sages, gave no evidence of being pathogenic for rhesus monkeys in repeated tests. The MEF virus, originally rhesus-pathogenic, apparently suffered a progressive loss of its pathogenicity for rhesus monkeys during serially maintained rapid mouse passages. The Y-SK virus, finally, seemed to have undergone but little change in its pathogenic power for rhesus monkeys as the strain was passed rapidly through mice. It is realized that the described phenomena may not always be duplicated in different laboratories since the Lansing virus has frequently been reported by others as being fully rhesus-pathogenic. Yet the authenticity of our strains is attested by the fact that all three murine strains had fully preserved their biological and immunological characteristics. The observed differences in the degree of pathogenicity for rhesus monkeys and in the extent to which murine virus returns to mice from paralyzed monkeys may be due to individual differences among strains or reflect an attainment of different levels of perfection in the adaptive process.

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Monkey Pathogenicity of Various Strains of Murine Poliomyelitis Virus. II. Experiments with Col. SK-MM Group.* (17490)

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In the preceding paper(1) data have been presented to show that the Lansing, MEF and Y-SK virus, upon serial passage in mice, may undergo various degrees of deterioration in their ability to produce flaccid paralysis in intracerebrally infected rhesus monkeys. This reduction in monkey pathogenicity, however, was not associated with any demonstrable changes in other biological or immunological properties. It became next of interest to investigate whether the Col-SK-MM group of viruses—agents which since their initial transfer to mice have shown little or no paralyzing power in rhesus monkeys—would be capable of inducing paralytic infection, with central nervous system lesions, in cynomolgus (Java) monkeys.

The cynomologus monkey was chosen for this purpose since the response of this animal to poliomyelitis virus differs in many respects from that observed in rhesus monkeys. Whereas the rhesus monkey, as a rule, is susceptible only to intracerebral infection and the virus remains strictly localized in the central nervous system, the cynomolgus monkey may be infected by peripheral portals of entry, including the gastrointestinal tract, with the production of a systemic disease characterized by wide extraneural distribution of the virus and by fecal excretion. Moreover, isolation of poliomyelitis virus from fresh human or nonhuman sources often succeeds better with the cynomolgus than with the rhesus monkey.(2)

The following communication deals with a study of the pathogenicity for cynomolgus monkeys of current mouse passages of Col-SK and MM virus. Included are also experiments with 2 viruses of as yet uncertain identity but with close serological relationship to Col-SK and MM virus. These are the virus of so-called encephalomyocarditis (EMC virus)(3) which was isolated from chimpanzees suffering from unknown myocardial disease, and the Schultz High Lansing strain, presumably a "high" variant of the original Lansing vi-

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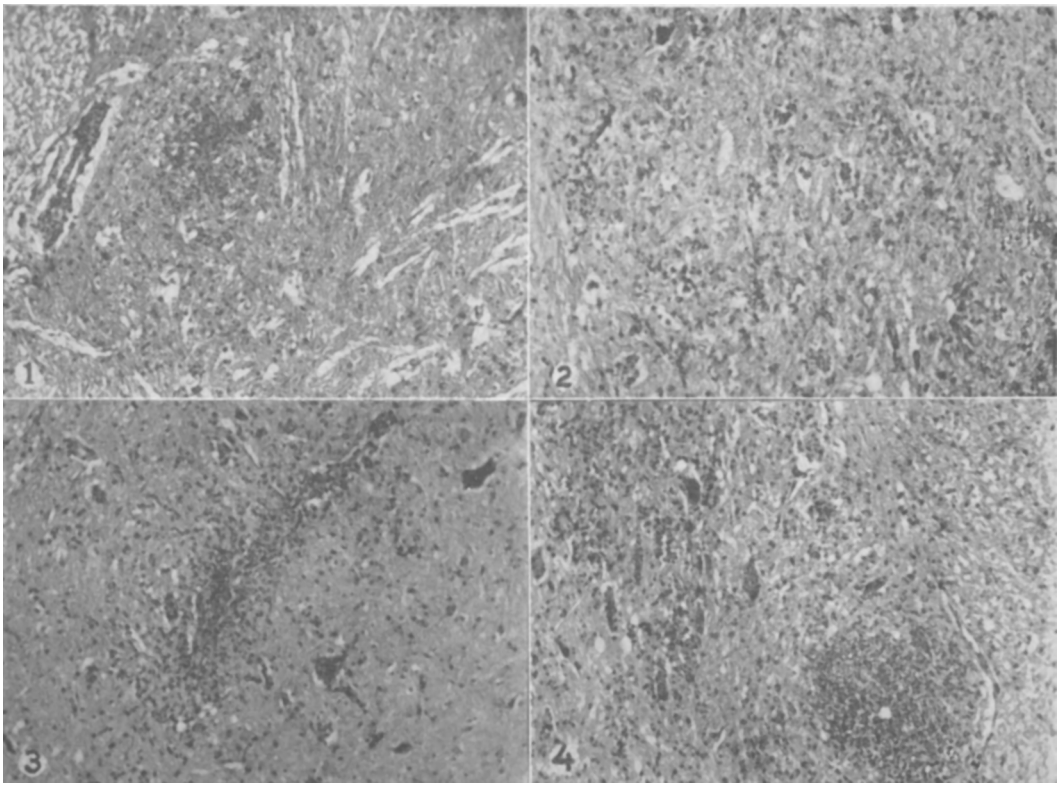


