TABLE I.
Average Weights and Standard Errors at 100 Days of Age.

Injection	Cond. of eye	No. of animals	Body, g	Testes, g	Sem. V., mg	Adr., mg	Thym., mg	Thyr., mg	Pit., mg
None	Nor.	20	224 ± 4	3.0	681 ± 91	34.2	257 ± 9	13.2 ± .3	9.2 ± .2
"	Bl.	20	207 ± 3	2.8	511 ± 78	34.2	261 ± 9	14.8 ± .4	7.4 ± .2
Saline Ext.	Bl.	10	222 ± 5	2.9	735 ± 56	34.0	238 ± 16	12.7 ± .9	7.6 ± .4
B. Carotene	Bl.	10	206 ± 1	2.8	496 ± 24	32.8	269 ± 9	15.0 ± .5	8.2 ± .2
Post. Lobe	Bl.	10	202 ± 1	2.8	504 ± 54	32.2	261 ± 8	14.2 ± .5	7.1 ± .1
Ether Extr.	Bl.	9	190 ± 4	2.5	515 ± 33	29.8	271 ± 17	15.8 ± .5	7.8 ± .1

sufficiently consistent to warrant this report.

Reference to Table I indicates that at 100 days of age the males from the saline extract series more nearly approached the body and organ weights of normal males of the same age than did the animals in any other experimental group. This is especially true of the testis, seminal vesicle, and thyroid weights. Fig. 1 indicates that the males which received saline extracts averaged consistently heavier than bilateral enucleated controls. Injections of sterile saline only into blinded males had no alleviating influence on weight curves.

Five-day Series. One male of each pair autopsied at 5-day intervals received the saline extract beginning day 12, while the blinded litter-mate male received none. Litter-mate pairs were contrasted and revealed: (1) that injected males were heavier in body, testis, seminal vesicle, adrenal, liver, thyroid, and pituitary weights up to age 70 days, (2) that there was no marked difference in thymus weights, (3) that spermatozoa were present in the testes of injected animals by 45 days, and in non-injected males by 50 days. Spermatozoa are present in normal animals of this strain by 35-40 days of age. (4) Although seminal vesicle weights were essentially the same up to day 45, by 65 days, the seminal vesicles of non-injected males were only 10 times, injected animals were 18 times, and normal animals were 20 times the 45-day weight.

Summary. Normal male rats, and bilateral optic enucleated male rats injected with a saline extract of bovine retinas are quite similar in body and most organ weights. Retinal extracts of bovine eyes apparently have an alleviating effect upon the weight retardation and delayed sexual maturity characteristic of optic enucleated male rats.

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Studies on Purification of Poliomyelitis Virus.* 2. pH Stability Range of MVA Strain.

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While it is known that poliomyelitis virus activity is preserved for long periods of time in infected tissues[1] and in sewage,[2] little has been published regarding its stability under conditions where the virus is exposed to the more extreme hydrogen ion concentrations.

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1 Flexner, S., and Lewis, P. A., *J. Am. Med. Assn.*, 1910, **54**, 45; Römer, P. H., and Joseph, K., *Münch. med. Woch.*, 1910, **57**, 347; Flexner, S., Clark, P. F., and Amoss, H. L., *J. Exp. Med.*, 1914, **19**, 205; Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, **25**, 539; Rhoads, C. P., *J. Exp. Med.*, 1929, **49**, 701.

2 Paul, J. R., and Trask, J. D., *J. Am. Med. Assn.*, 1941, **116**, 493.

The experiments of Sabin[3] on the extraction of virus with dibasic phosphate and those of Amoss[4] suggest a stability to solutions as alkaline as pH 8 or 9. Howitt[5] has found virus activity after exposure to pH 4.4 and heating to 58°, and more recently Schultz and Robinson[6] have reported that the virus survives exposure to pH 2.2 in the presence of cysteine and to pH 10.4 in the presence of sodium peroxide. Treatment with a stronger sodium peroxide solution at pH 11.4, however, caused a loss of virus activity.

The above mentioned experiments suggest that poliomyelitis virus possesses an unusual stability to the extreme ranges of hydrogen ion concentrations. As such experiments have been carried out on relatively crude virus extracts, however, it is not certain that virus freed from the various tissue components would show the same behavior. Additional information of this nature would be of value not only in providing further characterization of the virus and in showing the relationship between the various strains but might also prove useful for purification purposes. In the present paper experiments are presented in which the stability of the MVA strain[7] was studied over the acid range from pH 1 to 4 and over the alkaline range from pH 8 to 11.

Experimental. The virus preparations used in every case consisted of samples that had been purified by ultracentrifugation.[8] It has been shown that such preparations produce poliomyelitis in rhesus monkeys in from 50 to 100% of the animals injected intracerebrally with 5×10^{-9} g of purified virus nitrogen. This amount was, therefore, estimated to contain between 1 and 10 minimal infective doses. The method used to determine whether or not virus activity was destroyed by a given hydrogen ion concentration was to

TABLE I. Summary of pH Stability Experiments.

