

bohydrate solution was derived from the organism used, and contained arabinose. Similar antibacteriolytic power was shown in an experiment in which galactose-containing organisms and carbohydrate were used.

*Conclusions.* The vibrios which have been studied in this endemic area for cholera (Bengal) appear to fall into 3 groups on the basis of their carbohydrate content and agglutinability. In the majority of vibrios derived from cholera, galactose is the characteristic sugar. A second smaller group of vibrios, also from cholera, contain arabinose. These do not appear to differ from the first in agglutinability or in virulence, to judge from the type of case in which they have been found. The third group consists of non-agglutinable vibrios derived from water, some of which differ from *Vibrio cholerae* only in their failure to react with cholera agglutinating serum. All of these are arabinose-containing vibrios.

Polysaccharides containing both arabinose and galactose have been isolated from "rice-water" stools of cholera cases.

Both types of carbohydrate have an antibacteriolytic effect.

## 7147 P

### Experimental Poliomyelitis. Active Immunization with Neutralized Mixtures of Virus and Serum.\*

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Work on the nature of virus-antiserum combinations has indicated that although the virus in such mixtures is completely inactivated, the effect on the virus is not a destructive one, but more in the nature of a "neutralization" phenomenon. It is possible to recover active virus from neutral virus-serum unions by such means as dilution, and cataphoresis.<sup>1-5</sup> It is therefore not surprising that

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<sup>1</sup> Andrews, C. H., *J. Path. and Bact.*, 1928, **31**, 671.

<sup>2</sup> Douglas, S. R., and Smith, W., *Brit. J. Exp. Path.*, 1928, **9**, 213.

<sup>3</sup> Long, Perrin H., and Olitsky, Peter K., *J. Exp. Med.*, 1930, **51**, 209.

<sup>4</sup> Olitsky, Peter K., and Long, Perrin H., *J. Exp. Med.*, 1929, **50**, 263.

<sup>5</sup> Olitsky, Peter K., Rhoads, C. P., and Long, Perrin H., *J. Exp. Med.*, 1929 **50**, 273.

mixtures of virus neutralized by serum have been shown to possess antigenic properties.<sup>6, 7, 8</sup>

Utilizing these facts, with a view toward ultimate development of a safe and practical method for active immunization against poliomyelitis, applicable to humans, investigations were undertaken to determine the conditions under which a high incidence of immunity could be obtained in the experimental animal.

To that end, 2 series of animals, whose serums had previously been tested by the neutralization test to rule out the presence of neutralizing substance, were inoculated subcutaneously and intramuscularly, with neutralized mixtures. In the first series, varying proportions of virus and serum, the potencies of which were only roughly approximated, were used. The second series of animals received inoculations of accurately neutralized mixtures based on careful titrations of virus and serum. Two such balanced mixtures of serum and virus were used in this series; one containing 16 cc. of 5% virus suspension to 1 cc. of human convalescent serum, the other mixture containing 20 cc. of virus to 1 cc. of serum.

Since the purpose of this attempt at immunization was to determine the number of animals that would give a demonstrable response following injection of neutralized mixtures of virus and serum of known titre, the neutralization test was selected as the most desirable criterion for immunity. This is in accord with conclusions reached by Stewart and Rhoads<sup>9</sup> and by Brodie and Goldbloom,<sup>10</sup> that the neutralization test (using small doses of virus) is a more sensitive test for immunity than is the intracerebral test.

All monkeys were bled 4 to 6 weeks following the last inoculation, and the serums tested for the presence of immune substance by the neutralization test. The serums of the second series (12 animals) were tested against 10 infective doses of the titrated virus.

The results of these experiments are summarized in Table I.

The results indicate that a high incidence of immunity ( $\frac{3}{4}$  of the animals treated), as indicated by the neutralization test, follows inoculations with balanced neutral mixtures of serum and virus.

Experiments are now being conducted to determine the degree of this immune response and to what extent this can be developed.

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<sup>6</sup> Andrews, C. H., *J. Path. and Bact.*, 1929, **32**, 265.

<sup>7</sup> Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 185.

<sup>8</sup> Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 115.

<sup>9</sup> Stewart, F. W., and Rhoads, C. P., *J. Exp. Med.*, 1927, **49**, 959.

<sup>10</sup> Brodie, Maurice, and Goldbloom, A., *J. Exp. Med.*, 1931, **53**, 885.

TABLE I.

Series No.	No. monkeys	Inoculum virus-serum	Tot. amt. inoculated, cc.	No. inoculations	Interval between inoculations, days	Results of neutralization tests on serums of treated monkeys
1	6	1:1	5	1		.5 cc. serums of 2 monkeys neutralized .5 cc. of a 5% virus suspension.
		3:1	5	1	*	Serums of 3 monkeys failed to neutralize.
		4:1	10	1		One animal died of tuberculosis.
		5:1	12	1		
2†	3	16:1	30	4	7	.5 cc. serums of 2 of 3 monkeys failed to neutralize 10 infective doses of titrated virus.
	3	16:1	60	7	7	
	3	20:1	30	4	7	
	3	20:1	69	7	7	.5 cc. serums of 3 monkeys neutralized at least 10 infective doses of titrated virus.

\* Each animal received the 4 inoculations at weekly intervals.

† The potency of the serum and virus used in this series was determined by titration and was kept constant throughout the experimental period.

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