TABLE II.
Effect of Nicotinic Acid and Tryptophan on the Toxicity of 3-Acetylpyridine.

3-Acetylpyridine γ per egg	Nicotinic acid γ per egg	Tryptophan γ per egg	No. of eggs* injected	Fraction dead in 24 hr
1500	0	0	6	1.00
1500	2000	0	6	0.50
1500	2500	0	6	0.67
2000	0	0	7	1.00
2000	2000	0	7	1.00
2000	2500	0	7	0.88
600	0	0	7	0.86
600	0	7500	7	0.00
650	0	0	7	1.00
650	0	7500	7	0.00
700	0	0	7	1.00
700	0	7500	7	0.14

* All eggs incubated at 37°C and were 4 days old at time of injection.

a very limited capacity to convert nicotinic acid to the corresponding amide. 4. Sublethal levels of 3-acetylpyridine are shown to cause a mal-development of the chick embryo.

16338

Effect of pH on MM Virus.

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MM virus was originally isolated from a hamster which had been injected intracerebrally with cord and medulla of a fatal case of poliomyelitis. Typical and severe poliomyelitic lesions are induced by it in the central nervous system of hamsters and albino mice.[1]

Serologically MM virus appears to be similar to the SK virus of Jungeblut and Sanders[2] but differs from the Lansing-like poliomyelitic strains and the Theiler virus.[3]

The virus is highly virulent for mice by the intraperitoneal as well as the intracerebral route. Thus it affords an opportunity for testing the effects of antibiotic agents on small amounts of a virus which induces poliomyelitic lesions in the test animal. In relation to a problem of this kind[4] an investigation was made of the effect of pH on MM virus.

Procedure. Mouse brains from the 45th to the 48th generation stored in 50% glycerol were ground, made up in a 10% suspension usually in salt solution containing 10% of infusion broth or occasionally in distilled water, and centrifuged to remove large particles. The supernatant fluid was used at once or after

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1 Jungeblut, C. W., and Dalldorf, G., *Am. J. Pub. Health*, 1943, **33**, 169.

2 Jungeblut, C. W., and Sanders, M., *J. Exp. Med.*, 1940, **72**, 407.

3 Dalldorf, G., to be published.

4 Schatz, A., and Plager, H., *Bull. Torrey Bot. Club*, 1948, **75**, 256.

storage in the dry-ice box. Albany standard strain mice† weighing 10 to 12 g were injected intraperitoneally. By this route of inoculation the majority of animals given 0.05 ml of a 0.5 x 10^{-6} dilution of mouse brain were usually dead or paralyzed by the seventh day. Unless otherwise specified, animals were observed over a period of one week after which experiments were discontinued. Five mice were employed for each individual test.

The buffers were Michaelis' veronal acetate,[5] Sorenson's glycocoll sodium hydroxide,[6] and the phthalate, phosphate, borate, and hydrochloric acid mixtures of Clark and Lubs.[6] These were made up approximately to the desired pH and sterilized by heating for 10 to 15 minutes in a water bath or by autoclaving for 15 minutes at 15 pounds' pressure. In most instances, 0.5 ml of a 10^{-3} distilled-water dilution of virus-infected mouse brain was added to 4.5 ml of buffer. The effect of distilled water and of physiologic saline adjusted to different reactions with acetic or hydrochloric acid was also determined. After the desired periods of incubation at room temperature, dilutions were made in broth-salt solution and tested in mice.

Final pH values for the suspensions were determined electrometrically on corresponding normal mouse-brain mixtures, except for the first test with veronal acetate and glycocoll solutions in which the effect of buffer dilution was duplicated with distilled water instead of normal mouse-brain suspension. Bromphenol and bromthymol blue indicator tests with 10^{-4} dilution of virus mouse brain and normal mouse brain in veronal acetate buffer at pH 4.0 and 7.0 and also in aqueous suspensions at pH 7.4 revealed no difference in reaction between the normal and infected mouse-brain suspensions. For solutions at pH 9.0 and above, corrections for sodium-ion

TABLE I. Survival of MM Virus in Veronal-acetate and Glycocoll-NaOH Buffers. 10^{-4} Virus Dilution Exposed at Room Temperature.

