due to one of two factors or to both, (a) dilution with extracellular water, (b) depolymerization or dissociation of a highly polymerized hyaluronic acid by hyaluronidase.

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Inactivation of Poliomyelitis Virus in Relation to Gastric and Intestinal Digestion.*

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Whether or to what extent gastric and intestinal digestion inactivates poliomyelitis virus is a problem that has an important bearing on intestinal entry of infection and on the source of virus in the intestinal contents and stools. This problem has not been studied as closely as might be desired in its relation to the conditions of normal digestion, although such studies as are available indicate that the virus displays a considerable degree of resistance to inactivation in the gastrointestinal tract. Thus, Flexner, Clark and Dochez[1] found active virus in the intestine 2 hours after administration by stomach tube; and by the same method of administration Clark, Schindler and Preston;[2] Clark, Roberts and Preston;[3] and Levaditi, Kling and Lepine[4] found active virus in the stools within 48 hours. The effects of H-ion concentration upon virus in the range with which we are here concerned has been studied by Loring and Schwerdt[5] but their experiments were limited to periods of 1-2 hours and were done at room temperature. So far as we know no studies on the inactivating effects of individual digestive enzymes on poliomyelitis virus have been recorded.

In the present experiments the test conditions were intended to approximate those obtaining in the human stomach in respect to temperature, time, pH range, and presence of pepsin. In children over one year of age (the group most susceptible to poliomyelitis), Cutter[6] has shown that the fasting gastric contents 10 minutes after stimulation with histamine display an average acidity of pH 1.5 and occasionally reach pH 1.2. In young adults, the level is pH 1.0-1.2.[7] Hammon and Izumi[8] showed that poliomyelitis virus is not inactivated at levels of pH 4.0 and up. Gastric evacuation begins a few minutes after ingestion.[9] At 4 hours, after ingestion of various types of food, the stomach is nearly empty.[9] On the basis of such data, we have selected for our studies a range of pH 1.0-4.0 and exposure periods of 5 minutes to 4 hours. All observations were made at 37°C, or approximate body temperature.

The Lansing (Armstrong) mouse-adapted strain of poliomyelitis virus was used. Stock 10% and 20% suspensions of infected mouse cord in physiological NaCl solution were clarified at 18,000 r.p.m. for ½ hour, then

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2 Clark, P. F., Schindler, J., and Roberts, D. J., *J. Bact.*, 1930, **20**, 213.

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4 Levaditi, C., Kling, C., and Lepine, P., *Bull. Acad. Med.*, 1931, s. 3, **105**, 190.

5 Loring, H. S., and Schwerdt, C. E., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 173.

6 Cutter, R. D., *J. Pediat.*, 1938, **12**, 1.

7 Helmer, O. M., and Fouts, P. J., *Am. J. Clin. Path.*, 1937, **7**, Tech. Suppl., 41.

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

9 Best, C. H., and Taylor, N. B., *The Physiological Basis of Medical Practice*, 3rd Ed., 1943, Baltimore, The Williams and Wilkins Co.

stored in ampoules on dry ice, and appropriately diluted with saline solution at the time of use. Swiss mice, all, with a few exceptions, of the Webster strain from the Carworth Farms, were employed, using a standard intracerebral inoculum of 0.03 cc. Positive results were determined either by the appearance of paralysis or, in the case of mice found dead without observed symptoms, by histological examination. While differing from the current practice of judging results by the gross fatality rate, our method somewhat increases the accuracy and significance of the results by eliminating deaths from nonpoliomyelitic causes. In the tables, the numerator expresses the number of mice showing poliomyelitis and the denominator, the number of mice tested. pH determinations were made electrometrically, both before and after incubation of each preparation. Mixtures were adjusted with HCl within the range pH 1.0-3.0; for pH 4.0, an acetic acid-NaOH mixture was used. At the end of incubation at 37°C, each preparation was returned to approximate neutrality by the addition of NaOH. In the pepsin experiments, acid solutions of Lilly's pepsin were made up to contain 12% pepsin in the final mixture with virus and adjusted to the desired pH The proteolytic activity of each pepsin preparation was tested on coagulated egg albumin. At the end of each incubation period, enzyme activity was stopped by the addition of NaOH to approximate neutrality. For the controls, pepsin inactivated at 70°C for ½ hour was used in the same amounts as in the tests. (Results shown in Tables I and II).

TABLE I.
Inactivation of Lansing Virus at Varying pH Levels.
1:500 suspensions.

	Incubation time					
pH	5 min	¼ hr	½ hr	1 hr	2 hr	4 hr
1.0	0/7	0/7	0/7	0/7	0/7	0/6
1.5	3/6	0/6	0/6	0/6	0/6	0/6
2.0	6/7	4/7	5/7	4/7	3/6	0/6
					(0/13)*	
3.0	6/8	6/7	6/8	6/7	5/8	8/8
					(14/19)*	
4.0	7/8	7/8	4/8	5/8	5/8	6/8

* Another series of tests.

This group of experiments shows complete inactivation of virus in 5 minutes at pH 1.0. At pH 1.5, partial inactivation occurs at 5 minutes, and complete at 15 minutes, while at pH 2.0, partial inactivation begins at about 2 hours and is complete at 4 hours. At pH 3.0 and pH 4.0, no inactivation is found at 4 hours. In other tests not tabulated, inactivation was complete at pH 3.0 in 22 hours, but incomplete at pH 4.0 in the same period.

