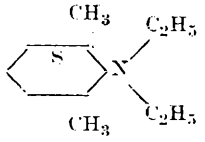
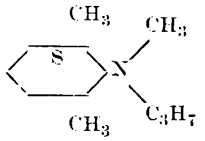
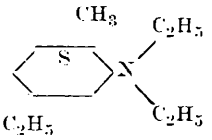
TABLE I (Continued)

No.	Formula: Cation	Anion	Mol wt of base	Control (C): LD_{50}, mM/kg	Control (C): f*	Control (C): No. of animals	Nembutal (N), 66 mg/kg: LD_{50}, mM/kg	Nembutal (N), 66 mg/kg: f*	Nembutal (N), 66 mg/kg: No. of animals	Protection ratio, N/C
11.		Br	170.3	.100	1.27	48	.114	1.24	48	1.14
12.		Br	170.3	.220	1.22	48	.304	1.28	48	1.38†
13.		Br	184.3	.104	1.32	42	.189	1.33	48	1.82†

* The "f" of a term is the factor by which the term is multiplied and divided to determine its confidence limits at $P = 0.05$.

† Significant increase in LD_{50}.

‡ The S in the ring indicates complete saturation (piperidinium ring).

quaternary ammonium derivative, reduces the toxicity of all but 4 compounds. These 4 compounds have methyl branches on 2 of the alpha carbons and at least one of the other substituents on the nitrogen is an ethyl group.

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Inhibition of Hemagglutination of Columbia-SK Virus by Human Polio-convalescent Sera.* (18888)

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Discovery of the fact that Columbia-SK virus causes hemagglutination of sheep red cells(1) permits use of the phenomenon to determine the presence of hemagglutination-inhibitory antibodies in human sera against this virus and related strains (MM, EMC, Mengo, F). A standard method was recently developed by Gard and Heller(2) for the assay of such antibodies against MM virus in the sera of patients suffering from various neurotropic infections. When applied to a series of 569 human sera this test yielded 12.5% strongly positive reactions in a group of 384 sera collected from patients with a diagnosis of paralytic or non-paralytic poliomyelitis, aseptic meningitis or encephalitis. By contrast, only 0.7% similarly positive reactions were encountered in a control group

* Aided by a grant from the Sister Elizabeth Kenny Foundation.

1. Hallauer, C., *Proc. IVth Internat. Congr. Microbiol.* (July 1947), Copenhagen, 1949, p257; Verlinde, J. D., and de Baan, P., *Ann. Inst. Pasteur*, 1949, v77, 632; Olitsky, P. K, and Yager, R. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 719.

2. Gard, S., and Heller, L., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 68.

of 185 sera, including 39 samples from patients with various diseases showing no involvement of the central nervous system and 146 samples from healthy individuals. None of the positive human sera were capable of neutralizing the virus in mice. We have undertaken to repeat these experiments with a small number of convalescent sera from cases of poliomyelitis or of encephalitis and with "normal" human sera, using Col-SK virus as the test agent and employing a somewhat different but comparable technic(3). The results of this investigation are reported herewith.

