and patient R.A.S. was given a dosage intermediate between that given to adults A.F. and R.L. Typhus fever in children is generally so mild that evaluation of any therapeutic agent is extremely difficult even when large numbers of cases are investigated. It was our impression that Chloromycetin therapy produced no untoward effects in these children and may have been of some benefit.

Conclusion. On the basis of these limited observations, it may be concluded that the administration of Chloromycetin to patients with typhus fever is a relatively safe procedure. Furthermore, the chemotherapeutic effect obtained in the few patients treated was sufficiently encouraging to warrant further tests with the drug. It is suggested that chloromycetin be employed in oral treatment of the next group of patients to be studied according to the following schedule; an initial dose of 40 mg per kilo body weight followed by a total daily dose of 35 mg per kilo body weight, given in divided amounts at 2-hour intervals, until obvious improvement in the patient's condition is noted; subsequently maintenance dose of 20 mg per kilo body weight per day given in divided amounts at 4-hour intervals, until the 13th or 14th day after onset.

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Active and Inactive Murine Poliomyelitis Virus as Interfering Agents Against Poliomyelitic Infection in Monkeys.*

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It has previously been shown that live mouse-adapted human poliomyelitis virus has the ability of interfering with the propagation of simian poliomyelitis virus in rhesus monkeys. Such interference is demonstrable (a) by the intracerebral injection of a mixture of murine (SK, MM) and simian (Aycock, RMV) virus, or (b) by the peripheral administration of large amounts of murine virus during the early incubation period of poliomyelitic infection.[1] The mechanism of this antagonism is not known even though several hypotheses have been advanced as possible explanations.[2,3]

In an attempt to throw more light on this problem it seemed of interest to investigate whether the interfering effect can be elicited with inactivated or attenuated murine virus as well as with active virus. The following report deals with an exploration of this question. The murine virus used was the MM strain, the simian virus was the Aycock strain. The methods chosen for inactivation were (a) desiccation, (b) exposure to radioactive sodium, (c) ultraviolet irradiation, and (d) chlorination.

Methods: Desiccation. Brains from infected mice were harvested at the height of paralysis, placed in an open Petri dish, and dried in a desiccator over sodium hydroxide at 37°C., 20-22°C or 4°C for various lengths of time. The procedure was carried out either *in vacuo* or in nitrogen atmosphere. It was found that the virus preserved its full virulence (10^{-9} to 10^{-10} i.c. or i.p.) for the longest period tested, *i.e.* 20 days, when dried in the icebox at 4°C. At room temperature (20-22°C.) the virus suffered a progressive loss of virulence

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1 Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407; Sanders, M., and Jungeblut, C. W., *J. Exp. Med.*, 1942, **75**, 631; Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1942, **76**, 127.

2 Jungeblut, C. W., *J. Exp. Med.*, 1945, **81**, 275.

3 Gard, S., *Acta Med. Scand.*, 1944, **119**, 27.

which began at 5 days and became more marked at 10-15 days; after 29 days drying the remaining potency was such that mice could only be infected intracerebrally with a 10^{-1} dilution. This inactivation, however, could be largely prevented by drying in nitrogen atmosphere. When desiccation was carried out in the incubator (37°C) a rapid loss of virulence occurred so that virus dried for 5 days was barely infectious in a 10^{-1} dilution by intracerebral test; the same dose produced no symptoms after intraperitoneal inoculation. Virus thus attenuated was used for the later experiments.

Exposure to radioactive sodium. A 10% virus suspension, prepared from freshly harvested infected mouse brains, was combined in equal volumes (1 ml) with radioactive sodium (Na^{24}) in isotonic sodium chloride solution at pH 5-6.† The amounts of radio-sodium used varied from 20 to 120 mg NaCl and the radioactivity from 1 to 10 millicurie, as measured with the Geiger-Muller counter at the time of initial contact with the virus. After various periods of contact, ranging from 24 hours to one week in the icebox, the preparations were sampled and viral potency was determined. Within the first 48 hours there was little if any loss of virulence. After 96 hours contact, some of the preparations had deteriorated to the extent that only 10^{-1} dilutions were infectious for mice, while other preparations apparently had retained their full virulence. No further change occurred after longer contact up to one week. Virus titrations could only be carried out by the intraperitoneal route, since intracerebral injections proved toxic for mice. Since the procedure gave extremely irregular results in repeated tests and failed to yield preparations which were non-infectious in a 10^{-1} dilution by intraperitoneal test in mice, none of these preparations were employed in later experiments. One more reason for abandoning these preparations was the fact that virus attenuated by radioactive sodium proved capable of causing a lethal burn in the brain of a monkey following intracerebral injection, even though the mixture, through standing for 4 days, had lost the greater part of its initial radioactivity.

Ultraviolet irradiation. Through the courtesy of Dr. S. O. Levinson of the Michael Reese Research Foundation, Chicago, an opportunity was afforded to test several murine virus preparations which had been inactivated by flash exposure to extreme ultraviolet irradiation with the high power, low pressure lamp used by the Chicago group of workers. The exposure time varied from 0.2 to 0.5 second. Preparations irradiated for 0.3 second proved uniformly non-infectious for mice in 10^{-1} dilution by i.c. or i.p. test.

Chlorination. Solutions of sodium hypochlorite were prepared which contained various amounts of the substance ranging from 42 parts per million to 4200 parts per million. Equal volumes of the NaClO solution and of a non-centrifuged 10% viral mouse brain suspension were combined and samples were tested at different lengths of contact for infectivity in mice. It was found that concentrations of 42, 420, 1250 and 2100 ppm had caused no deterioration of potency. However, concentrations of 3150 ppm regularly inactivated the virus completely after 30-45 minutes contact in the icebox, as determined by i.c. and i.p. tests in mice. Material thus prepared, without neutralization of the residual chlorine, was used for later experiments in monkeys.

