Reproducible Oral Infection Rates in Monkeys with the Mahoney Strain (Type 1) of Poliomyelitis Virus.* (20498)

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Reproducible and consistent rates of infection of monkeys with the virus of poliomyelitis given orally have not in the past been successful. A variety of vehicles for suspension of the virus have been tried, including water, physiological sodium chloride, milk and butter, milk-soaked bread, bananas, etc. The virus-vehicle mixtures were either fed by means of a syringe or offered to the monkeys for voluntary consumption(1-6). Bodian and Howe(7) using 4 poliomyelitis virus strains (Lansing, Brunhilde, Kotter, and Per) were unable to infect either *M. mulatta* or cynomolgus monkeys by giving virus orally by means of a syringe. Using the Y-SK strain (Type 2) of poliomyelitis virus, Sabin(8) was able to infect 54% of the monkeys when milk was given prior to oral virus feeding. The virus was given twice daily for 3 days. Of 10 controls receiving no milk and one-half as much virus as above, none developed paralytic poliomyelitis. Subsequent studies by Bodian(9) failed, however, to confirm the above data. In these studies, 5 strains of virus were used (Per, Y-SK, Leon, Mahoney, and Wfd) representing all 3 antigenic types. Infection rates were, however, not consistent, varying from 0 to 50%.

With increasing interest in chemoprophylactic and chemotherapeutic studies in experimental poliomyelitis, and considering the fact that the intracerebral and intranasal routes of infection are too severe to screen compounds, a more suitable method for gastrointestinal infection was highly desirable.

Methods. The Mahoney strain (Type 1)† of poliomyelitis virus was used. Cynomolgus monkeys from the Philippine Islands were utilized. In one experiment, bread soaked with a milk and virus mixture was given in regular feeding pans. In the remainder, virus was given by stomach tube attached to a syringe, using a Fr. No. 16 urethral catheter, after 48 hours of fasting. Water was offered *ad lib.* during the fasting period

Results. A comparison was first made on the efficacy of voluntary feeding of bread soaked with a mixture of virus and milk, with the same quantity of virus in milk given by stomach tube. The animals were fasted for 24 hours prior to the virus being given. Normal feeding was resumed 24 hours after the virus. The results presented in Table I show that virus given by stomach tube produces a higher infection rate than by simple *ad lib.* virus feeding. Also the problem of "virus splashing" about the cage is eliminated and more accurate dosages can be given by stomach tube.

An attempt was made to titrate the Mahoney virus by the oral route of infection to determine, if possible, a reproducible PD_{50}‡ dose of virus. In this group of monkeys, virus was given in milk by stomach tube after 48 hours of fasting to eliminate as much as possible residual food in the small intestines. The results presented in Table II show that repeated daily doses of virus are superior to a single dose.

TABLE I. Comparison of Infection Rates of Monkeys Fed Virus in Milk and Virus-Milk Mixture Given by Stomach Tube.

No. monkeys	Virus	Fate	% paralyzed
4	Fed ad lib 5 ml 20% Mahoney virus in 25 ml milk soaked on half slice of bread	2/4*	50
5	5 ml 20% Mahoney virus in 25 ml milk given by stomach tube	4/5	80

* 2/4 = 2 monkeys developed paralysis out of 4 inoculated.

‡ PD_{50} = Dose of virus producing paralysis in 50% of the animals fed with virus.

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† A sample of this virus was kindly supplied by Dr. David Bodian, Johns Hopkins University.

The next group of animals was tested for the influence of alkaline chemicals and milk fat upon the rate of infection. All animals fasted for 48 hours. Chemicals, milk, cream, and virus were all given by stomach tube. Market milk was used (homogenized and pasteurized) with a fat content of 3.5%; the market cream used was a commercial product (Half and Half) with a fat content of 12%. The results presented in Table III suggest that milk fat rather than alkaline chemicals protects the virus during its sojourn in the stomach.

From the data obtained above (Table III) it was decided to determine whether the amount of milk fat (cream) influenced infection rates, when virus and cream mixtures were given by stomach tube. Thus, Half and Half (12% fat), table cream (18% fat), and whipping cream (33% fat) were used. Virus-cream mixtures were stirred with a glass rod attached to an electric motor, the speed being adjusted so as to prevent frothing. The virus-cream mixture was kept in an ice bath and stirred for 40 minutes. All animals fasted for 48 hours before virus was given.

TABLE II.
Titration of Mahoney Virus by Oral Route.

