delayed 5 or 6 days. The behavior of wounds in animals on the scorbutigenic diet for 7 and 12 days prior to wounding was very similar. It is important to record the observation that the contracting scorbutic wound margin at any time could be readily detached from the base, causing immediate distraction. The wound margin was much more firmly bound in the normal.

Discussion. It must be emphasized that the term "wound contraction" is used here to describe that early phase of closure of tissue defects characterized by the movement of preformed tissues, and does not describe the late changes in scar tissue shortening. This latter process is probably different in origin and dependent on collagen fibers of the new formed connective tissue (although direct evidence is presently lacking).

The fact that contraction, except for an initial delay, proceeds in an almost normal manner in scorbutus is consistent with the hypothesis that the actual movement inward of the wound edge is dependent upon directed cell migration as originally suggested by Abercrombie, Flint and James(1), and by Grillo, Watts and Gross(4,8). The markedly different strength of attachment of the wound margins in normal and scorbutic wounds suggests that the role of collagen in this process, if any, is to produce a temporary and continually remodelled tethering of the wound margin to the base. Thus, as the margin progresses inward, collagen fibers might be laid down in advance and removed or detached as the wound edges pass overhead, a phenomenon suggested by our previous observation(4) that normally firm attachment was found only at the wound margin and relatively little collagen could be demonstrated peripheral to the margin beneath the advancing skin.

Summary. Full thickness skin wounds in scorbutic guinea pigs contract at same rate during the first 2 weeks of healing as do those in normal animals. There appears to be a delay in initiation of contraction. Significance of these observations is discussed.

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Interference Between Certain Neurotropic Viruses in Tissue Culture.* (24908)

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Interference between Col SK virus and poliomyelitis virus, which occurs in monkeys, guinea pigs or in hamsters upon infection with both viruses, has previously been described (1-6). More recently, tissue culture methods have been employed in quantitative studies of interference reactions between related or unrelated viruses(7-13). Since several strains of the Col SK virus group can be propagated on certain cell lines with definite cytopathogenic effects(14), it became of interest to study interference phenomena between these agents and other neurotropic viruses on suit-

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able tissue culture substrates. As has been reported before(14c), tissue cultures of L cells, HeLa cells and KB cells support cytopathogenic growth of EMC virus but not of Col SK virus which cannot be maintained over serial passages on the corresponding tissue culture media; this discrepancy in growth requirements of the 2 viruses extends also to human amnion cells. Further experiments showed that, notwithstanding the absence of cytopathogenic growth, considerable amounts of Col SK virus are adsorbed on the cellular surface of HeLa cultures, provided the test dose of virus is kept within reasonable limits. This was determined by titration in mice of viral supernatant fluids, removed from cell sheets after variable periods of contact at 37°C, in comparison with the titration of viral fluids obtained from cell-free controls. The actual amount of a test dose of 10^5 LD_{50} of Col SK virus, which was adsorbed on about 5 million HeLa cells after an interval of 4 hours, was calculated to be of the order of 2 logs. Therefore, an opportunity presented itself to utilize non-cytopathogenic Col SK virus as interfering agent against subsequent challenge with other fully cytopathogenic neurotropic viruses on various cell lines.

Materials and methods. The following viruses† were used: Col SK virus, EMC virus, Poliomyelitis virus (Type I: Mahoney, Brunhilde; Type II: Y-SK, MEF_1; Type III: Saukett) and Coxsackie virus (Group B, Type 5). Col SK virus was employed as brain passage virus harvested from paralyzed mice. EMC virus was harvested from infected L cell cultures. The poliomyelitis and Coxsackie viruses were tissue culture fluids harvested from infected HeLa cell cultures. L cell cultures were used for interference experiments between Col SK and EMC virus; the medium for L cells was Hanks' solution with 40% horse serum and 20% chick embryo extract (plus antibiotics). Interference reactions between Col SK virus and poliomyelitis or Coxsackie viruses were studied on HeLa cells or on human amnion cells. The HeLa cells were available in 2 sublines I and II which differed in their sensitivity to cytopathogenic growth of EMC, poliomyelitis, or Coxsackie viruses, the titers of all 3 viruses being consistently at least one log higher with the more sensitive cell strain. HeLa and amnion cells were propagated in a medium consisting of 20% horse serum and 10% human serum in Hanks' balanced salt solution which contained 0.1% yeast extract (plus antibiotics). After 4-7 days growth the cells were trypsinized and suspended (100,000-200,000 cells per ml) in Scherer's maintenance solution containing 10-20% horse serum. All cell suspensions were then distributed, in 0.5 ml volume, into rubber-stoppered stationary tubes and 2-day-old sheets, after replacement of the medium, were used for interference tests. The basic experimental procedure adopted was as follows: For each experiment 7 groups of tissue culture tubes were set up, with 5 tubes in a group. A constant amount of Col SK virus, *i.e.* 0.1 ml of a 1:125 dilution, was added to each tube containing 0.4 ml of maintenance fluid; higher concentrations of Col SK virus were toxic for cells because of their content of mouse brain tissue. After contact of 4 hours at 37°C, the fluids were drained and replaced by 0.4 ml of fresh maintenance fluid; parallel tests showed that the results were essentially the same with cell sheets which had been washed 3 times. Following adsorption the cells were infected with 0.1 ml of the challenge virus in 7 serial dilutions progressing in 5-fold steps to the endpoint of cytopathogenicity. All tubes were then reincubated. The challenge virus was titrated simultaneously over the same range of dilutions under similar experimental conditions, except that a 1:125 suspension of normal mouse brain was added as first inoculum. Thus, 35 tubes were used for the test and an equal number for the control, making a total of 70 tubes for each interference experiment. All cell sheets were examined microscopically on the third and fourth day after infection, the endpoints of cytopathogenicity were determined by the Reed-Muench method, and the titers were ex-