Interference experiments in monkeys. A number of experiments were run in which rhesus monkeys, following intracerebral infection with 50-100 minimal paralytic doses of Aycock virus (0.5 ml 1:100 *glycerinated* cord suspension) received daily injections by peripheral routes (intravenous, intraperitoneal) of massive doses (10-15 ml of 10% viral mouse brain suspension) of murine virus inactivated by desiccation, by ultraviolet irradiation or by chlorination. These injections were commenced on the day of infection and maintained during the incubation period of the disease, but in no case exceeding 12 days.

† The sodium isotope used in these experiments was prepared in the Radiation Laboratories of the Physics Department, Columbia University, and was kindly supplied by Dr. Allan F. Reid.

TABLE I.

Comparison of the Efficacy of Active and Inactive MM Murine Virus as Interfering Agents Against Infection with Simian Virus in Monkeys.

Interference test.						Controls				
			Result						Result	
Interfering agent	No. of monkeys	Simian virus (glycerinated cord)	Com. P†	Partial P	No P	Type of control	No. of monkeys	Simian virus (glycerinated cord)	Com. P	Partial P	No P
Active MM virus*	24	0.5 ml Aycock virus 1:100 i.c.	3	5	16						
Inactive MM virus (Desiccation)*	6	"	5	1	0	Untreated	19	0.5 ml Aycock virus 1:100 i.c.	16	3	0
Inactive MM virus (Ultraviolet irradiation)*	5	"	4	1	0						
Inactive MM virus (Chlorination)*	16	"	11	0	5	Untreated	9	"	9	0	0
						Normal mouse brain	3	"	3	0	0
						Chlorinated normal mouse brain	5	"	4	1	0
						NaClO	2	"	2	0	0

* Dose administered: 10-15 ml 10% viral mouse brain suspension daily for a maximum period of 12 days. † P = Paralysis.

TABLE II.

Chlorinated MM Murine Virus Used as Interfering Agent Against Infection with Simian Virus in Monkeys.

Interference test						Controls				
			Result					Result		
Interfering agent	No. of monkeys	Simian virus (fresh cord)	Com. P†	Partial P	No P	No. of monkeys	Simian virus (fresh cord)	Com. P	Partial P	No P
Inactive MM virus (chlorination)*	8	0.5 ml Aycock virus 1:100 i.c.	8	0	0	1	0.5 ml Aycock virus 1:100 i.c.	1	0	0
	4	0.5 ml Aycock virus 1:1000 i.c.	4	0	0	1	0.5 " " " 1:1000 "	1	0	0
						1	0.5 " " " 1:2000 "	1	0	0
	4	0.5 ml Aycock virus 1:2000 i.c.	4	0	0	1	0.5 " " " 1:10,000 "	1	0	0
						1	0.5 " " " 1:50,000 "	1	0	0
						1	0.5 " " " 1:100,000 "	1	0	0

* Dose administered: 10-15 ml 10% viral mouse brain suspension daily for a maximum of 12 days.
† P = Paralysis.

An appropriate number of monkeys, infected intracerebrally with the same dose of Aycock virus, and left either untreated or treated with 10% normal mouse brain suspension, with chlorinated normal mouse brain suspension, or with sodium hypochlorite solution (3150 ppm), accompanied these experiments. To provide a basis for comparing the relative efficacy of the several inactivated virus preparations with that of active virus, a number of monkeys were included which received similar injections of unmodified 10% viral mouse brain suspension following intracerebral infection with the same dose of Aycock virus. The results are brought together in Table I.

It appears from the data given in Table I that monkeys which had received ultraviolet-irradiated or desiccated MM virus developed paralysis in the same manner as did the corresponding control animals. When chlorinated MM virus was used as interfering agent there was some evidence of slight but irregular protection. Thus, of a total of 16 monkeys treated in this manner, 5 animals (31.2%) escaped paralysis, whereas all of 19 control monkeys succumbed to the disease. By contrast it will be seen that in a group of 24 monkeys which had received unmodified murine virus, 16 animals (66.6%) remained free from paralysis as compared with a morbidity rate of 100% in a control group of 19 untreated monkeys. The latter figures are in close agreement with the experience previously collected in similar experiments.

In order to determine whether the results obtained with chlorinated murine virus could be duplicated in monkeys infected with a larger dose of simian virus, the experiment was repeated with another group of monkeys. The interfering agent was again MM virus, inactivated by chlorination (2600-3500 ppm sodium hypochlorite), but the infecting dose of Aycock virus ranged from at least 50-1000 minimal paralytic doses (0.5 ml of 1:100 to 1:2000 *fresh* monkey cord suspension). An adequate number of untreated control monkeys accompanied this experiment. The results are shown in Table II.

It will be seen from Table II that all of the treated monkeys developed the typical disease as did the corresponding control animals.

Conclusions. The sum total of the work reported leads to the conclusion that MM virus, inactivated by exposure to various physical and chemical agencies, is unable, as a rule, to prevent the development of paralysis in monkeys infected with simian virus under the conditions employed in these tests. The slight and irregular protection observed on occasion with the use of chlorinated murine virus is exceptional and operates only against limited doses of simian virus. The results are, therefore, in marked contrast with the high rate of non-paralytic survival that is obtainable when unmodified active virus is used as interfering agent in comparable amounts. While interference with active murine virus still offers the only successful approach—known at present—to a prevention of the experimental disease, the fact that inactivation of the virus causes a definite qualitative or quantitative alteration of the effective interfering factor opposes serious difficulties to a possible application of this method for human use.