Summary. 1. Five rats were given access to a 3% solution of sodium chloride, tap water, dry dextrose, and the saltless stock diet in 4 separate containers. 2. After adrenalectomy the rats greatly increased their intake of sodium chloride, decreased their intake of dextrose, but continued to eat about the same amount of saltless stock diet as before. They showed a small increase in water intake. 3. Daily treatment with desoxycorticosterone decreased the intake of sodium chloride solution and tap water to the normal levels and increased the dextrose intake nearly to the normal level. 4. Thus it was shown that adrenalectomized rats have a definitely decreased appetite for dextrose, as well as an increased appetite for sodium chloride.

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Armstrong Mouse Adapted Poliomyelitis Virus: Effect of pH of Inoculum, and of "Enteric Organism Filtrate."*

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pH OF THE INOCULUM. In reviewing the literature on experimental poliomyelitis, no reports have been found on the optimum pH of the inoculum for monkeys or other susceptible animals. Recently, Howitt noted that poliomyelitis virus suspended in broth of acid reaction appeared to accelerate the action of strains of poliomyelitis virus which were of low pathogenicity for monkeys.[1] At her suggestion, since we were working with the Armstrong adapted virus† in mice, experiments were made to determine definitely the possible effect of the pH of the medium in which the virus was suspended. By employing these inexpensive animals large numbers could be used and more conclusive results be obtained than by using monkeys.

Method. Virus suspensions were prepared by grinding a number of brain stems and cords from dying mice with alundum and saline to make a 20% suspension by weight. This was either employed immediately or in some cases was frozen in ampoules and stored

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1 Howitt, B., personal communication.

† The virus was obtained originally from Dr. C. Armstrong.

at –76°C as a stock suspension. The titer of this frozen virus was found to remain constant over a period of months.

Buffer solutions of pH 4.0, 5.0, 6.0 and 7.0 were prepared as follows:

pH 7.0—6.0 cc of M/15 $Na_2HPO_4 . 12H_2O$ and 4.0 cc M/15 KH_2PO_4 per liter of normal saline.

pH 6.0—1.0 cc of M/15 $Na_2HPO_4 . 12H_2O$ and 9.0 cc M/15 KH_2PO_4 per liter of normal saline.

pH 5.0—10.0 cc of N/10 CH_3COOH and 7.1 cc N/10 NaOH (both N/10 solutions in normal saline).

pH 4.0—10.0 cc of N/10 CH_3COOH and 2.0 cc N/10 NaOH (both N/10 solutions in normal saline).

These buffer solutions were autoclaved at 15 lb pressure for 20 minutes, then the pH was checked and any necessary adjustments made with sterile reagents.

Dilutions of virus were prepared by adding the required amount of buffer solution to the stock virus suspension. When low dilutions were required the pH of the final suspension was tested and correction made by adding 10% acetic acid.

When the pH 4.0 or pH 5.0 buffer was added to the virus suspension precipitation regularly occurred. Before inoculation resuspension was effected and inoculation performed quickly before separation occurred in the syringe. All inoculations were made intracerebrally under light ether anesthesia, employing a dose of 0.03 cc. A highly susceptible strain of Rockefeller Swiss mice‡ was used throughout. Other strains of Swiss mice were found to be unsatisfactory due to low susceptibility and irregularity of response. Mice were observed for 21 days and the time of paralysis and of death recorded. Those dying within 24 hours of inoculation were not included in the tabulation of results. In calculating the survival time for each group the survivors were considered as having lived only 22 days. In repeated titrations of virus, where groups of 12 or more mice were employed for each dilution, we found that within a relatively wide range of virus dilutions the mean length of survival, computed in the above manner is in most instances a satisfactory index of the amount of virus employed.

Experiments 1 and 2. Table I shows the combined results of 2 experiments in which groups of 6 mice were inoculated with virus suspensions in dilutions of 1:512 and 1:1024 in buffered saline of pH 4.0, 5.0, 6.0, and 7.0. Practically all mice died except at pH 7.0, where 4 of 18 survived. The mean length of survival is shown for

‡ Carsworth Farms, Inc., New City, N.Y.

TABLE I.
Experiments 1 and 2—Effect of Buffered Saline pH 4.0, 5.0, 6.0 and 7.0 and of Enteric Filtrate pH 4.5 on Mortality and Mean Survival Time of Mice.

		Dilutions			
		1:512		1:1,024	
Menstruum	pH	Mortality ratio	Mean days survival	Mortality ratio	Mean days survival
Buffered saline	4.0	12/12	7.6	12/12	9.1
Enteric Filtrate	*4.5*	*12/12*	*10.6*	*9/10*	*9.9*
Buffered Saline	5.0	12/12	12.1	6/6	13.3
" "	6.0	12/12	13.5	5/6	15.3
" "	7.0	9/12	14.6	5/6	18.5

each group. In groups of 6, certain irregularities may occur, but it is apparent from the table with the combined results of 2 experiments that the pH 4.0 suspension killed more rapidly than did any of the others and that there is a progressively increasing mean length of survival with increase of pH.

Experiment 3. In this experiment (Table II) a frozen stock virus was used, in dilutions of 1:500, 1:2,000, 1:8,000, 1:32,000, and 1:128,000. A buffer solution of pH 4.0 and one of pH 7.0 were used to prepare the dilutions and 8 mice inoculated with each suspension. In the lower dilutions where all mice died, the pH 4.0 suspension killed the mice in a shorter period of time. At a dilution of 1:8,000 of the pH 4.0 saline, 7 of 8 mice died, and 1 at pH 7.0. At dilutions of 1:32,000 and 1:128,000 of the pH 4.0 suspensions 3 and 2 mice died respectively, and none at the higher pH value.