FIG. 1. Col-SK virus (guinea pig) first monkey generation cynomolgus No. 7 lumbar cord.
FIG. 2. MM virus (mouse) first monkey generation cynomolgus No. 12 lumbar cord.
FIG. 3. High Lansing virus third monkey generation cynomolgus No. 32 cervical cord.
FIG. 4. EMC virus third monkey generation cynomolgus No. 30 lumbar cord.

rus.(4) For purpose of control, 2 highly virulent Theiler strains of spontaneous mouse encephalomyelitis, *i.e.* GDVII and FA virus, were also examined for possible pathogenic effects in cynomolgus monkeys. The EMC virus was obtained from Dr. J. Warren, Army Medical School, Washington, D. C., the Schultz High Lansing virus from Dr. Edwin Schultz, Stanford University, California, and the 2 Theiler strains from Dr. Max Theiler, Rockefeller Foundation, New York.

Methods. Ten per cent viral brain suspensions were prepared from freshly harvested current mouse passages of the above mentioned strains and 1 cc of the supernatant, after light centrifugation, was injected intracerebrally into young cynomolgus monkeys weighing between 1000 and 2000 g. In the case of Col-SK virus, in addition to mouse virus preparations, viral brain suspensions were also used from paralyzed cotton rats or guinea pigs. The injected cynomolgus monkeys were kept under close observation and symptoms noted. Animals showing paralysis were sacrificed at the height of their smyptoms and the cord was removed for histological study as well as for virus recovery. Freshly prepared monkey cord suspensions were further passed to new cynomolgus or rhesus monkeys (1 cc 1:10 intracerebrally) and, at the same time, were titered out in mice by both the intracerebral and intraperitoneal route. Serial monkey passages were maintained over 2, 3 or 4 generations depending upon the absence or presence of paralytic symptoms. The results obtained are brought together in Diagram I and in Table I and are further illustrated in a series of microphotograms (Fig. 1-4).

Col-SK Virus. It will be noted that Col-SK virus, whether harvested from paralyzed cot-

TABLE I. Symptoms, Pathology, and Viral Content (Cord) of Monkeys Paralyzed by Col-SK, MM, High Lansing (Schultz) or EMC Virus.

