DEN ENDE (1952) and by CASALS and OLITSKY (1951) as the result of passing the low virus through suckling mice or through suckling hamsters (MOYER et al. 1952; POWELL and CULBERTSON 1953). However, since the antigenic alterations described by the earlier workers remain unconfirmed, the possibility cannot be ruled out that they were brought about by inadvertent contamination with Col SK virus, even though the high Lansing and MEF strains were found by HALLAUER to possess no hemagglutinating properties. Because of the implied seriousness of the problem, the chances for laboratory contamination to occur were examined systematically by no less than three different authors (SCHULTZ and WHITE 1948; VERLINDE et al. 1952; VANELLA et al. 1956) under varying conditions of contact among infected and non-infected animals. The concensus is that natural virus transmission is possible through contamination with infected faeces or nasopharyngeal secretions, but it also becomes clear that when infected animals are kept in different cages, or are housed in different rooms, these chances, for all practical purposes, are reduced to a negligible minimum.

Of various serological methods the neutralization test and hemagglutination test are widely used for identification of the Col SK group of viruses, whereas complement fixation reactions, with the exception of some work reported by WARREN et al. (1949) and by GIFFORD and DALLDORF (1954) have been virtually

neglected; according to the latter authors, neutralizing, hemagglutination-inhibitory and complement-fixing antibodies persisted in rhesus antiserum against MM virus for over two years in good titer. The virus is a very powerful antigen and potent antisera can easily be produced by immunization of mice, guinea pigs, cotton rats, hamsters, albino rats, monkeys and rabbits with live or killed virus. ALLEN (1954), who reported successful immunization of mice carrying in their peritoneal cavity collodion sacs filled with Col SK virus, believes that the immunizing effects were due to a soluble antigen. The addition of bio-flavonoids, especially hesperidin, is said to further improve the antigenic capacity of Col SK virus vaccine (MOSS et al. 1956). Even when high-titered sera are used, neutralization by the intracerebral method is limited to one or two logs of virus at best but inactivation of 9 or 10 logs of virus by the same sera can be demonstrated by intraperitoneal injection of the virus-serum mixtures. Apparently, the union between virus and antibody is not very firm and dissociation takes place readily, as is also shown by the results of diluting a neutral virus-serum mixture beyond the range of antibody activity, but within the limits of virus potency. For this reason, the intraperitoneal method should be employed routinely in all tests aiming to discover relatively small amounts of antibody such as may be present in human or animal convalescent sera. The neutralization test may be run in two ways, i. e. by combining a fixed dose of virus, usually 100, 500, or 1000 m. f. d., with varying serum dilutions, or by setting up mixtures of a fixed dose of serum, usually a 1 : 5 dilution, with graded doses of virus. Both methods give comparable results and, when the test includes a virus titration to the end point of infectivity, permit calculation of antibody content in terms of the LD_{50} neutralization index. Results obtained with the hemagglutination-inhibition reaction are generally in fair agreement with the neutralization reaction, as indicated by DALLDORF's (1952) and JUNGEBLUT and BAUTISTA's (1954) comparative tests with Mexican sera (see Table 25). Quantitative serological

Table 25. *Comparison of Neutralization and Hemagglutination-Inhibition by Mexican Sera.*
I. Immune reactions against Col SK virus with Mexican sera.
(C. W. JUNGEBLUT and G. BAUTISTA: Am. J. Trop. Med. and Hyg. 3:466, 1954.)

Persons Tested	Urban							Rural						
	No.	Positive		Quest.		Negative		No.	Positive		Quest.		Negative	
		N	HI	N	HI	N	HI		N	HI	N	HI	N	HI
Polio. cases	13	0	0	0	1	13	12	5	4	3	1	1	0	1
Contacts	13	3	3	1	0	9	10	11	3	4	0	1	8	6
"Normal"	28	0	0	3	0	25	28	61	16		3		42	

N = Neutralization reaction. HI = Hemagglutination-Inhibition reaction.

II. Immune Reactions Against Col-SK Virus with Mexican Sera.
(G. DALLDORF: 1952: Quoted by KELLER and VIVELL, 1954.)

Hemagglutination Inhibition	Number of Sera	Neutralization	
		Positive	Negative
Positive	45	25	20
Negative	52	14	38

Table 26. *Protective Effect of Human Gamma Globulin against Col SK Virus Infection in Mice.*

Col. SK Virus 0.1 cc ip (3 Hours after Gamma Globulin or NMS)	Gamma Globulin (Undiluted) 0.5 cc ip											Control: Normal Monkey Serum 0.5 cc ip										
	Days after Infection*)										Total**)	Days after Infection*)										Total**)
	1	2	3	4	5	6	7	8	9	10		1	2	3	4	5	6	7	8	9	10	
10^{-4} (18 Mice)	0	0	0	6	11	12	12	12	14	14	14/18	0	0	0	9	16	16	18	18	18	18	18/18
10^{-6} (18 Mice)	0	0	0	3	3	7	7	7	7	7	7/18	0	0	0	7	14	16	17	17	17	17	17/18
10^{-8} (8 Mice)	0	0	0	1	1	2	2	2	2	4	4/8	0	0	0	4	6	6	7	7	7	7	7/8

*) Cumulative Mortality
**) Numerator = Paralyzed Mice
Denominator = Number of Mice Injected

tests carried out with the different strains of the Col SK virus group show extensive cross reactions, but homologous reactivity is sometimes slightly more pronounced than heterologous crossing, suggesting the presence of major and minor antigens in the virus complex. Protection of vaccinated animals, showing high antibody levels in the serum, against peripheral reinfection with living virus is possible but intracerebral challenge resistance can usually be demonstrated only in convalescent animals. Passive transfer of immunity from vaccinated Col SK virus mice to their young was described by OERSKOV and ANDERSEN (1948). Working with MMvirus ANDERSON and BOLIN (1946) as well as CURLEY and GORDON (1948) demonstrated the brief duration of such immunity in the young and proved that resistance was associated with the immunity of the mother which suckles the newborn mice (GORDON and CURLEY 1949). Transfer of antibody by way of ingested milk was postulated as the mechanism responsible for the passive protection. Infection and immunity in offspring of mice infected with Col SK virus was studied in detail by KNOX (1950) who found that litters of immune mothers were fully protected against intranasal infection between weaning and the age of 44 days whereas after 119 days the immunity had been lost. Furthermore, as shown by KNOX, the milk from immune mothers was capable of neutralizing the virus *in vitro* while normal mouse milk contained no antiviral substances. There was no evidence in this work to suggest that the offspring of infected mothers may acquire a state of active immunity through inapparent infection.