The data indicate a slightly greater degree of inactivation with pepsin than by acidity alone, but the difference between tests and controls is small. The optimum proteolytic effect of pepsin is stated to occur at pH 2.0-2.8.[10]

Effect of Trypsin. Supplementing the study of gastric digestion on virus, the effect of trypsin was investigated. For technical reasons, mice could not be used for this purpose and the experiments were performed on monkeys with a freshly isolated strain of poliomyelitis virus in its first passage. A suspension made up to contain 10% pancreatin (Parke-Davis) and 10% virus adjusted to pH 7.9 was incubated for 4½ hours at 37°C. The activity of the pancreatin was confirmed by visible digestion of egg albumin and by determination of pH and free acids in casein digest. At the end of incubation pancreatic activity was inhibited by lowering the pH to 4.2 by the addition of HCl. In 6 Rhesus monkeys, 0.5 cc of the virus-pancreatin mixture was instilled daily into each nostril for 5 consecutive days. Three monkeys developed typical paralytic poliomyelitis, and one, nonparalytic poliomyelitis as demonstrated by characteristic lesions in the cord. Two monkeys showed no evidence of infection. Of 6 controls given virus in saline suspension by the same procedure, none developed clinical signs of poliomyelitis and the olfactory bulbs of 2 showed no lesions. It would appear that exposure to trypsin not only fails to destroy the virus but may even increase its infectivity.

Comment. Our experiments indicate that at high levels of acidity such as prevail in

[10] Northrop, J. H., *J. Gen. Physiol.*, 1919-20, **2**, 113.

TABLE II.
Inactivating Effect of Pepsin.

	Virus conc.	1:20		1:200		1:500	
pH	Incubation time, hr	Active pepsin	Inactivated pepsin	Active pepsin	Inactivated pepsin	Active pepsin	NaCl-HCl controls*
2.0	½	9/10	10/10	3/10	6/10	9/10	
"	1	0/10	1/10	0/10	2/10	3/10	
"	2	0/10	0/10	0/10	0/10	0/10	
3.0	½					9/10	
"	1					7/10	
"	2					6/10	9/10

* For other comparative figures with NaCl-HCl-virus mixtures, see Table I.

the normal fasting gastric contents, virus is rapidly and completely inactivated. At somewhat lower levels (pH 2.0-3.0) pepsin has a slight inactivating effect. However, the actual effectiveness of this "barrier" in preventing the passage of virus into the intestine is certainly far from complete since the optimal conditions for inactivation are present in the stomach only at times. The ingestion of milk raises the pH of the contents and maintains it, at least in infants up to 19 months, at 3.2 or more for at least 2 hours.[11] With carbohydrate foods (Ewald test meals) on the other hand pH levels are frequently at the inactivating levels. Thus, Kahn and Stokes[12] found in 5 human subjects aged 4, 5, 6, 9 and 43 years old respectively with gastrostomies that after one hour, the pH was 1.32, 1.56, 1.57, 1.73 and 3.31. At the height of digestion of an ordinary meal containing meat, the acidity of the gastric contents is stated to lie in the range pH 1.3-2.5.[9] Mucus and saliva are said to raise the pH somewhat.[13] The rate of gastric evacuation is another factor of importance. After ingestion of most foods, evacuation begins in 1-7 minutes, and with fluids a considerable amount may leave the stomach almost at once. On the other hand, emptying requires 3 hours or more, being especially rapid with carbohydrates (95% at 3 hours), a little less so with proteins (80-85% at 3 hours) and quite slow with fats (30-50% in 3 hours).[9]

Taking the varying conditions of gastric acidity and motility into consideration it seems clear that when virus is swallowed, some of it is destroyed in the stomach and some enters the intestine intact, but the proportions cannot be well estimated. Conditions of high acidity, such for instance as occur after ingestion of carbohydrates and meats, favor inactivation while highly buffered foods, particularly milk, tend to prevent it. Virus contained in that part of the ingesta which leaves the stomach too soon to be highly acidified is apt to escape inactivation.

Since the pH of the intestine has a range of about 6.0-8.0[14] and since, as we have shown, trypsin has no inactivating effect, any virus reaching the intestine is likely to remain intact, and to have the opportunity of causing infection. Virus can also become concentrated in the large bowel as water is abstracted from the contents and thus become easily demonstrable in the stools. While, as we[15] have mentioned elsewhere, we believe that primary intestinal entry is an exceptional event, we suspect on the basis of the frequency of lesions in the celiac ganglia[15] that secondary intestinal entry following pharyngeal entry is not uncommon.

Summary. At the pH levels prevailing in

11 Babbott, F. L., Jr., Johnston, J. A., Haskins, C. H., and Shohl, A. T., *Am. J. Dis. Child.*, 1923, **26**, 475.

12 Kahn, G., and Stokes, J., Jr., *Am. J. Dis. Child.*, 1926, **32**, 667.

13 Marriott, W. McK., and Davidson, L. T., *Am. J. Dis. Child.*, 1923, **26**, 542.

14 Mann, F. C., and Bollman, J. L., *J. Am. Med. Assn.*, 1930, **95**, 1722.

15 Faber, H. K., and Silverberg, R. J., *J. Exp. Med.*, 1946, **83**, 329.

the stomach when only gastric juice is present, and at the height of digestion of carbohydrate and mixed meals containing meat, poliomyelitis virus is rapidly inactivated. Pepsin contributes slightly to inactivation. Since the pH levels necessary for virus inactivation are present in the stomach only part of the time, and since part of the gastric contents are evacuated before such levels can be attained, a certain proportion of ingested virus has the opportunity to escape intact into the duodenum; here and in the rest of the intestine the pH is too high to inactivate it and trypsin has no inactivating effect. Virus entering the bowel therefore must be presumed to remain active, thus providing conditions suitable for secondary intestinal entry of infection, and also for concentration of virus in the contents of the large intestine.

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