Monkey No.	Virus injected	Monkey generation	Symptoms	Pathology, CNS	Virus content of cord titered in mice	
					i.c.	i.p.
Cynomolgus C2	Col-SK cotton rat No. 5	I	Generalized paralysis, 4 days	Ganglion cell necrosis	10^{-5}	10^{-5}
Rhesus AS48	"	I	Paresis both legs, 7 days	Died 16 days. Poliomyelitis with repair	Neg. 10^{-1}	Neg. 10^{-1}
Cynomolgus C4	Col-SK mouse No. 422	I	Paralysis left arm and both legs, 3 days	Recovered	N.d.†	N.d.†
" C5	"	I	Paralysis right leg, 3 days	Typical poliomyelitis	10^{-5}	10^{-5}
" C1	Col-SK guinea pig No. 81	I	Paralysis left arm and leg, 6 days	" "	10^{-3}	10^{-3}
" C6	" No. 83	I	Weakness 5th day, died 6th day	" "	10^{-3}	10^{-1}
" C7	" No. 83	I	Paralysis right leg, 13 days	" "	10^{-1}	Neg. 10^{-1}
Rhesus AS50	Col-SK monkey C5	II	Paralysis right leg, 6 days	" "	Neg. 10^{-1}	Neg. 10^{-1}
" AS51	"	II	Paralysis right arm, 13 days	" "	10^{-1}	Neg. 10^{-1}
Cynomolgus C21	"	II	Weakness 3rd day died 3rd day	Ganglion cell necrosis	10^{-5}	10^{-5}
" C22	"	II	Generalized paralysis, 4th day	" " "		
Rhesus AS60	" C7	II	Paralysis left arm, 7 days	Typical poliomyelitis	10^{-3}	10^{-1}
" AS62	" AS51	III	Paralysis left arm, 8 days	" "	10^{-3}	10^{-1}
Cynomolgus C11	MM mouse No. 138	I	Generalized paralysis, 3 days	Ganglion cell necrosis	Neg. 10^{-1}	Neg. 10^{-1}
" C12	" " " "	I	Paralysis both legs, 4 days	Typical poliomyelitis	10^{-3}	10^{-3}
" C19	High Lansing mouse No. 9	I	Generalized paralysis, 3 days	" "	10^{-5}	10^{-5}
" C20	High Lansing mouse No. 9	I	Generalized paralysis, 3 days	Ganglion cell necrosis	N.d.†	N.d.†
" C25	High Lansing monkeys C19-C20	II	Paralysis left leg and arm, 4 days	" " "	10^{-5}	10^{-5}
" C26	High Lansing monkeys C19-C20	II	Paralysis left leg and right arm, 6 days	Typical poliomyelitis		
" C31	High Lansing monkeys C25-C26	III	Generalized paralysis, 5 days	" " "	10^{-5}	10^{-5}
" C32	High Lansing monkeys C25-C26	III	Generalized paralysis, 4 days	" " "		
" C17	EMC mouse No. 70	I	Paralysis both legs, 3 days	" " "	10^{-5}	10^{-5}
" C18	"	I	Paresis left leg and arm, 4 days	Died 24 days. Not examined	N.d.†	N.d.†
" C23	EMC monkey C17	II	Generalized paralysis, 5 days	Typical poliomyelitis	10^{-5}	10^{-5}
" C24	"	II	Paralysis left arm and leg, 5 days	Atypical		
" C30	EMC monkeys C23-C24	III	Paralysis left arm and both legs, 4 days	Typical poliomyelitis	10^{-5}	10^{-5}

† N.d = not done.

ton rats, mice or guinea pigs, proved to be highly pathogenic for cynomolgus monkeys in paralyzing 5 of 6 such monkeys following intracerebral injection; on the other hand, paralysis occurred in only one of 2 injected rhesus monkeys. When carried from the first to the second monkey generation, the number of "takes" was greatly diminished, only one of 5 injected cynomolgus monkeys and 3 of 7 injected rhesus monkeys developing paralysis. In the third monkey generation there was a further reduction of monkey pathogenicity in that paralysis occurred in one rhesus monkey only within a group of 5 rhesus and 2 cynomolgus monkeys. No paralysis was observed in the fourth monkey generation consisting of 2 rhesus monkeys.