The presence of specific antibodies against the Col SK group of viruses in human or animal sera is discussed in more detail elsewhere (sections on epidemiology and clinical observations). Suffice it to say here that pooled human gamma globulin contains considerable quantities of neutralizing antibodies against Col SK virus as shown by BIELING in Germany (1956), by VERLINDE in Holland (1953) and by us in the United States. In our experience,

human gamma globulin (Lederle sample with no preservative) neutralized, undiluted and in dilutions of 1 : 5, about 2 logs of Col SK virus (ip test). When used in a protective test in mice, given 3 hours before infection, it protected about 50% of the animals against at least 100 LD_{50} of virus (Table 26).

Viral diseases, with notable exceptions (vaccinia, lymphogranuloma), are usually not accompanied by a state of hypersensitivity which can be measured by local or systemic allergic tests. It is therefore not surprising that previous contact with Col SK virus failed to induce in guinea pigs sensitization demonstrable by the classic anaphylactic test upon reintroduction of the homologous virus. Whenever hypersensitivity did occur, it was due not to the virus but to the anaphylactogenic action of animal tissues (JUNGEBLUT 1948). It is of interest, though, that viral infection apparently had not altered the immunological specificity of the tissues on which the virus had been propagated.

Non-specific serological changes, arising as the result of human infection with poliomyelitis virus, Coxsackie virus and possibly Col SK virus, or through immunization of experimental animals with viruses of the Col SK group, have been observed by several authors. Thus, abnormally high levels of sheep hemagglutinins in the sera from patients with the above clinical diagnoses were first noticed by JUNGEBLUT and HORVATH (1951) as well as by VIVELL, SCHMITT and GERSTNER (1952); the groups studied included no cases of infectious mononucleosis. Extensive studies by DEIBEL (1953) confirmed these preliminary observations by reporting 28% positive patients sera against 7.7% positive control sera; it was further determined that these sheep hemagglutinins were of the Forssman type and that the presence of hemagglutinating antibodies was not correlated with the titer of specific antiviral antibodies. Subsequent work by us on the same subject is in complete agreement with DEIBEL's data and showed furthermore that heterophile hemagglutinins are frequently encountered in the sera of monkeys following infection with Col SK virus. There are no grounds for speculating that these hemagglutinins may be a non-infectious component of the virus itself and the reason for their formation, early during infection, is obscure unless one were to assume the existence of an overlapping heterophile antigen in the virus and in the red cell. Increased titers of cold hemagglutinins against human 0 cells were described by SIEGEL (1951) as occurring in the sera of rabbits after immunization with Col SK, EMC or MM virus, but not with Mengo virus. The differences were impressive enough to suggest to SIEGEL a different antigenic make-up for Mengo virus.

Clinical Observations (Including Serological Tests with Human Sera).

Little definite information is available on the ability of the Col SK group of viruses to produce disease in man although subclinical infection, with antibody formation, seems to occur not infrequently, especially in some semitropical and tropical countries (Mexico, Surinam, Africa). The isolations of these viruses from human sources that have been reported from time to time have often occurred under circumstances which permitted no concomitant antibody studies with the patients' serum; this includes three fatal cases, i. e. MM, Faitz and Ortlieb. In other instances, these viruses were apparently recovered in combination with poliomyelitis virus (SK, ES) so that one cannot be certain of their rôle as true etiological agents of the disease process. There remain a small group of cases which yielded only Col SK virus on isolation and in which specific virucidal or hemagglutination-inhibitory antibodies, occasionally in rising titer, could be demonstrated in the patients' serum. These are the four isolations reported by KOCH and BIELING, two by VERLINDE and VAN TONGEREN, and one by

DICK et al. The group of four cases reported by the German authors showed the clinical picture of a meningo-encephalomyelitis, characterized by a brief febrile period of meningeal symptoms with nuchal rigidity and lymphocytic pleocytosis in the spinal fluid; the clinical symptoms included also a transient bronchitis or pharyngitis. The two cases published by the Dutch authors were of the meningeal or encephalomyelitic type. Finally, the laboratory infection described by DICK et al. took the course of a polioencephalomyelitis with fever, headache, transient paresis of the trapezius muscle and involvement of the acoustic nerve. None of these cases presented the typical signs of an encephalitic process with hallucinations, convulsions, ataxia etc., but all resembled more the picture of aseptic meningitis or non-paralytic poliomyelitis.

In addition to the cases discussed above, studies are on record of human illnesses in which no virus was isolated from the patient but specific antibodies against the Col SK virus group, sometimes with rising or falling titer during convalescence, could de demonstrated in the serum. The best studied cases in this category are those reported by SMADEL and WARREN (1946) during an outbreak of so-called "Three Day Fever" in Manila among American soldiers; typical cases of poliomyelitis were not uncommon among Army personnel in this area at that time. The group includes a total of 44 patients of whom 17 had significant antibody levels against EMC virus; in three cases a considerable increase in EMC virus antibody titer was demonstrable during convalescence over a period of three weeks whereas antibodies against other viruses (Jap B encephalitis, lymphocytic choriomeningitis, mumps) were absent. Following this episode more or less extensive reports have come from other parts of the world (USA, Mexico, Germany, Holland, Sweden) on the occurrence of Col SK-MM virus antibodies in the serum of patients suffering from various neurotropic viral infections; in some instances the serum was also examined early and late during the disease. The results of these investigations are brought together in Table 27. The data show clearly that a small but definite percentage (10-20%) of positive serological reactions, demonstrable especially by neutralization but also by hemagglutination-inhibition, occurs in a group of patients with the diagnosis of aseptic meningitis, poliomyelitis, or encephalitis, whereas only sporadic positive reactions are noted with sera from patients ill with other diseases or with sera from healthy individuals, except during pregnancy. A more detailed analysis of the figures reported by KELLER and VIVELL (1954), with reference to diagnosis and degree of immune reaction (HI), is shown in Table 28. KELLER and VIVELL (1954) who conducted systematic serological studies for three years also noticed a seasonal increase in positive reactions of patients sera during the summer months (Fig. 19) and give

Fig. 19. Seasonal incidence of positive immune reactions (Ha-I) with patients' sera against MM virus. (W. KELLER and O. VIVELL: Erg. inn. Med. Kinderheilk. 5, 47, 1954.)