† Poliomyelitis viruses and Coxsackie virus were obtained through courtesy of Dr. H. Winsser, Albany, N. Y. We are indebted to Dr. J. Syverton, Minneapolis, and to Dr. B. Mandel, N. Y. City, for supplying the 2 lines of HeLa cell cultures; L cells and the stable strain of human amnion cells were kindly provided by Dr. M. Holden of this Department.

TABLE I. Interference between Col SK Virus and EMC Virus on L Cells.

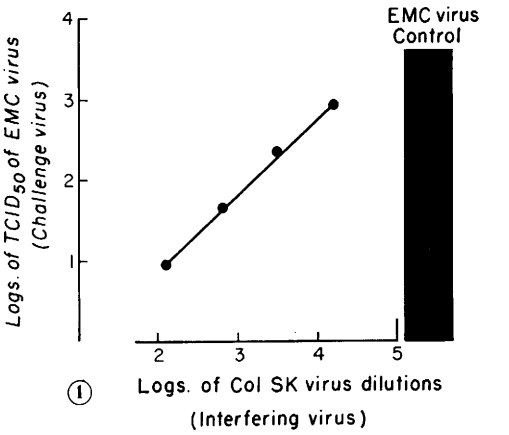
Interfering virus dilutions	Challenge virus 1:125-1:390625	L cells: EMC control	L cells: Interference test	L cells: Rate of interference
		$TCID_{50}$ (logs)		
Col SK 1:125	EMC	3.60	.97	2.63
1:625	"	"	1.67	1.93
1:3125	"	"	2.34	1.26
1:15625	"	"	2.90	.70

pressed as TC ID_{50} per 0.1 ml of culture fluid. It was therefore possible to calculate rate of interference as logs of challenge virus suppressed in each of the various interference systems. With the Col SK-EMC and Col SK-Mahoney interference systems the basic procedure was extended to include further quantitative tests in which Col SK virus was used as interfering agent in serial 5-fold dilutions over a range from 1:125 to 1:15,625. The results of these experiments are brought together in Tables I and II.

Results. The data given in Table I indicate that effective interference between Col SK and EMC virus occurred on L cells. The degree of this interference ranged from suppression of 0.7 log to 2.63 logs of challenge virus, depending on dose of Col SK virus used during the initial phase of the experiment. When the figures are plotted log for log, as in Fig. 1, it will be seen that interference between the 2 viruses proceeded as a first order reaction over the range of dilutions tested. None of the poliomyelitis viruses, when placed in contact with L cells, interfered with subsequent production of EMC virus.

It appears from Table II that effective interference occurred also between Col SK virus and poliomyelitis or Coxsackie viruses with HeLa cells, subline I. The degree of interference varied from suppression of about 1 log to almost 3 logs of challenge virus. In the case of the Col SK-Mahoney virus system, a similar quantitative relationship between amount of interfering virus and amount of suppressed challenge virus could be demonstrated as for the Col SK-EMC system (Fig. 2). Interference between Col SK and poliomyelitis virus was less marked on amnion cells since only about 1½ logs of challenge virus were suppressed. Minimal interference, or no interference at all, was observed with the more sensitive subline II of HeLa cells.

