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Observations on the Intranasal Route of Infection in Experimental Poliomyelitis.*

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It is generally recognized that while experimental poliomyelitis may be induced with regularity in monkeys inoculated with a potent virus by the intracerebral route, infection results much less frequently when the virus is instilled into the nasal passages. Since the natural infection in man occurs chiefly, if not entirely, by the nasal route, it is sometimes desirable, especially in certain experimental studies, to imitate closely the natural mode of infection. To be really serviceable any method employed must produce infection with considerable regularity. While the results of different investigators with the intranasal route of inoculation have been but meagerly reported, the general experience seems to have been that while infection by the intranasal route is possible, it is seldom realized in more than one-half of the monkeys so inoculated. At times less than a third of the animals develop the disease. In exceptional instances only have somewhat better results been reported. Rhoads[1] and Weyer, Park and Banzhaf[2] have recently reported quite uniform results following 3 or more intranasal instillations of virus, *administered on successive days.* The total number of monkeys employed by these investigators is not specifically stated, nor do they describe in detail the procedure. The reasons for their more uniform results are therefore not apparent. Having ourselves worked with what were evidently the same strains of poliomyelitis virus, we are unable to attribute their exceptional results altogether to the employment of viruses of unusual virulence. It has become obvious to the writers that while the virulence of a virus, as well as the number of instillations, are undoubtedly of great importance in establishing infection by the intranasal route, factors other than these apparently also influence the results, including possibly such factors as the general anatomical and physiological status of the mucous membranes; the properties of the nasal secretions; the reaction (pH) of the aqueous

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1 Rhoads, C. P., *J. Exp. Med.*, 1931, **53**, 115.

2 Weyer, E. R., Park, W. H., and Banzhaf, *J. Exp. Med.*, 1931, **53**, 553.

vehicle of the virus, etc. Amoss and Taylor[3] reported that washings of nasopharyngeal mucous membranes of normal persons may at times possess the power to inactivate the virus of poliomyelitis. This ability to inactivate the virus was not constantly exhibited by secretions obtained at different times from the same individual. Flexner and Amoss[4] later revealed that the nasal mucosa of individual normal monkeys may also possess virus inactivating properties. The exact nature of the substance responsible for the inactivation was not determined, but appeared to be distinct from the specific immune substances which make their appearance in the blood of poliomyelitis convalescent animals and man. It is conceivable, therefore, that the condition of the nasal mucous membranes and of the nasal secretions of individual monkeys may influence to some extent the possibility of establishing infection by the intranasal route.

Needing a dependable extracerebral route of infection in some studies which we wished to complete, we made a more comprehensive inquiry into the possibilities of the intranasal route of infection. Working with the so-called Aycock strain, we had earlier found that ordinary nasal instillations, even though repeated on several successive days, failed to give more than 50% infection; nasal tampons saturated with virus, plus ordinary virus instillations, proved even less effective. Several monkeys inoculated by submucous injections of virus failed to develop poliomyelitis. Following this another strain of poliomyelitis virus (MV) was obtained from Dr. Simon Flexner and our studies were carried on with this strain. The results proved distinctly superior to those we had realized with the Aycock strain. Of a total of 9 monkeys given 3 intranasal instillations of this virus, all instillations being made on the same day, 7 (77%) developed the disease. Since a higher incidence of infection than this was desired, we endeavored to determine the effect of washing thoroughly the nasal passages immediately before each intranasal instillation of virus, employing a M/15 phosphate solution (primary potassium phosphate adjusted to pH 5.0 with secondary sodium phosphate), the nasal passages being thoroughly irrigated before each virus instillation with about 25 cc. of this solution. Since we have applied these preliminary nasal washes the number of infections seem to have been definitely increased. Up to the present out of a total of 42 monkeys inoculated according to this procedure 40 (95%) have developed poliomyelitis. The aver-

[3] Amoss, H. L., and Taylor, E., *J. Exp. Med.*, 1917, **25**, 507.

[4] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1920, **31**, 123.

age incubation period in this series of animals was about 8 days. The individual virus instillations, 0.5 cc. in amount each, were made at 3 hour intervals on the same day, a 10% virus suspension in physiological saline (pH 6.3) being employed. Just what effect the *acid* rather than alkaline virus suspension had in giving us a higher percentage of infection, was not determined. The virus suspensions were allowed to stand at refrigerator temperature (2°C.) from one to 4 days before the intranasal instillations were made. They were in each instance prepared from sections of cords and medullas derived from 4 or 5 monkeys; the sections being *pooled* and ground thoroughly in a motor driven mortar for at least one hour.

To determine whether the results realized were due merely to the washing itself, or to both the mechanical effect of washing and the reaction (pH) of the solution used, preliminary intranasal washings were carried out with phosphate buffer solutions of different pH values.† Of 3 monkeys receiving intranasal washes with M/15 phosphate buffer of pH 7 only 1 developed poliomyelitis; of 2 washed with M/15 phosphate buffer of pH 8.3 neither developed the disease. Of 4 washed with Sorensen's borate buffer, pH 8.9, 2 developed the typical disease; one developed an abortive form of poliomyelitis 17 days after inoculation and one failed to develop the disease.

Summarizing our observations, we may state that our results indicate that the incidence of infection by the intranasal route may be definitely increased by preliminary intranasal washes with acid buffer solution. Alkaline solutions appear less effective. Our results also show that with a virus of high infectivity, 3 intranasal instillations, preceded by intranasal washes, will result in infection in about 95% of the animals so treated. These virus instillations may all be made on the same day, thus enabling one to date the infection with more accuracy than is true when the instillations are made on successive days over a period of 3 days to a week, as is the common practice.

† We have observed that the nasal secretions recovered by washing the nasal passages of monkeys may shift the physiological saline from pH 6.5 to pH 7.15, a shift from the acid to the alkaline side.