Table 27. *Neutralizing or HA-Inhibitory Antibodies Against Parapoliomyelitis Virus (COL SK, MM, EMC) in Sera from Patients with Poliomyelitis, Aseptic Meningitis or Encephalitis (also control sera from patients with other diseases or from healthy individuals).*

Author	Test	Poliomyelitis, Aseptic Meningitis, Encephalitis (Acute or sub-acute)		Other Diseases (No CNS Involvement)		Healthy Individuals		Locality
		No of Sera	% Positive	No of Sera	% Positive	No of Sera	% Positive	
Hallauer and Renz 1945	N	66	33.3*					Switzerland
Smadel and Warren 1947	N	44	38.6	1	100			Philippines
Warren, Smadel and Russ 1949	N	8	100			291	0	U. S. A.
Jungeblut 1950	N	127	21			71	16**)	Greater New York
		5	20					Saltillo, Mexico
Jungeblut and Horvath 1951	HI	57	16	12****)	0	80	2.5	Greater New York
Gard and Heller 1951	HI	384	12.5	39	0	146	0.7	Sweden
Keller and Vivell 1952	HI	242	12.4	270	3.7	133	4.5	Germany
Bieling and Koch 1952	N	?	33	?	2.3			Germany
Hofmann and Jungeblut 1952	N; HI	23	8.7; 17.3			30***)	16.7; 0	Germany
Jungeblut and Bautista 1954	N; HI	13	0			13***)	12	Puebla, Mexico (Urban)
		5	80; 60			11***)	30; 40	Gorospe, Mexico (Rural)
Van Tongeren and Verlinde 1954	HI	8	63					Holland
Tiggelman - v. Krugten and Collier 1955	HI	61	26.2			338	13	Suriname

*) Minimal Reactions.
**) Includes 2 Sera from Individuals with a recent history of abortive or paralytic Poliomyelitis.
***) All Sera were from Contacts with Poliomyelitis.
****) Jap. B. encephalitis.
N = Neutralization.
HI = Hemagglutination—Inhibition.

a comparison of the age distribution of MM virus antibodies with that of other viral antibodies. While the serological data furnish some evidence that Col SK viruses may play a role in the production of an as yet ill-defined syndrome of human illness resembling aseptic meningitis or non-paralytic poliomyelitis, no etiological relationship between these viruses and non-bacterial myocarditis in man has so far been established. Occasional clinical and pathological reports of fatal encephalitis in young children with associated myocarditis may be found

Table 28. *Hemagglutination-Inhibitory Antibodies Against MM Virus in Sera from Patients with Various CNS Infections, other Diseases, and Healthy Individuals.* (KELLER and VIVELL, 1954.)

Diagnosis	Number of Sera	Result		HA-I Titer (Serum Dilution inhibiting 4 HA Units)						
		Negative	Positive	1:5	1:10	1:20	1:40	1:80	1:100	1:320
Aseptic Meningitis	213	187	26 (12.2%)	5	7	6	4	4	0	0
Poliomyelitis	191	163	28 (14.7%)	4	6	8	3	4	2	1
Encephalitis	146	143	3 (2.1%)	0	1	1	1	0	0	0
Other CNS Diseases	141	110	4 (3.5%)	1	0	1	2	0	0	0
Other Diseases	224	217	7 (3.1%)	1	2	2	1	0	0	1
Healthy Individuals	72	72	0	0	0	0	0	0	0	0
Pregnant Women	64	58	6 (9.4%)	0	3	2	1	0	0	0

wherein the authors have suggested an implication of Col SK or EMC virus (BRENNING 1951; STOEBER 1947-1952; CHIARI 1952), but, since no virus studies were done, the suggestion remains unsupported. In fact, recent observations seem to indicate that outbreaks of "epidemic myocarditis of infants" are due, not to Col SK virus, but to Coxsackie Virus Type B (see section on pathology).

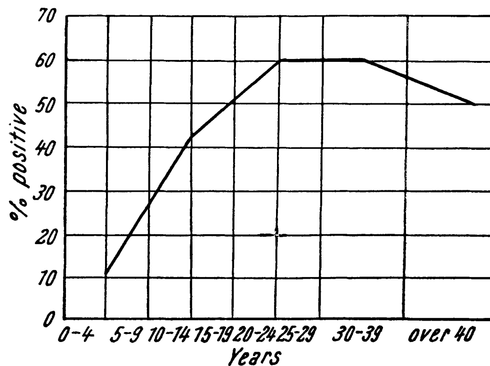


Fig. 20. Antibodies against Col SK virus in mexican sera. (G. DALLDORF: Quoted by KELLER and VIVELL, 1954.)

Serological tests with "normal" human sera indicate a very low immunity rate for Col SK virus in most of the populations studied and serve to point up the rarety of subclinical infection in man, except under conditions of contact with an active case (KOCH 1950; JUNGEBLUT 1950; VIVELL 1952; VERLINDE and VAN TONGEREN 1953). This is also shown by the extensive surveys made in Africa by DICK (1953), SMITHBURN (1952), MCNAMARA (1952), and PELLISSIER (1954); however, recent observations by BARSKI and LAMY (1955) indicate up to 50% positive neutralization reactions

(Mengo virus-T.C. technique) among the sera from healthy African pygmies native to the tropical forest (see Table 29). A different situation apparently exists in certain semitropical countries of Central and South America, i. e. in Mexico and in Surinam, where several authors encountered an unusually high incidence of positive immune reactions against Col SK virus among healthy individuals living in more or less isolated rural areas (JUNGEBLUT and BAUTISTA 1954; DALLDORF 1952; GAJDUSEK 1955; COLLIER 1955) (see Table 29). The age distri-

Table 29. *Neutralizing or Hemagglutination-Inhibitory Antibodies Against Col. SK Group of Viruses in Sera from Healthy Individuals Residing in Semi-Tropical Countries.*