Diluent	Exp. No.	pH	Period of exposure, days: 1 — Virus dilutions inoculated into mice: 10^{-4}	1: 10^{-5}	1: 10^{-6}	2: 10^{-5}	2: 10^{-6}	7: 10^{-4}	7: 10^{-5}	7: 10^{-6}
Veronal-acetate buffer	2	2.02	0/5	0/5	0/5					
		3.48								
" "	2	4.05	2/5*	0/5	0/5			0/5	0/5	
" "	1	4.15		0/5	1/5	2/5	0/5	0/5	0/5	
" "	1	5.17		0/5	0/5	0/5	0/5	0/5	0/5	
		6.18								
" "	1	7.15		5/5	5/5	5/5	5/5	5/5	5/5	4/5
" "	1	8.04		5/5	5/5	5/5	5/5	5/5	5/5	5/5
" "	1	8.95		5/5	4/5	5/5	5/5	5/5	5/5	5/5
Glycocoll-NaOH buffer	1	8.99		5/5	5/5	4/5	3/5	5/5	5/5	5/5
"	1	9.95		5/5	5/5	5/5	4/5	3/5	1/5	0/5
Control (distilled water)	1			5/5†	5/5‡	5/5	5/5		5/5	5/5
" "	2	7.40		5/5	3/5				5/5	3/5

Numerator = number of mice which died; denominator = number of mice injected. Test period = 7 days.
* 2 survivors paralyzed on 7th day. † 2 paralyzed mice sacrificed on the 3rd day. ‡ 1 paralyzed mouse sacrificed on the 3rd day.

† A strain used at the Division of Laboratories and Research, New York State Department of Health, Albany, New York.

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

[6] Clark, W. M., *The Determination of Hydrogen Ions*, Baltimore, Williams and Wilkins, 1928, 717p.

TABLE II. Survival of MM Virus in HCl, Phthalate, Phosphate, and Borate Buffers. 10-4 Virus Dilution Exposed at Room Temperature.

Diluent	Exp. No.	pH	Period of exposure, days: 1 — Virus dilutions inoculated into mice: 10^{-4}	10^{-5}	10^{-6}	2 — Virus dilutions inoculated into mice: 10^{-5}	10^{-6}	7 — Virus dilutions inoculated into mice: 10^{-4}	10^{-5}	10^{-6}
HCl	3	1.12	0/5	0/5	0/5					
	3	2.10	1/5	0/5	0/5					
Phthalate buffer	3	3.00	0/5	0/5	0/5					
	3	4.00	1/5	0/5	0/5					
	3	5.00	0/5	0/5	1/5					
Phosphate buffer	4	6.00		3/5	1/5	2/5	0/5	3/5	0/5	
	4	7.01		5/5	5/5	5/5	5/5	5/5	5/5	5/5
	4	7.75		5/5	5/5	4/5	3/5		4/5	0/5
Borate buffer	4	7.71		4/5*	5/5	4/5	4/5		5/5	4/5
	4	8.75		5/5	4/5	5/5	5/5		5/5	5/5
	4	9.55		5/5	4/5	3/5	3/5		0/5	0/5
Control (distilled water)	3	7.01		4/5	3/5				5/5	2/5
	4	7.47		5/5	5/5	5/5	3/5		5/5	3/5

Numerator = number of mice which died; denominator = number of mice inoculated. Test period = 7 days.
* 1 survivor paralyzed. † ± 0.05.

concentration were taken into account. Inoculation of mice with virus-free buffers at the extremes of pH indicated no toxicity for the solutions *per se*. When streaked on blood-agar plates, the mixtures gave no growth.