Materials and methods. The poliomyelitis sera were obtained in the Fall of 1950 from paralytic and non-paralytic cases during the acute or subacute stage of their illness; the encephalitis sera were from patients convalescent from an attack of Jap B encephalitis in 1950.† The "normal" sera were collected from healthy donors at the Blood Bank of the Presbyterian Hospital, New York City. Prior to their use in the hemagglutination-inhibition test all sera had been absorbed with packed sheep red cells (1 hour at 37°C and overnight in the icebox) in order to remove any heterophile hemagglutinins. This procedure was adopted routinely because a majority of the polio-convalescent sera contained abnormally high levels of cold agglutinins against sheep erythrocytes(4). The virus was obtained from the brains of paralyzed passage mice. Several freshly harvested viral brains were pooled and ground with veronal-buffer(5) so as to make a 10% suspension. This suspension was lightly centrifuged and the supernatant fluid further diluted serially with the same diluent. Concurrent with the inhibition tests the hemagglutinating activity of each virus pool was titrated by combining 0.2 cc of the various virus dilutions with 0.2 cc of a 0.25% suspension of washed sheep red cells in veronal-buffer and adding 0.2 cc of veronal-buffer to each tube. The sheep cells had previously been collected in Alsever's solution and were stored in the icebox for a period not exceeding 2 weeks. The hemagglutinating system was placed in the icebox and the reactions were read the following morning. With the use of veronal-buffer and the choice of suitable sheep erythrocytes viral hemagglutination resulted not in pattern, but in compact aggregation of cells which could be broken up only after shaking the tubes. No such reactions were obtained with normal mouse brain. The smallest amount of virus which produced clumping, clearly visible by macroscopic observation, was designated as one hemagglutinating unit. Under the conditions of our tests the hemagglutinating activity of the several virus preparations was remarkably constant, one unit usually being equivalent to a 1:640 to 1:1280 brain dilution. For the hemagglutination-inhibition test the absorbed sera were serially diluted (1:5-1:1280) with veronal-buffer and 0.2 cc of the various serum dilutions were mixed with a fixed dose of virus, *i.e.* 0.2 cc of a 1:160 viral brain dilution calculated to equal 4 or 8 hemagglutinating units. The serum-virus mixtures were held for 1 hour at room temperature and 0.2 cc of a 0.25% sheep red cell suspension was added to each tube. The reactions were read after overnight refrigeration together with those of the virus titration. Controls with normal and immune monkey sera, as well as cells alone in veronal-buffer, were included in each test. Whereas normal monkey sera never inhibited viral hemagglutination, many of the human sera gave partial hemagglutination inhibition. This necessitated a somewhat arbitrary evaluation of the results in order to separate definitely positive reactions from reactions of doubtful significance. A standard code was finally adopted under which absence of hemagglutination was considered "negative" when it occurred only in serum dilutions below 1:10, "questionable" when it occurred only in serum dilutions below 1:20,

3. Horvath, B., and Jungeblut, C. W., *Fed. Proc.*, 1951, v10, 359.

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4. Jungeblut, C. W., Unpublished data.

5. Mayer, M. M., Croft, C. C., and Gray, M. M., *J. Exp. Med.*, 1948, v88, 427.

TABLE I. Code for the Interpretation of Hemagglutination-Inhibition of Col-SK Virus (4-8 H.U.) by Human Sera.

Sera	Serum dilutions 1:5	1:10	1:20	1:40	1:80	1:160	1:320	Result
Human serum 1	*1*	4	4	4	4	4	4	Negative
" " 2	0	*1*	4	4	4	4	4	"
" " 3	0	0	*1*	4	4	4	4	Questionable
" " 4	0	0	0	*1*	4	4	4	Positive
" " 5	0	0	0	0	0	*1*	4	"
Normal cynomolgus serum	4	4	4	4	4	4	4	Negative
Immune Col. SK cynomolgus serum	0	0	0	0	0	0	1	Positive

0 = no hemagglutination, 1 (2-3) = partial hemagglutination, 4 = complete hemagglutination.

TABLE II. Hemagglutination-Inhibition of Col-SK Virus by Sera from Normal Persons and by Sera from Acute Cases of Poliomyelitis or Japanese B Encephalitis.

Sera	No.	Hemagglutination-Inhibition Negative	Questionable	Positive
		%	%	%
Normal sera (New York City)	80	74 (92.5)	4 (5)	2 (2.5)
Poliomyelitis sera (Jersey City)	40	39 (80)	4 (8)	6 (12)
Poliomyelitis sera (Minneapolis)	8	4 (50)	1 (13)	3 (37)
Total poliomyelitis sera	57	43 (75)	5 (9)	9 (16)
Jap B encephalitis sera (Tokyo)	12	12	0	0

and "positive" when it occurred in serum dilutions of 1:20 or above. An illustration of the basic principle is given in Table I.

Results. The results of the inhibition tests (Table II) show that a small percentage (16%) of the polio-convalescent sera gave strongly positive reactions, whereas the Jap B encephalitis sera were uniformly negative. The control series of "normal" sera showed an insignificant incidence (2.5%) of strongly positive reactions. Of the 14 positive or questionable poliomyelitis sera 5 were positive (Index 100-1000) and 9 were negative when tested for neutralization of Col-SK virus in mice. None of the Jap B encephalitis sera had neutralizing antibodies for Col-SK virus but all, except 2, neutralized Y-SK virus.