Country	Author	Number of Sera tested	Result	
			Number of Positive Sera	Percent Positive
Africa:				
Mengo District....	SMITHBURN, 1952	28	3	10.7%
Tanganyika	SMITHBURN, 1952	296	0	0
Uganda/Mengo....	DICK, 1952/53	247*	4	1.6%
Nigeria	MCNAMARA, 1952/53	251	10	3.9%
Belgian Congo.... (Middle Congo, Oubangi-Chari, Tschad)	PELLISSIER, 1954	860	29	3.3%
Belgian Congo.... (Bemi-Bombossa) Pygmies	BARSKI and LAMY, 1955	112**	63	56.0%
Central America:				
Mexico........... (Gorospe Village)	DALLDORF, 1952	97	11	11.0%
Mexico				
Puebla City	JUNGEBLUT and BAUTISTA, 1954	28***	0	0
Gorospe Village.	JUNGEBLUT and BAUTISTA, 1954	61***	16	25.7%
Mexico........... Sierra Madre Occidentalis Native Indians	GAJDUSEK, 1955	56	3	5.5%
South America:				
Suriname	TIGGELMANN—v. KRUGTEN and COLLIER, 1955	338	46	13.0%

*) Includes 2 Sera from two Lab. Infections.
**) Neutralization of Mengo Virus by Tissue Culture Method.
***) Exclusive of Sera from Poliomyelitis Contacts.

bution of these antibodies in a group of Mexicans, as given by DALLDORF (Fig. 20), is different from that of the antibody curve against poliomyelitis virus in that it rises more slowly, suggesting that infection with the two viruses occurs under different circumstances; on the other hand, a comparison of the age distribution of Col SK virus immune reactions with that of positive Weil-Felix reactions in the same rural area of Mexico shows that the risk of exposure during childhood

and adolescence is somewhat similar for both, Col SK virus and the virus of *Rickettsia mooseri* (see Fig. 21). One would naturally expect that the primitive

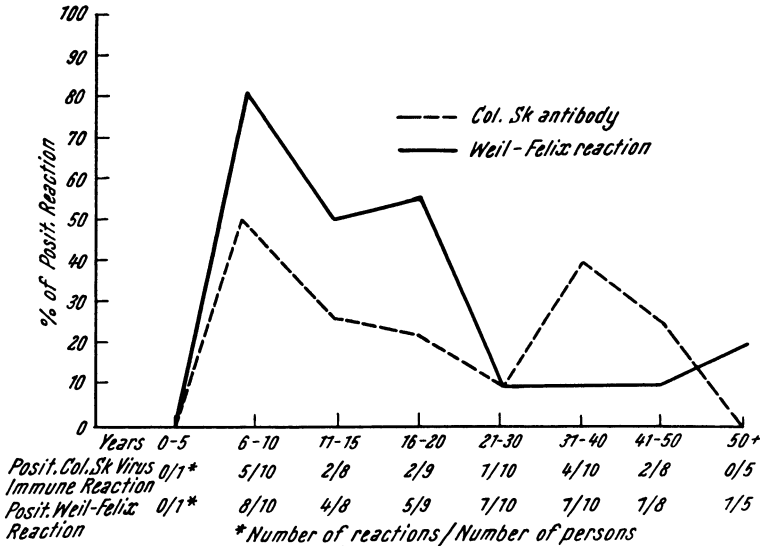


Fig. 21. Age distribution of positive Col SK virus immune reaction and of positive Weil-Felix reaction in sera from residents of Gorospe (Mexico). (C. W. JUNGEBLUT and G. BAUTISTA: Am. J. Trop. Med. and Hyg. 3, 466, 1954.)

hygienic conditions under which these populations live, would favor the rapid spread of a great many infectious diseases, with a correspondingly high and precocious development of specific immunity indices. Of special interest in this connection are the data reported by GAJDUSEK (1955) who carried out extensive serological tests with a sizeable group of Tarahumara Indians living in the Sierra Madre occidentalis of North-West Mexico. As will be seen from Table 30 the

Table 30. *Neutralizing Antibodies in the Sera of Tarahumara Indians Against 13 Neurotropic Viruses.*

Virus	Adolescents		Adults	
	Number	Number Positive	Number	Number Positive
Eastern Equine Encephalomyelitis	36	0	6	0
Western Equine Encephalomyelitis	50	0	6	0
St. Louis Encephalitis	38	0	6	0
California Encephalitis	50	0	6	0
Venezuelan Equine Encephalomyelitis	13	0	2	0
Colorodo Tick Fever	36	0	6	0
Dengue I	49	0	6	0
Yellow Fever	49	0	4	0
Lymphocytic Choriomeningitis	49	0	6	0
Encephalomyocarditis	50	2	6	1
Poliomyelitis Type I	41	32		
Poliomyelitis Type II	41	37		
Poliomyelitis Type III	41	26		

(C. GAJDUSEK: Pediatrics 16: 819, 1955.)

only positive reactions against a group of 13 neurotropic viruses examined occurred with poliomyelitis virus and with EMC virus; by contrast, antibodies against other common viruses, like mumps and herpes, were uniformly present in high percentages in this population. The exclusive occurrence, side by side, of antibodies against the Col SK viruses and the poliomyelitis viruses in the isolated, primitive population studied by GAJDUSEK seems to us not a matter of chance but rather an indication of some close biological relationship between the two viruses (see section on classification).

In conclusion, it may be said that direct evidence, based on virus isolations, is so far hardly sufficient to incriminate the Col SK virus group as important human-pathogenic agents. On the other side, one must recognize the fact that the presence of specific antibodies against this virus in the sera from patients with CNS viral infection, and their focal occurrence in the sera from healthy individuals in certain parts of the world, cannot be explained unless one assumes the existence of a distinct infectious disease entity which may present a clinical syndrome similar to that of poliomyelitis but which possesses dissimilar epidemiological features. That such a disease occurs in man, for which the name "parapoliomyelitis" has been tentatively proposed, is postulated by several European workers who have also stressed the rural and endemic character of the infection, suggesting a possible connection with an extrahuman virus reservoir in rodents (see section on epidemiology).

The situation is currently summarized by GAJDUSEK as follows: "These findings provide sufficient grounds to predict that cases of EMC virus infection in childhood may be found in this country (USA) if sufficiently sought for. The pediatrician must now consider this virus among the other possible etiological agents in children suffering from the syndrome of aseptic meningitis and it might be well to suspect this group of viruses in cases of GUILLAIN-BARRÉ syndrome of polyradiculitis and other paralytic and non-paralytic, non-bacterial CNS infections."

Epidemiology.