No. monkeys	Virus	Fate	% paralyzed
5	Single dose. 5 ml 10% virus in 25 ml milk by stomach tube	1/5*	20
4	3 doses same day. 5 ml 5% virus in 25 ml milk by stomach tube	2/4	50
4	3 doses same day. 5 ml 2.5% virus in 25 ml milk by stomach tube	2/4	50

* 1/5 = One monkey developed paralysis out of 5 given virus.

TABLE IV. Effect of Cream on Infection Rate.

No. monkeys	Virus	Fate	% paralyzed
5	5 ml 20% virus in 25 ml half and half cream. Single dose	3/5*	60
5	10 ml half and half cream 1½ hr before virus; then 5 ml 20% virus in 25 ml half and half cream. Single dose	0/5	0
5	5 ml 20% virus in 25 ml table cream. Single dose	2/5	40
5	5 ml 20% virus in 25 ml whipping cream. Single dose	2/5	40

* 3/5 = 3 monkeys developed paralysis out of 5 given virus.

The results presented in Table IV suggest that Half and Half cream (12% fat) and virus mixtures is optimal for reproducible infection rates when virus is given orally in a single dose.

In subsequent chemoprophylactic experiments using the Half and Half cream-virus (Mahoney) mixture (5 ml of 20% virus in 25 ml cream) given by stomach tube, 28 out of 50 control monkeys (representing 5 individual experiments) became paralyzed. This is a consistent and reproducible infection rate of 56%. These results are presented in Table V.

Discussion. From the data presented, cream (12% fat)-virus (Mahoney) mixtures given orally in a single dose, by means of a stomach tube, produce consistent infection rates in cynomolgus monkeys. Multiple doses of virus, as carried out by other workers, have yielded not too constant results. Multiple doses given over periods of 2 or 3 days makes

TABLE III. Effect of Alkaline Chemicals and Cream on Infection Rate.

No. monkeys	Treatment	Virus	Fate	% paralyzed
4	Stomach wash with 2% $NaHCO_3$ 5 min. before virus	5 ml 20% virus in 25 ml milk + 250 mg Al $(OH)_3$. Single dose	2/4*	50
5	250 mg Al $(OH)_3$ in 5 ml H_2O one hr before virus	5 ml 20% virus in 25 ml milk. Single dose	2/5	40
5	———	5 ml 20% virus in 25 ml half and half cream. Single dose	3/5	60

* 2/4 = 2 monkeys developed paralysis out of 4 treated.

TABLE V. Reproducible Infection Rates in Cynomolgus Monkeys by the Oral Route of Virus Inoculation.

No. monkeys	Virus 20% single dose of 5 ml Mahoney virus in 25 ml cream (12%)	Fate	% paralyzed
10	Virus given by stomach	5/10*	50
10	tube 48 hr after	6/10	60
10	fasting	5/10	50
10		7/10	70
10		5/10	50

* 5/10 = 5 monkeys developed paralytic poliomyelitis out of 10 monkeys inoculated.

it difficult to predict the day of infection. In prophylactic as well as therapeutic studies, the day of infection is of considerable importance. Thus virus given orally in one inoculation which produces a consistent infection rate enables one to more nearly predict the time of infection.

Summary. A method of orally infecting cynomolgus monkeys is presented. Consistent and reproducible infections (56%) have been obtained using the Mahoney strain (Type 1) poliomyelitis virus mixed with cream (12% fat) and introducing the virus-cream mixture into the stomach by means of a Fr. No. 16 urethral catheter in a single dose.

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Selective Inactivation of Esterase by Alcohol. (20499)

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In the course of studies on the distribution of esterases in the organs of the dog, tissues were fixed in 2 different fixatives: a mixture of equal parts of absolute acetone and 95% alcohol, and neutralized 10% formalin, both chilled. Formalin was washed out thoroughly under the tap, and ice cold acetone was used as the first dehydrating agent. Previous experience has shown that failure to wash out the formalin or the use of alcohol for initial dehydration may result in distinct weakening of enzymatic activity. It was soon found that in formalin-fixed tissues the overall intensity of the alpha naphthol acetate reaction(1) was definitely higher and its distribution more uniform than in the acetone-alcohol-fixed ones. The latter often showed a patchy distribution of activity, and the surface layers of the tissues were more intensely positive than the deeper portions. On closer inspection of the slides it became obvious that the activity of some structures remained practically the same, regardless of fixation, while that of other structures such as the hepatic parenchyma, the renal tubules, and the bronchial mucosa became profoundly depressed. Evidently, the acetone-alcohol mixture had a tendency to destroy the enzyme at certain sites.

To determine the nature of the noxious agent, pieces of tissue were fixed in absolute acetone, 90% acetone, absolute alcohol and 95% alcohol. After fixation in acetone, either absolute or 90%, the pictures obtained were almost as good as after formalin. Absolute

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