Experimental Results. Tables I and II indicate the pH tolerance of MM virus in the different buffers at room temperature. These results and other data not presented here show that under the conditions of the experiment the virus when exposed at 10^{-4} dilution was stable in the neutral and alkaline solutions. There was a definite loss of activity within twenty-four hours in the veronal-acetate and phosphate buffers below pH 7. On the other hand, the virus survived for at least 2 days in glycocoll and borate solutions at pH 9.95 and 9.55, respectively, but a decrease in activity was evident when tested on the 7th day. The veronal-acetate buffer, at approximately pH 7.8, contained infective virus in the 10^{-6} dilution after seven days at room temperature followed by refrigeration for 8 days. There was no significant difference between the phosphate and phthalate buffers at pH 6.0. However, the results did indicate that virus survival was somewhat better at around pH 8.0 in the borate than in the phosphate medium; at 10^{-4} dilution, active virus was present in the former but not in the latter buffer after 7 days at room temperature followed by 10 days at 4°-6°C.

In a more detailed experiment, ten-fold virus dilutions from 10^{-2} through 10^{-6} or 10^{-7} in broth salt solution, were exposed to equal amounts of veronal-acetate buffers. Similar dilutions prepared with normal mouse brain had pH values of 4.35, 5.25, 6.35-6.45, 6.85-6.95, 7.75-7.8. After incubation for 1- and 7-day periods, suitable ten-fold dilutions were inoculated into mice. The results indicated, in general, a less deleterious effect of unfavorable pH in the more concentrated virus suspensions. The virus stability was definitely greater at pH 7.8 than at pH 6.9. In this experiment and in several others, there was occasional evidence of some virus survival at around pH 4.0 as compared to that at pH 5.0. While these results are suggestive of greater virus stability somewhere

in the vicinity of pH 4.0 than at acid reactions immediately above or below, more detailed studies would be required to ascertain whether this is actually so.

Whether the increased virus survival in the lower dilutions and the apparent occasional survival at approximately pH 4.0 were due to a protective action of the mouse-brain constituents is not known. No visible flocculation of the mouse-brain suspensions occurred in the buffers with 10^{-4} dilution. However, at a dilution of 10^{-2}, maximum precipitation was observed at pH 3 to 5. This is in agreement with a similar observation of Wenner[7] on flocculation in mouse-brain virus suspensions at pH 4.0 to 5.5.

The effect of acidifying MM virus suspensions immediately before inoculation was also studied. Hammon and Izumi[8] using the Armstrong mouse-adapted poliomyelitis virus and Wenner with the Lansing strain reported that the mortality rate for mice inoculated with mouse suspensions at pH 4.0 and 4.6 respectively, was higher than the death rate of animals given neutral or alkaline inocula. In order to determine whether the MM strain was affected by readjustment before inoculation, 10^{-3} and 10^{-5} dilutions of the virus were exposed to veronal-acetate buffers of pH 4, 6, and 8; the broth-salt control was at pH 7.2. After incubation for 1- and 24-hour intervals, a portion of each virus-buffer suspension was adjusted with 0.4% NaOH to about pH 7.5. These alkalinized suspensions, as well as samples of the corresponding unadjusted virus-buffer mixtures, were inoculated into mice within an hour. The results indicated that readjustment to the favorable reaction range had no beneficial or potentiating action. In these experiments light was not excluded during the exposure periods.

In other tests, however, in which distilled water, physiologic saline, and buffer solutions were compared, precautions were taken to protect the test material from possible effects of light. Under these conditions, results with

TABLE III.

Survival of MM Virus in Acidified Distilled Water and Saline and in Veronal-acetate Buffer. 10^{-4} Virus Dilution Exposed at Room Temperature.