Since experimental infection of certain hosts with Col SK viruses may lead to viremia and fecal excretion of the virus, even in the absence of paralytic symptoms, multiple modes of transmission are open for the natural spread of the disease. Present evidence seems to exclude blood-sucking insects as vectors but conveyance of the virus by fecal contamination appears to be a possible and plausible route of infection. No data on the fate of Col SK virus in man are available which would either point to a prolonged intestinal carrier state, as in poliomyelitic infection, or which would provide any other clues for a likely mechanism of human transmission. Since the two viruses are rodent-pathogenic and are discharged with human faeces, the chances for their survival in wild rodents feeding on sewage seemed reasonable enough to look for an extrahuman virus reservoir in rats. The search for active virus in rats trapped in epidemic areas has been equally unproductive with Col SK virus as well as with poliomyelitis virus (JUNGEBLUT and DALLDORF 1946), although DE SOUSA (1954) recently reported the isolation from the faeces of sewer rats in Lisbon of a neurotropic, rodent-pathogenic virus with apparently new antigenic properties. On the other hand, specific neutralizing antibodies in the sera of wild rats have been found at least once for Lansing virus (BROWN and FRANCIS 1947) and may be demonstrated quite frequently for Col SK virus in certain localities. Thus, extensive studies by WARREN et al. (1949) with sera from 442 wild rats *(Rattus nor-*

vegicus, Rattus alexandrinus) trapped in various parts of the United States, showed that a very high percentage (30 to 87 %) of samples collected in the South contained EMC-MM-Mengo virus antibodies, whereas samples of similar size from Northern areas gave either completely negative returns or yielded a much lower incidence of positive sera[1]. No antibodies were found in the sera from various other wild animals caught in the same areas where the positive rat sera had originated (Table 31). The sharp dependence of immunity on local environment

Table 31. *I. Neutralizing Antibodies against EMC Virus in Sera of Wild Rats (U.S.A. and Canada).*

(J. WARREN et al. Proc. Soc. Exp. Biol. and Med. 71: 376, 1949.)

Locality	Number tested	Number Positive	% Positive
British Columbia	22	0	0
California	100	13	13
Florida	93	9	9.7
Georgia	57	6	10.5
Louisiana	9	0	0
Maryland	43	5	11.6
Michigan	11	0	0
Mississippi	49	43	87.0
New Mexico	6	0	0
North Carolina	2	0	0
South Carolina	21	0	0
Texas	23	7	30.5
Washington	6	0	0

Note: Practically all the wild Rats were *Rattus Norvegicus*; some from California were R. ALEXANDRINUS, all from South Carolina were cotton rats. (SIGMODON HISPIDUS.)

II. Neutralizing Antibodies against Mengo Virus in Sera of Wild Rats and Monkeys (Uganda).

(Ann. Rep. Virus Research Inst., Nairobi, 1953.)

Animal	No. of Sera Tested	Number Positive	% Positive
Rattus Rattus Kiyabius (Allen) (Probably R. ALEXANDRINUS)	82	20	24
R. (Mastomys) Coucha Ugandae (De Winton) (Multimammate Rats)	35	2	6
Other Wild Rodents	142	0	0
Rhesus Monkeys (At Entebbe Research Institute)	79	31	39

[1] A number of positive and negative samples were kindly sent to us by Dr. WARREN for retesting in our Laboratory. The results of these retests were in complete agreement with the original findings.

is quite different from the almost universal dissemination of THEILER's virus in albino mice, in which the disease seems to have been established over a long period of time. By contrast, it suggests that the Col SK viruses have been introduced rather recently into rat populations under the controlling influence of locally operating factors, possibly through contamination from human sources (see also section on EMC virus). Additional data, corroborating WARREN's findings in the USA, are contributed by serological surveys of wild rodents in Africa which were carried out at the Virus Research Institute in Entebbe, Uganda (1953) and subsequently reported by DICK (1953). As may be seen from Table 31, a fairly high percentage of positive specimens was again encountered with black rats *(Rattus rattus)* and in a smaller percentage also with multimammate rats *(Rattus coucha)*, whereas sera from 17 other species of wild rodents or small mammals contained no antibodies against EMC virus. Similar observations, although on a much smaller scale, were made in our own studies in Mexico (JUNGEBLUT and BAUTISTA 1954) which yielded three positive specimens among 19 wild rat sera collected in Mexico City. A possibility that domestic animals, through contact with infected wild rats, may also function as virus carriers is suggested by TIGGELMAN-VAN KRUGTEN and COLLIER's investigations in Surinam (1956) who found antibodies against Col SK virus in cattle (6 of 111), swine (10 of 102) and dogs (6 of 35) while sera from horses, sheep, goats and cats were negative. The present state of our epidemiological knowledge of Col SK virus infections may be briefly summarized by quoting from the Annual Report of the East African Virus Research Institute in Entebbe (1955): "It would appear that black and Norway rats may have importance chiefly as indicator hosts of the presence of endemic virus rather than serving as important reservoirs of infection. Being relatively insusceptible, most of these rats survive infection and develop neutralizing antibodies. Field rats and possibly other small rodents, exemplified by the multimammate rat, may prove to be hosts of greater importance in regard to transmission. Not only can multimammate rats pass virus in faeces and occasionally in urine, but also the whole carcass of such an animal contains virus, as judged by amounts recovered from skeletal muscle, blood, gut, etc. Thus, in case of ingestion by a predator such as mongoose or by cannibalism, it would serve as a source of infection. EMC virus, an agent remarkably resistant to environmental changes, might well persist in dust, faecal and other discharges. Experiments on albino rats indicated that infection can be readily induced by inhalation. Presence of a relatively low viremia in animals studied and failure of the agent to proliferate in mosquitoes, suggest that these insects probably are not vectors of EMC virus. Recovery of virus from mosquitoes caught in monkey runs may well be explained by their having recently fed on infected animals."

The information which has come from epidemiological studies of Col SK virus infections suggests certain similarities with THEILER virus and with lymphocytic choriomeningitis virus in that all three viruses may maintain an endemic reservoir in rodents, either in mice or in rats. However, while human infections with THEILER virus as far as is known do not occur, even though evidence of antigenic stimulation may exist (SEELIGMAN and JUNGEBLUT 1943; KELLER and VIVELL 1955), Col SK virus, like lymphocytic choriomeningitis virus, apparently is capable of adapting occasionally from rodents to man with the production of disease. It is also conceivable, of course, that the adaptation proceeds in the reverse direction from an originally human-pathogenic agent to a rodent virus, leading to the emergence of transient intermediate forms with mixed characteristics [CHEEVER et al. (1949, 1950); DANIELS et al. (1952); RHODE (1953)]. Whatever the true facts may be, Col SK virus offers an excellent example of the inherent variability of

viruses in their phylogenetic cycle of adaptation from man to animals, or from animals to man.