After the basic phenomenon was established, further experiments were concerned with a more detailed study of the mechanism of the interference reaction. The Col SK-Mahoney virus system on HeLa cells was



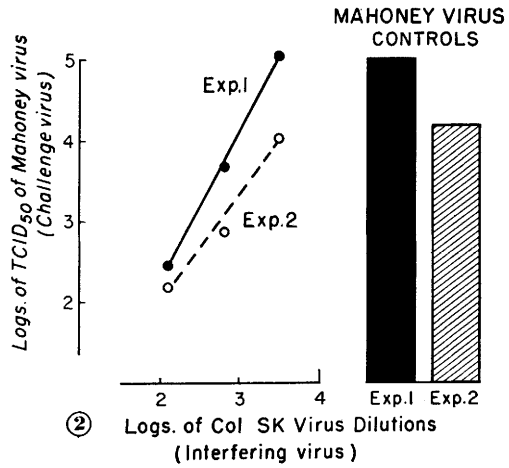


FIG. 1. Interference between Col SK and EMC virus in L cell tissue culture.

FIG. 2. Interference between Col SK and poliomyelitis virus in HeLa cell tissue culture.

TABLE II. Interference between Col SK Virus and Poliomyelitis or Coxsackie Virus on Various Tissue Culture Substrates.

Interfering virus	Challenge virus	HeLa cells (subline I)			HeLa cells (subline II)			Amnion cells		
		Control	Interference test	Rate of interference	Control	Interference test	Rate of interference	Control	Interference test	Rate of interference
		$TCID_{50}$ (logs)								
Col SK 1:125	Mahoney	4.47	2.44	2.03	5.60	5.25	.35	5.24	3.84	1.40
"	"	4.37	1.57	2.80						
"	"	4.53	1.63	2.90						
"	Brunhilde	4.53	3.26	1.27	5.24	5.24	0			
"	MEF_1	2.80	1.91	.89	3.99	3.34	.65	1.86	.34	1.52
"	Y-SK	4.63	1.74	2.89						
"	Sauckett	4.89	3.03	1.86	5.60	5.60	0	3.03	1.63	1.40
"	Coxsackie B_5	4.61	2.10	2.51				4.19	3.03	1.16

chosen as a model for this investigation, using Col SK virus in a constant dose of 1:125 for interference against challenge with Mahoney virus over a range from 1 to 100 TC ID_{50}. Several interesting observations were made. Thus, it was found that Col SK virus (1:125) which had been heated for 10 min at 53°C —a procedure that reduced the infectivity titer in mice from 10^{-9} to less than 10^{-3}— gave no evidence of interference. This result agrees with the observation that a dose of between 7 and 8 logs of active Col SK virus is required for suppression of about 100 TC ID_{50} of Mahoney virus. In other tests in which length of time allowed for contact between Col SK virus and cells varied over a range from 15, 30, 60, 120 minutes; 4 hours; 24 hours and 3 days, no interference at all occurred before 4 hours; the reaction was maximal at this time and equal protection was obtained at later intervals, up to 3 days. This finding is in harmony with rate of adsorption of Col SK virus on HeLa cells. The problem as to site of interference was studied by examining the effect of receptor-destroying enzyme on the reaction. It was found that previous treatment of HeLa cells with *Vibrio cholerae* RDE (1:50 dilution for 1 hour at 37°C) completely abolished the interfering action of Col SK virus. The outcome of this experiment is in line with what might have been expected, namely that interference does not take place unless Col SK virus has been adsorbed on the cells(15). However, it leaves open the question whether interference occurs on the cellular surface, or intracellularly. Finally, a number of tests were run with bottle cultures of HeLa cells in an effort to follow the interfering ability of Col SK virus at various intervals after its adsorption. In these experiments HeLa cell sheets were covered with 7 cc of a 1:100 dilution of Col SK virus in maintenance fluid. After contact between virus and cells for 4 hours at 37°C, the supernatant was removed and cells were thoroughly washed, including washing with specific antiserum, to remove any traces of unadsorbed virus. The cells were then covered with fresh maintenance fluid and the bottles were reincubated. The original virus di-

TABLE III. Loss and Re-appearance of Interfering Ability of Col SK Virus following Adsorption of Virus and after Its Subsequent Release from HeLa Cells.

Interfering virus—Col SK		Challenge virus—Mahoney		
Preparation	Infectivity LD_{50} (logs)	Control	Interference test	Rate of interference
		$TCID_{50}$ (logs)		
Original virus dilution	8	4.77	3.13	1.64
Supernatant after 4 hr contact with cells		"	4.77	0
" " washing cells	1	"	"	0
" " 6 hr re-incubation	5	"	"	0
" " 12 hr "	6	"	3.93	.84
" " 24 hr "	8	"	3.37	1.40

lution before adsorption as well as supernatants withdrawn at several points after adsorption were titrated by standard methods in HeLa cell tubes for interference against Mahoney virus; fluids collected after washing the cells were also titrated in mice for infectivity. Table III gives the results of one representative experiment.