Discussion. Our observations are in essential agreement with those reported by Gard and Heller(2) from Sweden and with the experience of Keller(6) in Germany. They indicate that a small percentage of sera from poliomyelitis patients, collected during the early stage of their illness, are capable of bringing about definite inhibition of hemagglutination of sheep red cells by Col-SK virus. This property was not destroyed by heating the sera for ½ hour at 56°C. Similarly positive reactions were absent in a small group of sera from patients convalescing from another neurotropic virus infection (Jap B encephalitis) and were only found sporadically among sera from healthy individuals. Yet, the data do not permit the assertion that this reaction reflects a specific response to infection with this virus because serological reactivity was not followed through the course of the disease. Moreover, hemagglutination-inhibition and neutralization reactions, though occurring together in some sera, showed no constant relationship. It is impossible, at present, to say whether hemagglutination-inhibition and neutralization measure different antibodies in the human sera or whether the discrepancy between the *in vitro* and *in vivo* methods is due to quantitative differences in the operation of the same antigen-antibody reaction. Nevertheless, the data are in general agreement with our earlier observations, according to which significant neutralizing

6. Keller, W., Personal communication.

antibody levels against Col-SK virus could be demonstrated in 28 (21%) of 132 sera from patients, residing in Greater New York, who had been diagnosed as paralytic or non-paralytic poliomyelitis(7). Recent studies of poliomyelitis occurring in certain areas of Mexico revealed an even higher frequency of neutralizing and hemagglutination-inhibitory sera among cases and contacts(8). Barring any other plausible explanation for such differences, the possibility should be considered that geographic variation in the distribution of the virus may be a factor concerned with the selectivity of the serological results.

Summary and conclusions. In a series of 57 sera collected from poliomyelitis patients during the acute or subacute stage of their illness, 9 (16%) gave strongly positive reactions, 5 (9%) gave questionable reactions and 43 (75%) gave negative reactions when tested for the presence of hemagglutination-inhibitory substances against Col-SK virus. All of 12 sera from patients convalescing from Jap B encephalitis gave negative reactions. In a control group of 80 sera from healthy individuals 2 (2.5%) gave positive reactions, 4 (5%) gave questionable reactions, and 74 (92.5%) gave negative reactions. Of 14 positive or questionable poliomyelitis sera, 5 contained significant neutralizing antibody levels against Col-SK virus as determined by mouse test. The significance of these findings is discussed.

7. Jungeblut, C. W., *Arch. Ped.*, 1950, v67, 519.

8. Jungeblut, C. W., and Bautista, G., In preparation.

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Isolation of an Active Polysaccharide Fraction from Plague Organisms.* (18889)

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An attempt to isolate an active polysaccharide from the supernatant of the Haffkine plague vaccine was made by Shrivastava(1). The substance reacted with antiplague horse and rabbit sera but the yield from a large quantity of supernatant (200 litres) was extremely low. The protein-free casein hydrolysate medium of Mueller and Johnson(2) as modified by Seal and Mookerji(3) offered an opportunity to reopen the investigation. The results of the trials to isolate the polysaccharide fractions from 3 sources, namely, (a) supernatant of the Haffkine plague vaccine prepared in the casein hydrolysate broth, (b) bacterial debris of the same vaccine, and (c) specific soluble protein of plague bacilli (Seal) (4) are reported below.

(A) *Isolation from the supernatant of the Haffkine plague vaccine. Strains used:* 1) Virulent plague strains 337/L and 139/L obtained from human plague cases; 2) Avirulent protective plague strain 53H/av originally obtained from a human plague case and made avirulent in the laboratory; 3) Avirulent non-protective plague strain TRU isolated by Schutze(5) from Java human strain Tjiwidej R(4) *Past-pseudotuberculosis* strains, PR/I and PR/IV, obtained from the National Type Cultures Laboratory, London.

Methods. Seven liters of bacteria-free filtrate of 3 weeks' growth, at 28°C, of the virulent plague strain 337/L in casein hydrolysate broth at pH adjusted to 7.2-7.3, was re-

* This work was originally carried out in 1942-43 at the Haffkine Institute, Bombay.

1. Shrivastava, D. L., *Annual Rep. Haffkine Inst., Bombay,* for 1938, p. 40.

2. Mueller J. H., and Johnson, E. R., *J. Immunol.,* 1941, v40, 33.

3. Seal, S. C., and Mookerji, S. P., *Ann. Biochem. and Exp. Med.,* 1950, v10, 79.

4. Seal, S. C., *Annual Rep. Haffkine Inst., Bombay,* for 1940-41, p. 48; *J. Immunol.,* 1951, in press.