Classification and Nomenclature.

Because Col SK virus shares many fundamental properties with human poliomyelitis virus and with related viral agents which produce poliomyelitis-like diseases in man or animals, its taxonomy is inextricably tied up with that of the viruses in that group. The fact that both, Col SK and MM virus, were originally recovered from material which also contained poliomyelitis virus is of some importance in that respect since the possibility of a double infection cannot be excluded; in fact, in view of the experience with the Dutch ES strain such a possibility seems to be a very real one, indeed. If this were true, then these particular strains may well differ in their antigenic make-up from EMC and Mengo virus, which were isolated as apparently pure agents, in that the coexistence of Col SK and poliomyelitis virus may have yielded virus populations containing some recombinants with antigenic properties of both viruses. Also, the position of Col SK virus as a human-pathogenic agent is still obscure and the classification and nomenclature of this group of viruses is admittedly of greater theoretical than practical importance. Nevertheless, as information concerning the mechanism of infection in human and experimental poliomyelitis accumulates and the properties of the virus itself are better understood, it seems advisable to review critically the basic characteristics of poliomyelitis virus in conjunction with those of Col SK virus for the purpose of arriving at some tentative system of classification for this virus group.

If one evaluates what is known of the intrinsic properties of the viruses which — in a broader sense — make up the poliomyelitis family, two major trends become apparent in the evolution of these agents: 1) an extension of the host spectrum from rodents to man, or vice versa, and 2) a specialization of tissue affinity from viscerotropism to neurotropism, also reversible. A schematic illustration of this process, as visualized by KELLER and VIVELL (1954), is given in Fig. 22. As is true for yellow fever (urban

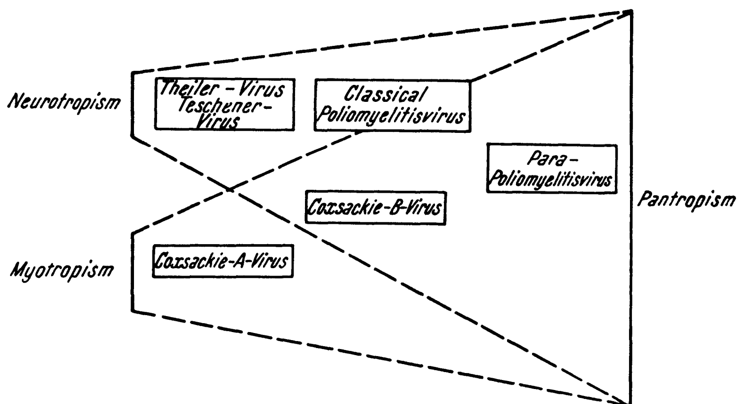


Fig. 22. Schematic relationship of poliomyelitis and poliomylitis-like viruses according to tissue tropism. (VIVELL and GAEDEKE: Erg. Hyg. 27, 512, 1952.)

versus jungle), it has been postulated by LÉPINE (1949) that human poliomyelitis virus may have had an ancient forerunner in the form of a rodent-pathogenic virus causing disease in wild rodents and vicariously in primates indigenous to the tropical belts of the

world (Africa, South America). It is obvious that the epidemiology and the general characteristics of Col SK virus answer the description for such a hypothetical agent. Granting the correctness of this speculation, one would expect Col SK virus at the present time to occur in nature much less frequently as an isolable agent than the more recently established human poliomyelitis viruses; in fact, one would probably have to be content with finding an imprint of its essentially saprophytic existence in form of specific antibody levels in the serum of animals and man. In this, and in other respects, the situation is quite analogous to the dearth of isolations of the GD VII or FA type of THEILERS virus from the brains of spontaneously paralyzed mice in contrast with the common occurrence of the intestinal TO type.

It has been suggested from time to time that viruses should be classified according to their morphology, physical characteristics, mode of reproduction, chemical composition, susceptibility to physical and chemical agencies, host spectrum, tissue tropism, pathogenic properties *in vivo* and *in vitro*, as well as other measurable qualities. Among these various characteristics, morphology and physical properties are, perhaps, the most stabile and reliable criteria, followed by susceptibility to physical and chemical agencies. The biological properties of viruses are more subject to change through adaptation, variation or mutation. This is especially true for the multitropic poliomyelitis viruses which may lose their monkey pathogenicity as the result of adaptation to mice (Lansing, Mahoney and Brunhilde strains). Yet, the virus-cell relationship, encompassing a range of reactions which lead from adsorption to growth and ultimately to cell destruction, appears, in principle at least, sufficiently well defined to help in setting up valid lines of demarkation.

A comparison (Table 32) of the important basic characteristics of four viruses, i. e. 1) Col SK virus, 2) the virus of human poliomyelitis, 3) the Coxsackie viruses and 4) the virus of THEILER's mouse encephalomyelitis shows that all have similar size, possess similar physical properties and, in so far as is known, cause similar pathological responses in neural and extraneural tissues including excretion through the intestinal tract. A difference exists between human poliomyelitis virus on the one hand and Col SK and THEILER virus on the other with respect to hemagglutination which occurs with the latter two viruses but so far has not been demonstrated with poliomyelitis virus. However, this difference carries no great weight because: 1) the hemagglutinating properties of a given virus may change between isolation and animal passage, as is true for influenza virus and for Jap B encephalitis virus; 2) virus strains, which are similar in all other characteristics, including antigenic structure, such as the GD VII and FA strain of THEILER's virus, may or may not possess hemagglutinating properties; 3) Col SK virus may lose entirely its hemagglutinating capacity when harvested from different tissues of the same animal or from the CNS of different animal species; 4) poliomyelitis virus, although showing no clumping of red cells, nevertheless becomes adsorbed on the cellular surface under certain conditions of contact (see section on hemagglutination).