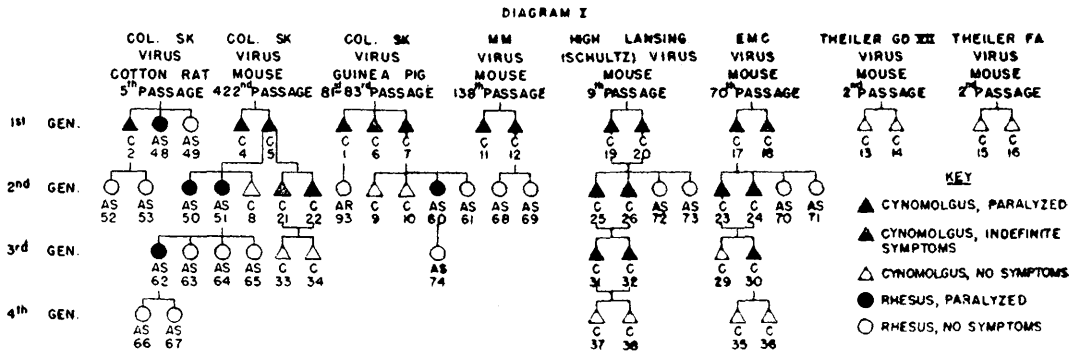
The lesions observed in the spinal cord of paralyzed cynomolgus or rhesus monkeys are described in Table I. In 8 out of 12 cases they were those of severe acute poliomyelitis, consisting of widespread ganglion cell necrosis in the anterior horn, with numerous examples of neuronophagia, extensive inflammatory reactions around the blood vessels and occasional areas of hemorrhage. In 3 cases the pathology was limited to more sharply defined localizations of ganglion cell necrosis in the anterior horn or central grey with poorly developed neuronophagia and but little inflammatory response. One case, finally, showed a mild poliomyelitic process with evidence of beginning repair characterized by extensive gliosis. A constant feature of the severely damaged cords was the close proximity between perivascular infiltration and ganglion cell necrosis. Pathological examination of the brain of 2 cynomolgus monkeys (not listed in Table I), which had developed paralysis following intracerebral injection with 1 cc of a 10^{-2} and 10^{-3} dilution of Col SK mouse virus, showed occasional lesions in the brain stem and a few scattered collections of cells in the motor cortex, near the tract of inoculation. A typical picture has been selected for illustration in microphotogram 1.

The results of virus titrations in mice injected with cord suspensions from the paralyzed monkeys are given in Table I. In general, the virus obtained from paralyzed monkeys was considerably less mouse-pathogenic than virus harvested from paralyzed mice but higher titers were usually observed in cynomolgus rather than in rhesus monkeys. With successive monkey passages virus recoveries, especially from rhesus monkeys, were of the order commonly observed with low-titred murine strains belonging to the Lansing, MEF and Y-SK group. However, when virus harvested from the original group of injected mice was further passed to a new set of mice, complete restoration of potency to the titer characteristic of Col-SK mouse passage virus, *i.e.* 10^{-9} i.c. and 10^{-8} i.p., became at once apparent. The loss of viral potency suffered by monkey passage was therefore merely a transient depression of virulence and the virus had not been transformed into a permanently low strain.

MM Virus. Two cynomolgus monkeys injected with the mouse virus became paralyzed within 3-4 days but attempts to carry the virus into the second monkey generation were unsuccessful. One of the paralyzed monkeys presented typical poliomyelitic lesions in the cord (Fig. 2), the other showed only ganglion cell necrosis with scanty inflammatory reaction. The virus recovered from the cords of both paralyzed cynomolgus monkeys proved of exceptionally low virulence in mice but could be restored to its original high level of murine potency in the next mouse passage.

High Lansing (Schultz) Virus. The mouse virus paralyzed 2 cynomolgus monkeys each in the first, second and third monkey generation but failed to transmit to the fourth generation. Severe poliomyelitic lesions (Fig. 3) were found in 4 of the 6 paralyzed monkeys, whereas in the remaining 2 animals only occasional areas showed ganglion cell necrosis without inflammatory reaction. The cords of all paralyzed monkeys yielded virus of moderately high virulence for mice.

EMC Virus. The mouse virus paralyzed one of 2 cynomolgus monkeys in the first generation, both cynomolgi of the second, and one of 2 in the third generation; no paralysis was obtained in the fourth generation. Typical poliomyelitic lesions (Fig. 4) were found in the cords of 3 animals, the remaining monkeys presenting an atypical picture of occasional chromatolysis of ganglion



MONKEY PATHOGENICITY OF THE COL. SK--MM GROUP OF VIRUSES

FIG. 5.

cells but no perivascular infiltration. No evidence of demyelination could be detected in the cords of the 4 monkeys examined. Virus recovered from the cords of paralyzed monkeys, in all 3 generations, proved consistently moderately pathogenic for mice.

Theiler Virus (GDVII and FA). Two cynomolgus monkeys were injected intracerebrally with each of the 2 strains harvested from fresh mouse passages. None of the 4 animals showed any signs of illness during one month observation. Two monkeys were sacrificed at that time and cord sections examined histologically. No lesions of any kind were found in these cords.

