rapid exchange of electrolytes. Oxygen uptake of slices of atrium *in vitro* is approximately the same as that of slices from the right ventricle(5).

MacLean, Hedenstrom, and Kim have studied the distribution of glass microspheres in the beating but empty canine heart(6). In their animals 6% of the spheres entering the coronary arteries were found in the atria. This agrees with the lowest values for Rb^{86} uptake observed in the present group. Two groups have measured the rate of blood flow into the cannulated left anterior atrial artery. Smith and Layton found an average flow of approximately 0.15 ml/cycle in the heart-lung preparation(7). Moir, Driscol, and Eckstein reported a flow of 4.4 ml/min or 72 ml/100 g of left atrium/min(8). This is compatible with the present isotope data.

Summary. The rate of clearance of Rb^{86} from blood by several areas of the atria was studied in 18 dogs because of evidence that Rb^{86} uptake and rate of coronary blood flow are closely related. Mean rates of clearance averaged 0.40 ml blood/g/min. These were not significantly different in the right atrium, left atrium and interatrial septum. Rb^{86} uptake by the atria formed a larger portion of total myocardial uptake when cardiac output was low. Frequently the clearance per gram of tissue exceeded that found simultaneously in the ventricles. This indicates that atrial flow is better maintained when the animal is in poor condition than is flow to the ventricles.

1. Love, W. D., Burch, G. E., *J. Clin. Inv.*, 1957, v36, 479.

2. Love, W. D., O'Meallie, L. P., *Clin. Res.*, 1963, v11, 55.

3. Love, W. D., Burch, G. E., *J. Clin. Inv.*, 1957, v36, 468.

4. Love, W. D., O'Meallie, L. P., *J. Lab. and Clin. Med.*, 1962, v60, 996.

5. Goh, K., Dallam, R. D., *Am. J. Phys.*, 1957, v188, 514.

6. MacLean, L. D., Hedenstrom, P. H., Kim, Y. S., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 786.

7. Smith, J. R., Layton, I. C., *ibid.*, 1946, v62, 59.

8. Moir, T. W., Driscol, T. E., Eckstein, R. W., *Circ.*, 1961, v24, 1000.

Received January 30, 1963. P.S.E.B.M., 1963, v112.

Inactivation of Hemagglutinating Capacity of Type A Influenza Virus By Trypsin Treatment.* (28209)

ROY CLEELAND AND JOHN Y. SUGG

Department of Microbiology and Immunology, Cornell University Medical College, New York City

The present communication describes the effect of certain environmental conditions on inactivation of influenza virus by crystalline trypsin, as determined by loss of hemagglutinating capacity. The aim of the study was not only to obtain information on factors influencing the virus-trypsin reaction, but also to determine whether the relative trypsin-susceptibility among a group of strains would vary with the conditions under which the tests were made. Only type A strains of human origin were used because in a previous study(1) neither any of 4 type B strains nor the one type A strain of swine origin examined showed reduction in hemagglutination titer following treatment with this enzyme.

* This work was supported by a PHS research grant from Inst. of Allergy and Infect. Dis., P.H.S.

Materials and methods. Viruses. The strains used were WS/33, PR8/34, BH/36, Cam/46, S-1/53 and Japan 305/57. All were adapted to growth in chick embryos.

Groups of 10-day-old embryonated eggs were inoculated with 0.2 ml of a 10^{-4} dilution of seed virus, incubated for 48 hr at 36°C, chilled and the allantoic fluids harvested. Infected fluids from each group were pooled and clarified by low speed centrifugation. Virus was then sedimented by centrifugation at 30,000 rpm for 30 minutes in a Spinco model L ultracentrifuge (# 40 rotor), resuspended to approximately 1/10 original volume in buf-

fer and again centrifuged at 2000 rpm for 30 minutes. The supernatant fluid was removed and where possible was adjusted to give a final hemagglutination titer of approximately 2048 in the test system.

For tests to determine the influence of pH on the reaction, virus was resuspended in distilled (deionized) water rather than buffer.

Buffer. Buffer, used in all experiments except those dealing with effect of pH on the reaction, was 0.1 M tris (hydroxymethyl-amino methane) containing sufficient 0.1 M HCl to lower the pH to 8.2 to 8.3 (tris-Cl).

Enzymes and enzyme inhibitor. Crystalline trypsin (2 X) and crystalline soybean trypsin inhibitor (5 X) were obtained from Nutritional Biochemicals Corp. The trypsin was dissolved in distilled water on the day of test and enzyme activity was checked on occasion by assay against N-benzoyl-L-arginine ethyl ester (BAEE) in M/15 phosphate buffer, pH 6.9, according to the method of Schwert and Takenaka(2). Soybean trypsin inhibitor was made up as previously reported (1).