Since the central nervous system has definite limitations in its ability to react to injury, a word is in order about the interpretation of neural lesions, the type of lesions, and the manner in which they are distributed throughout the spinal cord and certain areas of the brain. There can be little question that the reaction to the virus of individual motor ganglion cells in the anterior horn is strikingly similar for poliomyelitis virus, Col SK virus and the mouse and swine encephalomyelitis viruses, consisting of necrosis, neuronophagia, and subsequent gliosis. Also, the inflammatory response of the blood vessels, consisting of various degrees of perivascular cuffing, is found with all four viruses because of virus

Table 32. *Comparison of basic characteristics of Poliomyelitis, Col. SK Group, Coxsackie and* THEILER *Viruses.*

Characteristics	Poliomyelitis Viruses	Col. SK Group Viruses	Coxsackie Viruses	Theiler Viruses
Morphology:				
Ultrafiltration*)	10—15 mμ	10—15 mμ	6—9 mμ; 15—23 mμ	10—15 mμ
Electronmicroscopy	25—30 mμ (27 mμ)	25—30 mμ (27 mμ)	20—35 mμ (26 mμ)	20—30 mμ (28 mμ)
Sedimentation	130—150 S	130—160 S	135—155 S	162 S
U. V. Light Absorption	Max. 260 mμ; Min. 240 mμ	Max. 265 mμ; Min. 248 mμ	?	Max. 260 mμ
Physical Properties:				
Thermal Inactivation	50—55° C	50—56.5° C	60° C	50—55° C
Ether & Trypsin Resistance	Resistant	Resistant	Resistant**)	Resistant
Desiccation	Non-Resistant	Non-Resistant	Non-Resistant	Non-Resistant
Haemagglutination: (Sheep or Human Cells)				
Adsorption in Vitro	+	+	?	+
Agglutination	—	+	—	+
Experimental Host Range:				
Monkeys	+	+	— (±)***)	— (±)***)
Rodents (suckling; adult)	+	+	+; ±***)	+
Tissue Culture Range:				
Human	+	+	+	?
Monkey	+	+	+	?
Rodents	—	+	+	+
Embryon. Eggs	+***)	+***)	+***)	+***)
Tissue Tropism: (Exp. Lab. Animals)				
Neurotropism	+	+	+	+
Myotropism	+	+	+	+
Lymphotropism	+	+	+	+
Enterotropism	+	+	+	+
Lipotropism	+	—	+	?

*) Values for Ratio: Particle Size/Average Pore Diameter (Corrected by Elford Formula).
**) Certain B Strains are Ether-Sensitive.
***) Certain Strains or variants only.

transport by viremia. Differences do exist however, with respect to the localization of cerebral lesions which are definitely more confined to the motor cortex for poliomyelitis virus than for Col SK virus, or the viruses of mouse and swine encephalomyelitis. It would seem that these differences depend in a large measure on factors connected with the resistance of the host and the invasiveness of the virus than on fundamentally divergent properties of the individual strains. How could otherwise the polioencephalomyelitis virus isolated by PELLISSIER[1] in Brazzaville cause widely disseminated cerebral pathology in rhesus monkeys and yet be antigenically identical with Type I poliomyelitis virus? The same is true for THEILERS TO virus and the GD VII strain of encephalomyelitis virus which differ markedly in the extent of cerebral pathology in rodents but have similar antigenic structure (SHAW 1956). Comparable gradations are seen with the Col SK group of viruses in rhesus or cynomolgus monkeys. Thus, the EMC and F strain produce widespread involvement of the cerebral and cerebellar cortical layers, a pattern not found in the classical poliomyelitis process. On the other hand, the pathological picture produced by the Col SK and MM strains, especially following peripheral infection, more nearly resembles that of true poliomyelitis. The topography of the lesions corresponds well with the clinical symptomatology which may vary from an encephalitic syndrome with spasticity and ataxia to the picture of a unilateral flaccid paralysis, especially when the virus is introduced by a peripheral route. These differences have prompted KALM (1950-1955), as well as BIELING and KOCH (1952-1956), to insist on a formal separation of the Col SK group of viruses from the classical poliomyelitis virus group, a demand which seems justified from the pathologist's viewpoint. It is for this reason that we, too, have proposed a subdivision of the Col SK, group under the name of parapoliomyelitis or polioencephalomyelitis virus, within the family of poliomyelitis viruses.

As far as extraneural pathology is concerned, involvement in human poliomyelitis of the peripheral nervous system, i. e. nerve, motor end plate and contigous muscle fiber, is still under discussion. While there can be little disagreement that functional disturbances at the myoneural junction do occur in the paralyzed muscle, it is debatable whether these changes are primary or secondary. Structural damage located at the motor end plate or within the muscle fiber has been described from time to time in poliomyelitis patients and similar muscular lesions, consisting of necrosis and fragmentation of fibers with focal inflammatory response, occur in the heart muscle of fatal cases (see section on pathology).

[1] This virus was isolated by PELLISIER and TRINQUIER in 1953 from the stools of a 6 year old boy, with the clinical diagnosis of poliomyelitis, by transfer to monkeys. (Papio mandrillus, Cercopitheus cephus, C. aethiops and other species.) The virus produced in monkeys a diffuse meningo-encephalomyelitis with brain and cord lesions sufficiently different from the pathology of classical poliomyelitis to prompt the authors to name it "Virus humain encéphalomyélitique de Brazzaville". The virus was also pathogenic for mice, guinea pigs, occasionally even for rabbits, and could easily be cultivated in the embryonated hen's egg. Initial immunological studies revealed no antigenic relationship with any other known virus. However, reexamination by GOFFE, DICK and PELLISSIER in 1955, showed clearly that the strain (FELIX) was neutralized in tissue culture by Type I antipoliomyelitis serum, but not by antiserums against the other two serological types of poliomyelitis virus. The authors conclude with this statement: "Ces caractères biologiques rapprochent le virus de Brazzaville des virus encéphalomyélitiques isolés dans les régions tropicales et sont de nature à confirmer l'opinion de P. LÉPINE selon laquelle le virus poliomyélitique sérait d'origine tropicale."