Immunological Tests. The immunological properties of the various murine strains, following their passage through monkeys, were studied: 1) by examining a number of representative sera from paralyzed or symptomless monkeys for their ability to neutralize Col-SK and Y-SK murine virus in mice, 2) by determining the extent to which Col-SK monkey virus, harvested from infected monkey cords, could be inactivated in mice by known Col-SK and Y-SK antisera, 3) by reinfecting all surviving symptomless monkeys with simian poliomyelitis virus. Suffice it to say that neutralization of Col-SK murine virus was readily obtained by the majority of sera collected from cynomolgus or rhesus monkeys infected with Col-SK, MM, High Lansing or EMC virus (but not with Theiler virus), whereas the same sera failed to neutralize Y-SK murine virus. In harmony with this fact, Col-SK virus, prepared from infected cynomolgus cords, was fully inactivated by known Col-SK antiserum but not regularly by known Y-SK antiserum. In the reinfection experiment, finally, a group of 8 cynomolgus monkeys surviving asymptomatic infection with either Col-SK virus (C8, C10, C33, C34) High Lansing virus (C37, C38) or EMC virus (C29, C35), together with one normal rhesus control monkey, were challenged by intracerebral injection of simian poliomyelitis virus (1 cc 1:10 Aycock rhesus passage virus). All animals developed complete or partial paralysis within 1-3 weeks.

Discussion. The results obtained in this study leave little doubt that the murine strains at present classified as the Col-SK-MM-EMC-Mengo group of virus are highly pathogenic for cynomolgus monkeys and induce in this animal an experimental disease which is symptomatologically and pathologically similar to that produced by the Lansing-Y-SK group of murine virus in rhesus monkeys. African grey monkeys (Cercopithecus aethiops) have also been reported as developing paralysis more frequently than rhesus monkeys following infection with Mengo virus.(5) Although these facts seem well enough established,† significant differences in the degree of such monkey pathogenicity between the two

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† The fact that Col-SK virus paralyzes cynomolgus monkeys with the pathological picture of poliomyelitis in the spinal cord has been confirmed by Dr. J. D. Verlinde, Leiden, Holland, who will publish his findings elsewhere.

groups of virus must not be overlooked. For, whereas murine Lansing, MEF and Y-SK virus can often, if not always, be maintained in an unbroken series of monkey generations, murine Col-SK, MM, High Lansing and EMC virus produce paralysis in monkeys only for a limited number of passages. The abrupt extinction of paralyzing power for primates, despite the presence of a potentially virulent agent and the manifestation of extensive lesions in the central nervous system of the infected monkey, constitutes an as yet unsolved problem. The problem is further accentuated by the fact that monkey passage fails to bring about a permanent conversion of the "viscerotropic" Col-SK strain into the "neurotropic" Y-SK strain. If one contrasts the obvious depression of virulence in monkeys—a frequent phenomenon for the Col-SK-MM group of virus—with the ease with which the same viruses can be propagated in mice, one is confronted with several possible conclusions. Either different murine strains of human poliomyelitis virus possess different innate degrees of pathogenicity for monkeys and mice; or, some of these viruses have suffered an irreversible loss of their intrinsic monkey pathogenicity, subsequent to their experimental adaptation to rodents. A third consideration would be that in the case of the Col-SK-MM-EMC-Mengo group of viruses we are dealing with a serologically homogenous group of aberrant members of the poliomyelitis family which, though capable of producing paralytic poliomyelitis in monkeys and probably also in man,(6) are genetically not of human but of rodent origin in nature. This hypothesis might receive support from the recent demonstration of the regional occurrence of virucidal antibodies against EMC and MM virus in the sera of wild rats.(7) Obviously, further study of the complex problem is necessary in order to provide the correct answer.

Conclusions. 1) Col-SK, MM, High Lansing and EMC virus, harvested from remote rodent passages, produce typical poliomyelitis in intracerebrally infected cynomolgus monkeys. 2) Two strains of highly virulent Theiler virus, GDVII and FA, are non-pathogenic for cynomolgus monkeys. 3) The Col-SK-MM group of virus can be carried in cynomolgus monkeys only for a limited number of passages. 4) Judging from serological and biological characteristics, monkey passage fails to bring about a permanent conversion of Col-SK virus into Y-SK virus.

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Artificial Kidney. II. Construction and Operation of an Improved Continuous Dialyzer.* (17491)

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The insertion of a continuous dialyzer (artificial kidney) in the circulatory system of an animal or a human in uremia has been shown to be capable of removing nitrogenous waste products(1-10) and to result in the adjustment of electrolyte equilibrium.(1,3,8,

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