that the presence of sulfathiazole tends to increase and of sulfamerazine to decrease the lethal effect of combined sulfonamides on gram negative bacteria. On the other hand, the addition of sulfadiazine increases the bactericidal potency for gram positive ones. The combination producing the greatest depression of antibacterial activity for all organisms, regardless of their tinctorial reaction, appears to be one containing sulfamerazine, sulfadiazine, and sulfathiazole.

Conclusions. 1. The antibactericidal activity of various mixtures of sulfonamides is unpredictable. 2. The observed effect varies markedly with different species and with various strains of the same species of organism. 3. Depression of bactericidal potency against both gram negative and positive bacteria frequently results from combining sulfonamides. 4. The greatest and most constant decrease in antibacterial effect is produced by mixing sulfamerazine, sulfathiazole, and sulfadiazine. 5. These results suggest that combinations of sulfonamides may have little clinical usefulness in many instances, since they are often not as effective as the single agents which compose them.

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Studies on Passive Immunity in Poliomyelitis. V. Lansing Antibody Levels in Humans after Gamma Globulin Administration.* (19678)

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(Introduced by R. C. Parker.)

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Gamma globulin has been used successfully in man not only for the prevention or attenuation of measles, but also mumps(1) and infectious hepatitis(2,3). There is thus some justification for an investigation of gamma globulin in the prophylaxis of poliomyelitis; this possibility has been discussed by Hammon(4), who concludes that the available evidence warrants a large scale trial. The present paper reports a study that has been carried out to determine poliomyelitis antibody levels in serum after inoculation of the doses of gamma globulin likely to be used in such a study. Advantage has been taken of the fact that gamma globulin contains antibody to the Lansing as well as the other types of poliomyelitis virus(5-7), so that tests for antibody can be carried out in mice.

Materials and methods. Persons inoculated. It was necessary to examine the sera of a considerable number of persons in order to find those whose serum contained no Lansing antibody. Fifty-one children were bled at the Home of the Imperial Order of the Daughters of the Empire, Toronto. These children were from one to 11 years of age, and were hospitalized for tuberculosis, some being convalescent. The use of such patients offered the advantage that temperatures were regularly recorded, and any reactions would be quickly noted. Sera were tested for Lansing antibody by a screening test, the details of which are given below. The sera of 19 children were found to be free of demonstrable Lansing antibody. The sera of over 300 adults attending the Blood Bank at The Hospital for Sick Children, Toronto, were tested by a similar method, and the sera of 30 were found to be free of demonstrable Lansing antibody. It may be stated that in no case did any patient complain of more than mild local discomfort after the inoculation; there were no febrile reactions. *Gamma globulin.* Gamma globulin prepared from pools of human plasma was supplied through the courtesy

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of the American National Red Cross by E. R. Squibb & Sons, New Brunswick, N. J. The lot used has been studied in this laboratory, and contains sufficient Lansing antibody to protect mice against a cerebral challenge(7).

Inoculation procedures. Six children were inoculated with gamma globulin by the intramuscular route in dosages of 5 to 15 ml, not more than 7.5 ml being given in one buttock. Blood samples were taken before the injection, 4 days thereafter, and at weekly intervals for 6 weeks. Fourteen weeks after the intramuscular injection, the children were again bled and were then given gamma globulin by mouth; 4 received the same dose as given intramuscularly, and 2 received double the previous dose. Blood was taken weekly for 6 weeks. Four adults were inoculated with 20 ml gamma globulin intramuscularly, 10 ml in each buttock. Blood was taken at 4 days and at weekly intervals for 6 weeks. At a time varying from 11-14 weeks after the intramuscular injection, 2 of the adults were given 20 ml of gamma globulin orally, and the other two 40 ml. Blood was taken for a period of 6 weeks. Full details are shown in Table I.

Tests for Lansing antibody. Tests were carried out by the technic used in these laboratories for several years(8,9). In brief, to serial dilutions of serum was added sufficient Lansing virus-infected mouse pool to give a final amount of 100 LD_{50}. Virus-serum mixtures were held at room temperature for 1½ hours and then injected into groups of 10 or 20 mice. Mice were observed for 4 weeks. Appropriate virus controls were included in each batch of tests. In the preliminary screening tests, serum was used undiluted and a smaller dose of virus (35 LD_{50}) was added; mice were observed for 3 weeks.