Again, the proper interpretation of these observations is open to some doubt. However, the fact that active virus may be recovered from the paralyzed muscle (JUNGEBLUT and STEVENS 1950) during the acute stage, and also from the heart (JUNGEBLUT and EDWARDS 1951) at autopsy, carries weight in favor of regarding these pathological manifestations in the muscle as being due to primary activity of the virus. This conclusion is further supported by the capacity of the virus to actively multiply on extraneural tissues in human or monkey tissue culture mediums. Myositic involvement is difficult to demonstrate in experimental poliomyelitic infection, but can readily by produced in cortisone-prepared hamsters or monkeys. The lesions thus produced are indistinguishable from those which regularly accompany infection of monkeys or rodents with Col SK or Coxsackie viruses. There can, therefore, be no question that all the viruses in this group have neurotropic as well as viscerotropic properties. With respect to Coxsackie virus, the customary segregation from poliomyelitis virus has been largely dictated by the fact that Coxsackie virus is not pathogenic for monkeys. However, many antigenically typical strains of poliomyelitis virus are isolated in tissue culture which fail to paralyze monkeys. Moreover, a recent report by CHUMAKOV et al. (1956) describes the fecal isolation — as single agents — from clinically diagnosed cases of poliomyelitis of several strains of Coxsackie group A virus, pathogenic for suckling mice as well as for adult cotton rats, which produce paralysis, with poliomyelitis-like lesions, in monkeys. The characteristics of one these strains were confirmed by HABEL and LOOMIS (1957); see also DALLDORF (1957).

In view of what has been said, it seems reasonable enough to place the four viruses mentioned above, and, because of its enteric excretion (GARD 1951), probably also the virus of swine encephalomyelitis (Teschen disease), in one broad family of entero-myo-neurotropic viruses, distinct from others by virtue of their special affinity for the entire length of the motor neuron; this course begins with the cerebral motor cortex, continues through the anterior horn cells of the spinal cord and terminates with the motor end plates of the peripheral nerve and adjacent muscle fibers. As early as 1951 we had proposed such an over-all approach to classification, emphasizing at that time the apparent evolution of host pathogenicity from man to animals, or from animals to man. The system followed — in general principles — a scheme originally advanced by GARD (1943) and was in agreement with the classification adopted tentatively in BERGEY's Manual published by the Society of American Bacteriologists. Similar thoughts were expressed by MOLLARET (1950) and in the discussion by others at the Second and Third International European Poliomyelitis Conferences. In 1953, the scheme was further elaborated by us at the Sixth International Microbiological Congress. Combining the essential earlier data with certain later modifications, the following classification would therefore logically emerge:

Family: Poliomyelitis Viruses.

- **Genus I:** Human Poliomyelitis Virus. — Subspecies: Serological types I, II, III.
- **Genus II:** Parapoliomyelitis (or Polioencephalomyelitis) Virus. — Subspecies: Col SK, MM; EMC, MENGO; others.
- **Genus III:** Pseudopoliomyelitis Virus. — Subspecies: Coxsackie viruses types A and B.
- **Genus IV:** Mouse Encephalomyelitis Virus. — Subspecies: Theiler TO; GD VII, FA.
- **Genus V:** Swine Encephalomyelitis Virus. — Subspecies: Teschen disease virus.

The unitarian viewpoint implied in such a system (see also Fig. 22) not only has the advantage of giving effect to gradations in viral activity which seem to develop in

fluid sequence; it also obviates the dilemma of having to provide constantly for new viral entities and new diseases as strains with aberrant properties are being isolated. As far as nomenclature is concerned, the term encephalomyocarditis virus is a strain name. When used in the generic sense, or applied to the entire group, the name is "not only devoid of respectability on a priority basis", as WARREN (1953) admits, but has no general significance because of the frequent occurrence of myocarditis due to poliomyelitis virus, Coxsackie Type B virus, or even unrelated viral infections. In harmony with MOLLARET's designation, the term parapoliomyelitis virus, or polioencephalomyelitis virus, seems preferable to us.

The basic concepts of the classification scheme, as outlined above, have been accepted by many European workers, notably KELLER and VIVELL (1954) [see also classification by SHDANOW (1953)]; but the approach has found so far little favor with American investigators. A proposal by RHODES (1953) that Col SK virus should be placed in a new family, together with the arthropod-borne encephalitis viruses, seems clearly untenable because of confirmed differences in particle size, mode of excretion, and behavior of the virus in the infected insect. Yet, VAN ROOYEN[1], in 1954, constructed a new formal classification scheme on this basis, the essential features of which are given below:

Classification by VAN ROOYEN (1954):

Family IV. Erronaceae Holmes (Encephalitis Group).

Genus I. Erro. — Species. Louping-ill, Russian spring-summer encephalitis, Japanese B encephalitis, West Nile infection, St. Louis encephalitis, South American encephalitis, Equine encephalomyelitis virus, Fox encephalitis, Avian encephalomyelitis, Borna-disease virus.

Genus II. Legio. — Species. Poliomyelitis virus Type I, II, III.

Genus III. Theilerella. — Species. Theiler TO, FA, Teschen disease virus.

Genus IV. Armlillia. — Species. Lymphocytic choriomeningitis, Pseudo lymphocytic choriomeningitis.

Genus V. Formido. — Species. Rabies, Pseudorabies.

Family V. Smithburniaceae (Encephalitis Group).

Genus I. Smithburnia. — Species. Bunyamwera encephalitis, Bwamba fever.

Genus II. Jungeblutia. — Species. Columbia SK-MM.

Genus III. Rocaea. — Species. Various mosquito isolates from South America, California, Africa. (Semliki Forest, Uganda S., Zika forest, Nitaya virus.)

Family VI. Dalldorfiaceae.

Genus I. Dalldorfia. — Species. Coxsackie Type A and B.

A more recent definition of the poliomyelitis group of viruses was proposed in 1955 by v. MAGNUS, GEER and PAUL, acting as a study group for the Virus Subcommittee of the International Nomenclature Committee. It includes as group members only human poliomyelitis virus *(poliovirus hominis)* and the TO type of Theilers mouse encephalomyelitis virus *(poliovirus muris)*, leaving

[1] In a personal communication (April 7, 1955) to the author Dr. VAN ROOYEN writes as follows: At the moment I do not see any reason why Col SK virus should not be classified as a separate virus in Family IV between Genus II Legio and Genus III Theilerella, with the necessary change in numeration.

open the question as to the proper allocation of the GD VII and FA strains of THEILER's mouse virus, of swine encephalomyelitis virus, of the Coxsackie viruses, and of the viruses of the Col SK group.

Acknowledgment.

Much of the experimental work done in this Laboratory on Col SK virus — and on poliomyelitis virus—would have been impossible to carry out without the financial support of the Sister Elizabeth Kenny Foundation. It is a pleasure to acknowledge, on this occasion, the generous assistance received over a period of years from this organization. If the study of the Col SK group of viruses has helped to better understand the nature of poliomyelitic infection, the rewards are also a tribute to Elizabeth Kenny for visualizing so early the systemic charakter of this disease.

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