Test procedure. Equal amounts of virus suspension, buffer, water and trypsin were combined in that order, trypsin being added after the 3 other components had been at the test temperature for 5 min. When the effect of sodium or calcium chloride on the reaction was studied, a solution of salt in 4 times the desired final concentration was added in place of water. A control preparation in which trypsin was replaced by either water or heat inactivated trypsin was included in each test and was carried through exactly the same procedure as was the test. Unless otherwise specified the pH of the reaction mixture was 7.9 to 8.1 and final buffer concentration was 0.05 M. All tests were repeated 3 or more times with each strain, using a different preparation of the virus on each occasion. The results obtained with the different preparations did not vary significantly.

Hemagglutination (HA) tests. HA titers of control and test mixtures were determined at the designated time intervals by transferring a 0.2 ml sample to a tube containing an equal volume of trypsin inhibitor solution of sufficient strength to neutralize the amount of trypsin originally added. Serial 2-fold dilutions were then prepared in 0.01 M phosphate buffered saline, pH 6.9, or when $CaCl_2$ had been added to the mixture, in 0.01 M tris-Cl buffered saline, pH 7.6. 0.2 ml of a 0.5% suspension of chicken erythrocytes was added to each tube, the contents mixed and the pattern of the sedimented cells recorded after 1 hr at room temperature. The reciprocal of the highest initial dilution showing definite agglutination was taken as the titer.

Results. Enzyme concentration. The initial experiments were designed to determine the minimum concentration of trypsin required to inactivate each of the 6 strains when tested under uniform and what had been found previously(1) to be favorable conditions, *i.e.*, with both virus and enzyme in tris-Cl buffer and incubation at 37°C. Incubation period was 15 min. Different preparations of the same strain showed minor variations in trypsin requirement, which may have been due at least in part to differences in virus content, although the preparations were comparable on the basis of HA titer. In any event these variations were of insufficient magnitude to explain the differences noted among the strains. Results of representative tests are recorded in Table I. It will be seen that the trypsin concentration required to inactivate the hemagglutinating activity of WS and Cam was 4 times that required for BH, PR8 and Japan/305, and more than 30 times the amount that sufficed with S-1.

In view of these findings and in order to avoid both an inadequate and an excess enzyme concentration in the test mixture, the

TABLE I. Minimum Trypsin Concentration Required to Inactivate Hemagglutinating Capacity of Different Strains of Influenza Virus.*

Trypsin conc, μg/ml	HA titer					
	S-1	BH	PR8	Japan/305	WS	Cam
0	2048	2048	2048	2048	2048	2048
7.8	8	—	—	—	—	—
15.5	<2	—	—	—	—	—
31	<2	64	16	8	—	—
62	<2	<2	<2	4	256	256
125	—	<2	<2	<2	32	64
250	—	<2	<2	<2	8	8
500	—	—	—	—	<2	<2

* Incubated at 37°C for 15 min.

TABLE II. Effect of Temperature and Time of Incubation on Inactivation of Hemagglutinating Capacity of Influenza Virus by Trypsin.*

Incubation		HA titer					
Temp (°C)	Time (min)	S-1	BH	Japan/ PR8	Japan/ 305	WS	Cam
	0	1024	2048	2048	2048	2048	2048
37	1	4	64	—	—	—	—
	2	<2	<2	16	16	64	512
	4	<2	<2	<2	<2	4	128
	8	<2	<2	<2	<2	<2	<2
25	4	2	32	512	64	2048	2048
	8	<2	<2	8	8	256	256
	16	—	<2	<2	<2	4	32
	30	—	—	—	—	<2	<2
4	16	2	—	—	—	—	—
	30	<2	512	1024	—	2048	—
	60	—	128	1024	32	2048	512
	90	—	64	1024	<2	1024	512
	120	—	8	64	<2	512	512

* Amount of trypsin used varied with the strain tested: S-1, 31 μg/ml; BH, PR8, Japan/305, 250 μg/ml; WS and Cam, 1000 μg/ml. No significant reduction in titers of controls was observed.

amount of trypsin used in all subsequent experiments was varied with the strain examined so as to provide a concentration 2 to 4 times the minimum required for inactivation as determined in the above tests. The actual final concentrations employed were: S-1, 31 μg/ml; BH, PR8 and Japan/305, 250 μg/ml; WS and Cam, 1000 μg/ml.