Results. Tests on the sera of the persons inoculated with gamma globulin by the intramuscular route showed that only a small amount of neutralizing antibody was detected in the undiluted serum; no evidence of antibody was found in any test in which dilutions of serum were employed. The results of testing undiluted serum-virus mixtures are shown in Table I. In practically every instance, all of 20 mice died when inoculated with a mixture of virus and undiluted serum obtained

TABLE I. Lansing Antibody Content of Serum after Administration of Gamma Globulin.

Initials	Intramusc. inoculation of gamma globulin: Amt/lb body wt (ml)	Mice surviving when inoculated with virus and serum obtained at following times*: Before inj.	Day 4	Week 1	2	3	4	5	6	Interval between intramusc. and oral administration (wk)	Oral administration of gamma globulin: Amt/lb body wt (ml)	Mice surviving when inoculated with virus and serum obtained at following times*: Before inj.	Week 1	2	3	4	5	6
Children																		
LC	.24	0/20	4/19	13/20	4/20	13/20	7/20	8/20	5/20	14	.24	1/16	0/10	0/10	0/10	0/10	0/10	0/10
JMi	.42	0/20	1/20	6/20	5/20	0/20	1/20	0/19	0/19	14	.42	0/20	0/10	0/10	0/10	0/10	3/10	2/10
JMo	.13	0/20	5/19	5/20	1/19	0/20	0/20	3/20	0/20	14	.13	1/16	0/10	0/10	1/10	0/10	1/10	—
ER	.22	0/20	8/20	7/19	1/20	3/19	0/19	0/19	1/20	14	.22	0/19	1/10	0/10	1/10	2/10	0/10	2/10
PG	.25	0/20	7/20	3/20	7/20	3/20	6/20	4/19	5/20	14	.50	0/20	0/9	1/10	0/10	0/9	0/8	0/9
DR	.12	1/20	8/20	2/20	8/20	1/18	1/20	1/19	2/20	14	.24	1/20	0/10	0/10	0/9	0/10	0/10	0/10
Adults																		
DED	.11	0/19	4/20	4/20	2/20	2/19	0/20	0/20	1/20	11	.11	0/7	0/10	0/10	0/9	0/9	0/9	1/9
JDM	.14	0/20	1/20	0/19	1/19	0/20	0/20	0/19	0/20	14	.14	0/18	0/9	—	0/9	0/10	0/10	0/10
TBB	.14	2/19	7/20	1/19	3/20	2/20	1/17	4/19	5/20	13	.28	3/20	0/9	1/9	1/10	1/9	1/10	3/9
BM	.15	0/19	13/20	3/19	4/19	5/19	3/20	2/19	4/20	12	.30	0/19	0/10	0/10	0/10	0/10	0/10	0/10

* All tests carried out with a final amount of 100 LD_{50} of Lansing virus and undiluted serum.

before inoculation, indicating absence of antibody. However, when the samples taken at 4 days and one week after inoculation were similarly tested some mice survived. With certain persons some protective capacity was found in serum taken 2 weeks or more after inoculation. It will be noted that sera obtained after oral administration did not protect any mice.

Discussion. It can be concluded that after the inoculation of gamma globulin by the intramuscular route in doses varying from 0.1 to 0.4 ml per pound body weight only small amounts of antibody appear in the serum. Neutralizing antibody was present for 2 weeks or sometimes longer. It is probable that by titrating the sera in tissue culture, a technic which is sensitive to smaller amounts of antibody, a longer period of persistence could be demonstrated. After the oral administration of gamma globulin no serum antibody could be detected. The dosages of gamma globulin investigated are as high as can be given in field trials. Antibody levels, decline logarithmically, and the half life of I^{131} homologous gamma globulin in man is 12-14 days(10). Accordingly, the neutralizing effect conferred on the serum by a more potent antibody-containing preparation passively administered should persist for several weeks. In this connection, it is of interest to note that low levels of serum antibody are apparently quite adequate to protect cynomolgus monkeys against challenge by the oral route(11). It is possible that quite small amounts of antibody may likewise serve to protect humans against virus entry through the pharynx or gastrointestinal tract.

Summary. 1. The sera of 51 children and over 300 adults were first tested for the presence of Lansing virus neutralizing antibody. 2. Of those whose serum contained no demonstrable antibody, 6 children and 4 adults were given gamma globulin intramuscularly in doses from 0.1 to 0.4 ml per pound body weight. 3. Blood was taken at frequent intervals for 6 weeks and tested for antibody. A small quantity of antibody was present in the serum, as shown by a protective effect of the undiluted sample in mice. This antibody was detected in some cases for a few weeks. 4. No antibody was found in the serum of the same 10 persons following the oral administration of gamma globulin.

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