TABLE II.
Experiments 3, 4 and 5—Effect of Buffered Saline and of Enteric Filtrate at Various Hydrogen Ion Concentrations on Mortality and Mean Survival Time.

		Dilutions									
		1:500		1:2,000		1:8,000		1:32,000		1:128,000	
Menstruum	pH	Mortality ratio	Mean days survival	Mortality ratio	Mean days survival	Mortality ratio	Mean days survival	Mortality ratio	Mean days survival	Mortality ratio	Mean days survival
Exp. 3											
Buffered Saline	4.0	8/8	6.7	8/8	7.5	7/8	14.5	3/8	20.1	2/8	21.1
Enteric Filtrate	*5.0*	*8/8*	*7.2*	*7/7*	*9.7*	*3/8*	*19.7*	*0/8*	—	*0/8*	—
Buffered saline	7.0	6/6	12.0	8/8	9.2	1/8	21.1	0/8	—	0/8	—
Exp. 4											
Enteric Filtrate	4.5	—	—	8/10	11.3	6/10	14.9	—	—	—	—
" "	7.6	—	—	6/10	16.9	3/10	19.2	—	—	—	—
Exp. 5											
Enteric Filtrate	4.5	—	—	8/9	12.5	7/10	14.4	—	—	—	—
Buffered Saline	4.5	—	—	9/10	12.3	5/7	17.7	—	—	—	—
Enteric Filtrate	7.6	9/9	12.9	5/8	20.5	—	—	—	—	—	—
Buffered Saline	7.6	11/12	13.5	6/10	16.7	—	—	—	—	—	—

Experiments with Monkeys. Too few monkeys have been used to draw definite conclusions, but work to date with the Armstrong mouse adapted virus (160th mouse passage) in *Macacus philippinensis* monkeys suggests that a virus suspension of pH 4.0 is more effective than one of pH 7.0. Monkeys undergo a fulminating, fatal infection with rapidly progressing flaccid paralysis and with a short incubation period, when inoculated intracerebrally with the acid virus suspension. The virus was recovered readily in mice by inoculation of the monkey cord.

"ENTERIC ORGANISM FILTRATE." Toomey[2, 3] reported that when typhoid-paratyphoid toxic broth filtrate was mixed with a suspension of poliomyelitis virus and inoculated intracerebrally in monkeys a shortened incubation period and a more fulminating disease process resulted. Later by means of virus suspended in a similar filtrate, Toomey and Takacs[4, 5] were able to adapt to the cotton rat Flexner's "M. V." strain and 3 other strains of poliomyelitis virus.

"Enteric filtrate" was prepared as described by Toomey[2] using strains of typhoid, paratyphoid A, paratyphoid B, and several strains of organisms of the colon group obtained from monkey stools. The pH was found to be 4.5 before any adjustment was made. Although Toomey adjusted the pH to approximate neutrality in his work with monkeys[3] and in most of his work with cotton rats,[6] our tests were made in a similar manner to those with buffered saline as described above, using the enteric filtrate at various hydrogen ion concentrations.

Tables I and II (Experiments 1, 2, and 3) present the results of tests made with suspensions of virus in enteric filtrate at a low pH, together with similar virus suspensions in buffered saline. No advantage was noted for the enteric filtrate other than that attributable to the low pH.

Experiment 4. This experiment was planned to determine if the enteric filtrate was more effective at a low pH than when adjusted to pH 7.6. Two dilutions, 1:2,000 and 1:8,000, were used at pH 4.5 and pH 7.6 and 10 mice inoculated with each suspension. Table II shows that the pH of 4.5 was more effective than that of 7.6, as had been the case when saline was used.

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2 Toomey, J. A., *J. Inf. Dis.*, 1934, **54**, 74.

3 Toomey, J. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1015.

4 Toomey, J. A., and Takacs, W. S., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 364.

5 Toomey, J. A., and Takacs, W. S., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 22.

6 Personal communication.

Experiment 5. To determine whether enteric filtrate was endowed with any toxic property or accelerating effect that would render it more effective than a buffered saline solution at the same pH, groups of mice were inoculated with virus suspended in each menstruum at pH 4.5 and 7.6, employing 2 suitable dilutions at each pH. Table II shows the results obtained. No significant difference is noted between the effect of enteric filtrate and of saline at either a low pH or neutrality, in fatality rate or mean survival time. Again, the effect of a low pH is clearly demonstrated.

Summary. It has been demonstrated that in the mouse, employing the Armstrong mouse adapted Lansing strain of virus, a definite quantity of virus can be rendered more effective in producing infection by suspending it in a menstruum of low pH. By progressively decreasing the pH from 7.0 to 4.0 the effectiveness of the virus was increased in a step-like manner. The virus suspension at pH 4.0 appeared to be as effective as from 4 to 16 times as much virus at pH 7.0. At present the mechanism is not understood, although numerous conjectures might be made both in respect to local and possible systemic effects. Experiments to investigate the mechanism are in progress.

We have not compared the effect of neutralized enteric filtrate to that of neutral saline in monkeys and cotton rats, as was done by Toomey and by Toomey and Takacs, since these animals were not readily available in sufficiently large numbers to permit clear-cut evaluation of results. However, in the mouse, the effect of the Armstrong virus was not altered by combining it with enteric filtrate. At either a low pH or at pH 7.6 buffered saline has been shown to produce results of an entirely comparable nature.