Time and temperature of incubation. With each strain, 3 identical virus-trypsin mixtures were prepared, one of which was incubated at 37°C, one at 25°C and the third at 4°C. At various times up to 2 hours thereafter samples were removed and HA titers determined. The results (Table II) show that the time required to eliminate hemagglutinating activity depended on both the strain tested and temperature of incubation. At 37°C the time required ranged from a low of 2 minutes in the case of S-1 and BH up to a high of 8 minutes in the case of WS and Cam. The significance of this difference is enhanced by the fact that the 2 strains that required the longest time for inactivation were also the 2 strains with which the highest enzyme concentration was used. Lowering incubation temperature to 25°C increased the time required for inactivation, but the increase was of the same order of magnitude (approximately 4 X) for all strains and did not change the order of relative susceptibility to the enzyme from that observed when incubation was at 37°C.

When incubated at 4°C the hemagglutinating capacity of all strains was reduced, but only with S-1 and Japan/305 was it completely inactivated at the end of the 2 hour incubation period. In these tests therefore Japan/305 appeared to be much more susceptible to trypsin than either BH or PR8, and to that extent the order of sensitivity to the enzyme was different from that observed at the higher temperatures.

Effect of pH. A series of buffers was prepared by addition of sufficient 0.1 M NaOH or 0.1 M HCl to 0.1 M tris to provide a pH range of 7.5 to 10.0, in increments of 0.3 to 0.6 pH, when combined with virus and trypsin. Mixtures prepared in this manner were incubated at 37°C and the HA titers determined at 2 time intervals: minimum time required for inactivation of the test strain at pH 8.0 (Table II), and twice this time period. The results with all strains were similar in that most rapid inactivation occurred with all of them at pH 8.0 to 9.0. Above pH 9.5 and in a few tests in which the pH was adjusted to below 7.5 either no or only partial reduction in HA titer had occurred at the end of the incubation period.

Effect of salt. Data presented previously (1) demonstrated that 0.146 M sodium chloride prevented inactivation of the hemagglutinins of the majority of influenza viruses when incorporated into trypsin-virus mixtures that otherwise would have resulted in complete loss of hemagglutinating activity. Further data on the effect of salt on the reaction are provided by the following experiment. Mixtures of virus and trypsin containing varying concentrations of either sodium or calcium chloride were prepared as described in *Materials and methods.* The mixtures were incubated at 37°C and HA titers determined at various times up to 2 hours. All concentrations of both salts had an inhibitory effect on the reaction with all strains, as shown by the failure of the enzyme to inactivate hemagglutinating activity, or if inactivation was obtained, by the increased time required to produce that result as compared with the time

TABLE III. Inactivation of Hemagglutinating Capacity of Influenza Virus by Trypsin in Presence of Various Concentrations of Sodium or Calcium Chloride.*

	NaCl (M)			$CaCl_2$ (M)			
Strain	.0125	.025	.05	.00625	.0125	.025	.05
S-1	+†	+	+	+	+	±	±
BH	+	+	+	+	+	+	+
PR8	+	+	±	+	±	0	0
Japan/305	+	+	±	+	±	0	0
WS	+	±	0	+	±	0	0
Cam	+	±	0	+	+	+	+

* Incubated at 37°C for 2 hr. See footnote, Table II, for concentrations of enzyme used.

† + = inactivation complete; ± = significant reduction in titer but inactivation not complete; 0 = no significant reduction in titer at end of incubation period.

required for the control to which no salt had been added.

In Table III are summarized the findings at the end of the 2 hour incubation period. The inhibitory effect of sodium chloride was the most pronounced with those strains (WS and Cam) that were the most resistant in the preceding tests, and, conversely, the least pronounced with those strains (S-1 and BH) that were the least resistant (Table II, 37°C). With sodium chloride therefore the order of trypsin-susceptibility was not altered from that observed in the absence of added salt (Table II, 37°C). However, in the presence of calcium chloride, a different pattern of susceptibility emerged, primarily because this salt had significantly less inhibitory effect on the reaction of trypsin with Cam than on its reactions with other strains. In fact, Cam was the only strain with which calcium chloride was less effective, on a molar basis, than sodium chloride as an inhibitor. The data are insufficient for satisfactory comparison of the 2 salts at equivalent ionic strengths, but they do indicate that with all strains save Cam the inhibitory effect was more closely associated with the concentration than with the type of ion present. With Cam, the type of ion was an important factor.

Comment. Data presented here and previously(1,3) indicate that the capacity to react with trypsin, with the resulting loss of hemagglutinating activity, is a property shared by all type A influenza viruses of human origin. But susceptibility to the enzyme, as determined in this study, is a characteristic of the strain that varies with experimental conditions. However, any change in conditions that alters the susceptibility of one strain would appear to alter the susceptibility of all strains in the same direction, although not necessarily to the same extent. When tested under a standard set of conditions, trypsin-susceptibility seems to be a stable property, no more subject to variation than other properties of the virus, and, as suggested previously(3), might serve as a convenient marker in genetic studies.