Exp. No.	Conc. in mg N per ml	Control in Ringer's	pH range ± 0.49									
			1	2	3	4	7	8	9	10	11
26	5×10^{-8}	++*			—	+—	+—	+—	+++—	++ (10.0)†	
	5×10^{-7}	++			++	+	++	+++			
		++						+++			
34	5×10^{-9}			+— (2.4)		++		+—	+++—		
35	5×10^{-9}	++—		+++ (1.6)	+++—		++		+—	++ (10.1)	—— (11.2)
				++— (2.0)							
36	5×10^{-9}	+—	—— (1.0)	+— (1.6)	+—				——	+ (10.0)	
	5×10^{-8}		—— (1.0)								—— (11.0)
	5×10^{-7}										+— (11.0)
38	5×10^{-9}	+—	—— (1.0)	+— (1.6)	+—				+—	+— (10.3)	—— (10.9)
	5×10^{-8}		—— (1.1)	+— (1.6)					+—	+— (9.8)	—— (11.0)

* Each + sign indicates that the monkey developed poliomyelitis.
Each — sign indicates that the monkey failed to develop poliomyelitis within 3 weeks, but later either proved susceptible or died from other causes.
† The numbers in parentheses are the actual pH values for the more extreme hydrogen ion concentrations.

[3] Sabin, A. B., *J. Exp. Med.*, 1932, **56**, 307.

[4] Amoss, H. L., in Rivers' *Filterable Viruses*, 1928, p. 159.

[5] Howitt, B. F., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 158.

[6] Schultz, E. W., and Robinson, F., *J. Inf. Dis.*, 1942, **70**, 193.

[7] Schultz, E. W., and Gebhardt, L. P., *J. Inf. Dis.*, 1942, **70**, 7.

[8] Loring, H. S., and Schwerdt, C. E., *J. Exp. Med.*, 1942, **75**, 395.

find if this same amount of virus would produce poliomyelitis after it had been exposed to the desired pH and the solution readjusted to neutrality. If the solution proved completely inactive after this treatment, it is evident that less than one MID of active virus remained and that the pH in question destroyed most of the virus activity. If the solution proved infectious for some of the animals, the only conclusion that could be drawn was that an appreciable amount of virus activity withstood the treatment. Although an untreated control of the same concentration as the test solution was also injected in most of the experiments, the number of animals used was not sufficiently large to provide a more exact description of the amount of virus which survived the treatment. In cases where no virus activity was found with a concentration of 5×10^{-9} g after exposure to a given pH, the amount of virus was increased ten or a hundredfold, and the activity of these solutions determined after exposure to the same pH. In this way a rough measure was obtained of the amount of virus above the range of 1 to 10 MID destroyed by the treatment.

Phosphate-lactate buffers were employed for the initial pH stability experiments. Their buffering capacities were found too limited at the extreme pH values, however, consequently Michaelis' veronal-acetate-hydrochloric acid buffer[9] was used for solutions at pH 1 to 9, and Sorensen's borate-sodium hydroxide buffer[10] for the solutions between pH 10 and 11. Both buffer solutions were sterilized by autoclaving.

Purified virus preparations were exposed to the various buffers as follows: 0.5 ml of virus in Ringer's solution, one-tenth the concentration of that desired for the final inoculum, was mixed with 3.0 ml of buffer and the mixture allowed to stand at room temperature for 15 to 20 minutes. One ml of this solution was used to determine the exact pH by means of a glass electrode. After the allotted time for exposure had elapsed, the remaining 2.5 ml were approximately neutralized with 0.1 N or 0.5 N hydrochloric acid or sodium hydroxide. A previous electrometric titration of the buffer employed facilitated the estimation of standard acid or alkali required to adjust the hydrogen ion concentration to neutrality. Finally the calculated amount of sterile distilled water was added to effect a final dilution of the virus suspension ten times that of the original preparation exposed to the buffer.

Within one to 2 hours after exposure to the desired pH and readjustment, the neutralized virus-buffer mixture was inoculated intracerebrally into rhesus monkeys. One ml of virus solution was used for each inoculation.

The results obtained with 5 different purified preparations after exposure to the various hydrogen ion concentrations are summarized in Table I. It may be seen from the control in which the same amount of virus in Ringer's solution was inoculated as in the test solutions that about 50% of the animals inoculated with a concentration of 5×10^{-9} g of purified virus nitrogen developed poliomyelitis. Similar results were found for solutions which had been exposed to pH 1.6 or to pH 10 $\pm$ 0.3 or to the various hydrogen ion concentrations tested between these values. Examination of the table also shows that in no case was the solution infectious after exposure to pH 1.0 or to pH 11 $\pm$ 0.2 when tested at either a concentration of 5×10^{-9} or 5×10^{-8} g of purified virus nitrogen. In one experiment in which the virus concentration injected after exposure to pH 11 was increased to 5×10^{-7} g, however, one of two animals injected developed poliomyelitis. It would appear therefore that about 1% of active virus remained in this case.

Summary. Experiments are presented in which the stability of the MVA strain of poliomyelitis virus to acid and alkaline solutions has been determined. It is concluded that this strain when in purified form is relatively stable in solutions as acid as pH 1.6 or as alkaline as pH 10 $\pm$ 0.3 but is unstable in solutions more acid than the former or more alkaline than the latter values.

[9] Michaelis, L., *Biochem. Z.*, 1931, **234**, 139.

[10] Clark, W. M., *The Determination of Hydrogen Ions*, The Williams and Wilkins Co., Baltimore, 1928.