Diluent	pH		Period of exposure, days									
			1					7				
			Virus dilutions inoculated into mice									
	1 day	7 days	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-4}	10^{-5}	10^{-6}	10^{-7}
Distilled water + HCl	3.6	3.7	4/5	4/5	3/5*	1/5	5/5	0/5	2/5	0/5
" "	5.65	5.85	5/5	5/5	1/5	0/5	4/5	0/5	0/5	0/5
Distilled water	6.1	6.1	5/5	5/5	5/5	2/5	5/5	2/5	0/5	0/5
Saline + HCl	3.75	3.9	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Saline	6.1	6.25	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Veronal-acetate buffer	4.0	4.2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
" " "	6.0	6.0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
" " "	7.25	7.2	5/5	5/5	4/5	1/5	5/5	3/5*	0/5	0/5

Numerator = number of mice which died; denominator = number of mice inoculated. Test period = 7 days.
* 1 survivor paralyzed.

[7] Wenner, H. A., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 104.

[8] Hammon, W. M., and Izumi, E. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 579.

veronal-acetate buffers were similar to those previously obtained. In the range tested, from about pH 4.0 to pH 6.0, physiologic saline had an effect similar to that noted with veronal-acetate buffer solutions. On the other hand, the survival of virus in distilled water over the same range was apparently greater. Material diluted to 10^{-6} after exposure for 24 hours in a 10^{-4} dilution at about pH 4.0 was lethal for mice. After exposure for one week virus had survived in the 10^{-4} dilution close to pH 4.0 (Table III).

Summary. In various buffers, MM virus was stable at room temperature only at neutral or alkaline reactions. When diluted virus was exposed at approximately pH 1.0 to neutrality the infectivity was diminished within twenty-four hours. There was a slower decrease in virus titer at pH 9 to 10. At pH 7 to 9, the virus was found to be stable for more than seven days. The deleterious effect of unfavorable pH was less for concentrated virus suspensions. MM virus was apparently more stable in distilled water acidified with hydrochloric or acetic acid than in acidified physiologic saline or in veronal-acetate buffer solutions of approximately the same pH value.

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Experimental Enterococcal Food Poisoning in Man.

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Several outbreaks of food poisoning of a mild type in which enterococci were the predominant organisms isolated from the suspected foods, are described in a previous paper.[1] The literature on food poisoning caused by non-hemolytic streptococci is also discussed and it is pointed out that, in several instances in which the inculpated organisms were closely identified, they were found to be enterococci.

The relatively small number of outbreaks reported as caused by *Streptococcus faecalis* is in sharp contrast to the widespread occurrence of this organism in nature.[1] Accordingly the studies reported in this paper were initiated in an attempt to determine whether *Streptococcus faecalis* can act as a food poisoning agent. They consisted of feeding experiments with *Streptococcus faecalis* employing kittens and human volunteers.

The literature contains the following references to experimental streptococcal food poisoning in man. Cary, Dack, and Meyers[2] reported that a volunteer who had eaten parts of several sausages similar to others which had been implicated in an outbreak of mild gastroenteritis, developed nausea which increased for 24 hours, with two periods of vomiting and epigastric pain, followed by marked exhaustion and constipation. A green "pleomorphic" streptococcus whose species designation is not determinable from the description given was isolated from the sausage. Later Cary, Dack, and Davison[3] reported abdominal distress, cramps, and diarrhea, in 5 of 7 volunteers who ingested "reasonable" doses of several strains of alpha streptococci which had been implicated in 2 outbreaks of food poisoning. On the other hand, Dolman[4] reported failure to produce food poisoning in any of 4 volunteers each of whom had eaten a meat pie heavily infected with *Streptococcus "viridans"* which had been isolated from

[1] Buchbinder, L., Osler, A. G., and Steffen, G. I., *Pub. Health Rep.*, 1948, **63**, 109.

[2] Cary, W. E., Dack, G. M., and Meyers, E., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 214.

[3] Cary, W. E., Dack, G. M., and Davison, E., *J. Inf. Dis.*, 1938, **62**, 88.

[4] Dolman, C. E., *Canad. J. P. H.*, 1943, **34**, 97.