The inhibitory effect of sodium chloride on the trypsin-virus reaction is of special interest because of the frequency with which this salt is incorporated into suspending media and diluents used for preparation of test materials. The data indicate that only a small percentage of type A strains would show any reduction in HA titer as a result of trypsinization in presence of 0.05 M or higher concentrations of sodium chloride. This fact should be considered in evaluating the observed effects of trypsin on any property of the virus.

Summary. The effect of trypsin on hemagglutinating capacity of 6 type A strains of influenza virus of human origin has been determined under different experimental conditions. With both virus and enzyme in tris-Cl buffer and incubation at 37°C the hemagglutinating capacity of all strains was rapidly inactivated. Minimum concentration of enzyme and minimum time required for inactivation was not the same for all strains. However, with all strains, lowering the incubation temperature or adding sodium or calcium chloride to the trypsin-virus mixtures either reduced the rate of the reaction or completely inhibited it. These changes in test conditions did not in all instances affect all strains to the same extent. With 5 of the 6 strains the effect of added salt appeared to be more closely associated with the concentration than the type of ion present, whereas with the sixth strain the type of ion was a significant factor. The findings illustrate the importance of both the strain examined and the experimental conditions employed in de-

termining the response of type A influenza virus to trypsin treatment.

1. Sugg, J. Y., Cleeland, R., *J. Immunol.*, 1962, v88, 369.

2. Schwert, G. W., Takenaka, Y. A., *Biochim. et Biophys. Acta*, 1955, v16, 570.

3. Cleeland, R., Sugg, J. Y., *J. Immunol.*, 1960, v85, 539.

Received February 13, 1963. P.S.E.B.M., 1963, v112.

Effect of Acid Solutions on Human Gustatory Chemoreceptors as Determined by Parotid Gland Secretion Rate.* (28210)

H. H. CHAUNCEY, R. P. FELLER AND I. L. SHANNON

Department of Dental Science, Tufts University School of Dental Medicine, Boston, Mass., and USAF School of Aerospace Medicine, Brooks AFB, Texas

In the past measurement of human responses to gustatory stimuli has been based on subjective evaluation. Recent studies(1) employing parotid gland secretion rate as a method for measuring response to gustatory stimuli in humans have offered an objective approach to quantitative measurement of the effect of these stimuli. Utilization of these techniques permits accurate assay of physiological responses to innumerable experimental conditions.

In this study the secretory response elicited by 17 organic acids was used to determine which chemical and physical properties of acids exerted an influence on their stimulatory action on human gustatory chemoreceptors, giving rise to the "sour" sensation.

Materials and methods. Solutions were applied using wetted cotton applicator sticks. One complete application consisted of running the wetted swab around the lateral edges of the tongue and a subsequent swabbing of the dorsum of the tongue from the circumvallate papillae to the tip. Applications were at the rate of 4 times per minute.

Acids were presented in groups of three. The 9 subjects (third year, male, dental students) utilized for this study were divided into 3 groups of 3. The first subject in each group received the acids in the order ABC, the second in the order BCA, and the third in the order CAB. Nose clamps were worn throughout the experiment to prevent olfactory stimulation.

Parotid saliva samples were obtained using a vacuum cap(2). The fluid was collected in 15 ml centrifuge tubes, graduated to 0.1 ml, and the volume read to the nearest 0.05 ml. Rate of secretion was determined by measuring volume secreted in a standard time period. A control series using only water was conducted to evaluate the response elicited by tactile stimulation. These control values were subtracted from all other experimental data so as to measure only the response elicited by gustation.

After initial placement of the vacuum cap, a 5-minute acquaintance interval of stimulation was allowed each subject. This collection was considered a clearance sample and was discarded. Upon completion of the acquaintance period, a 10-minute experimental sample was collected. This was followed by a 5-minute rest interval, during which each subject was given a drink of water. Using this procedure a total of 3 experimental samples were obtained during a single sitting. Total collection period, including the 2 rest intervals, was 55 minutes. All samples were collected preprandially.

Subjective evaluation of the degree of sourness was also undertaken. Participants swabbed their tongues in the manner previously described, using groups of 3 acids. Subjects were allowed to sample the acids as often as necessary and in any sequence so as to determine the relative sourness of the 3 acids.

* This investigation was supported in part by U.S.P.H.S. research grant from National Inst. of Dental Research, N.I.H.