The data show that the interfering power of the supernatant was totally lost after 4 hours contact between Col SK virus and cells but that it reappeared between 12 and 24 hours following reincubation. Comparative tests in mice did not permit demonstration of any loss of infectivity through adsorption because of the excessive dose of virus used; however, it is clear that reappearance of the interfering principle, after washing of the cells, was paralleled by a corresponding release of infectious virus. Interestingly enough, both interfering power and infectivity of a supernatant, harvested at the 24-hour interval and then placed in contact with a new sheet of HeLa cells, could be shown to go through another phase of adsorption, but not of release.

Discussion. Our observations indicate that non-cytopathogenic Col SK virus interferes with subsequent growth of cytopathogenic EMC virus on L cells in tissue culture. Furthermore, it appears that magnitude of the interfering effect is directly proportional to amount of Col SK virus used for adsorption. Our results compare with what is known of interference phenomena among other related viruses in tissue culture experiments and are quite similar to those reported by Dinter(12) on interference between non-cytopathogenic and cytopathogenic strains of foot and mouth disease virus. Of greater importance, perhaps, is that definite interference can also be demonstrated on HeLa cells between Col SK virus and the 3 serological types of poliomyelitis virus or Coxsackie Group B virus. This fact serves to amplify earlier observations by other workers on occurrence of interference between these viruses in animal or tissue culture tests.

The actual mechanism of this interference, especially its site on the host cell, is not known. However, since Col SK virus uses cell receptors which are different from those used by poliomyelitis virus—one being susceptible to destruction by RDE, the other resistant to the action of the same enzyme(15)—suppression of virus multiplication may not occur on the cellular surface but intracellularly by blocking certain intrinsic metabolites at an early step in the process of viral synthesis. It remains for further experiments to clarify this problem. Recent work by Isaacs(10) suggests that, when heat-inactivated influenza virus is used as interfering agent against active influenza virus on chick chorio-allantoic membranes *in vitro,* a non-infectious, non-specific product of the interference phenomenon ("interferon") is formed which is capable by itself of inducing protection. Our experiments with active Col SK virus yielded no evidence that interference can be ascribed to any factor other than virus itself.

Summary. 1) Interference between non-cytopathogenic Col SK virus and cytopathogenic EMC virus occurred on L cells in tissue culture, causing suppression of 0.7 to 2.63 logs of challenge virus depending on the dose of interfering virus used. 2) Interference also

occurred between Col SK virus and poliomyelitis or Coxsackie Group B virus on HeLa cells, causing suppression of about 1 to 3 logs of challenge virus depending on the dose of interfering virus used. 3) The mechanism of the interference reaction is discussed.

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Bussuquara, A New Arthropod-Borne Virus.* (24909)

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Sentinel monkeys have been used extensively by the Belém Virus Laboratory in attempts to isolate arthropod-borne (arbor) viruses. In this paper are described serologic studies that led to characterization of an agent (An 4073) thus isolated as a new, hitherto undescribed arbor virus, which it is proposed to name Bussuquara. *Circumstances of isolation and early studies.* Bussuquara virus was isolated in the forest of the Inst. Agronomico do Norte, Belém. Blood from a sentinel howler monkey (*Alouatta beelzebul*) was inoculated intracerebrally into 3-day-old mice. Beginning on sixth day, some inoculated mice showed signs of illness. Brain tissue emulsions from these sick animals were in turn pathogenic for new suckling mice on intracerebral inoculation. The agent isolated was serially propagated by intracerebral passage in suckling mice. It readily passed through Seitz filters, and cultures of the infectious brain emulsions proved sterile on bacteriological media. Three days later the virus was again isolated from blood of the monkey. Sixteen days after second sample of infected blood was taken, the monkey died, and as serum from the moribund animal was markedly icteric, the liver was submitted to Dr. Madureira Para for histopathological examination. He reported lesions compatible with a diagnosis of yellow fever. Although the isolated virus was not neutralized by a known yellow fever immune serum, serum samples from adult mice that had survived intracerebral inoculation of this virus reacted in a hemagglutination-inhibition (HI) test with several antigens of group B arbor viruses at dilutions up to 1:160. To study the immunologic relationship of the agent further, the following experiments were made.

Methods. Immunologic characterization. No special effort was made to determine biological properties of the agent beyond what was necessary to characterize it

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