The increased toxicity of methylglyoxal in oxythiamine treated mice as compared to pyrithiamine treated ones may be dependent on the known differences in action of these antagonists. Pyrithiamine inhibits the phosphorylation of thiamine. When administered to animals it causes a variable decrease in thiamine pyrophosphate concentration in certain tissues. Oxythiamine, however, is itself phosphorylated and then acts as a competitor with thiamine pyrophosphate for active enzyme sites(7). Jones(6) has shown that neither a thiamine deficient diet nor the addition of thiamine antagonists to the diet markedly inhibited pyruvate decarboxylation. However, he did note that oxythiamine had a specific, though limited, inhibiting effect on pyruvate decarboxylation. It has been shown that oxythiamine is a more potent inhibitor of lactic dehydrogenase than pyrithiamine (8). It is thus reasonable to expect that administration of a large dose of oxythiamine would more profoundly alter carbohydrate metabolism than an equivalent dose of pyrithiamine. Since methylglyoxal is oxidized *via* lactic acid, an alteration in carbohydrate metabolism resulting from previously administered oxythiamine would be likely to enhance its toxicity.

The failure to detect methylglyoxal in the brain, muscle or urine of mice made thiamine deficient by dietary means is in agreement with the observations of Liang(2) on rats. Our inability to detect it in the oxythiamine treated mice was somewhat disappointing in view of the toxicity studies. However, Drummond has shown that even in markedly deficient animals, the level of glyoxalase activity may not be reduced sufficiently to prevent its oxidation to lactic acid(1).

Summary. Intraperitoneal injection of methylglyoxal was toxic to mice, the LD_{50} in normal mice after 24 hours being 290 mg/kg. The cause of death with doses of 400 mg/kg and above appeared to differ from that produced by lower doses. Neither pretreatment with a thiamine deficient diet nor pyrithiamine significantly affected the toxicity of methylglyoxal. However, pretreatment with oxythiamine lowered the LD_{50} of methylglyoxal to 150 mg/kg. Methylglyoxal was not detected in the urine of normal mice given methylglyoxal, nor in the urine, brain or muscle of mice treated with pyrithiamine or oxythiamine or in mice fed a thiamine deficient diet.

1. Drummond, G. I., *J. Nutrition*, 1961, v74, 357.
2. Liang, C. C., *Biochem. J.*, 1962, v82, 429.
3. Eys, J. van, Judge, M. A., Judd, J., Hill, W., Bozian, R. C., Abrahams, S., *J. Nutrition*, 1962, v76, 375.
4. Litchfield, J. T., Wilcoxon, F., *J. Pharmacol. and Exp. Therap.*, 1949, v96, 99.
5. Haynes, F. W., Weiss, S., *Am. Heart J.*, 1940, v20, 34.
6. Jones, J. H., d'Angeli, E., *J. Nutrition*, 1960, v70, 537.
7. Koedam, J. C., *Biochem. et Biophys. Acta*, 1958, v29, 333.
8. Eys, J. van, *J. Nutrition*, 1961, v73, 403.

Received May 22, 1964. P.S.E.B.M., 1964, v116.

The Neuraminidase of Parainfluenza Virus (Type 2).* (29461)

RICHARD W. DARRELL AND CALDERON HOWE
(Introduced by Harry M. Rose)
Department of Microbiology, College of Physicians and Surgeons, Columbia University, New York City

The ability of the influenza viruses to elute from erythrocytes appears to be dependent upon the presence of active neuraminidase in the viral particle(1,2,3,4). The parainfluenza viruses are also able to agglu-

* These studies were supported in part by Nat. Inst. of Health Grant AI 03168 and The General Research Support Grant, and by the Office of the Surgeon General, Depart. of the Army, under the auspices of the Commission on Influenza, Armed Forces Epidemiological Board.

tinate and elute from erythrocytes(5), but direct evidence for neuraminidase has been lacking. This paper presents the results of investigation into the enzymatic properties of parainfluenza virus (type 2) in relation to infectivity, hemagglutinin and receptor specificity.

Materials and methods. A line of stable human amnion cells† was grown at 37°C in Eagle's basal medium with Hanks' balanced salt solution and 10-15% calf serum. Type 2 parainfluenza virus (CA virus) was obtained from The American Type Culture Collection. Washed monolayers in 32 ounce bottles were infected with 10^3-10^5 $TCID_{50}$ of virus, and the virus harvested 3-4 days later. Virus was concentrated from culture fluid by centrifugation at 50,000 to 100,000 *g* for 60 to 90 minutes, and resuspended to 1/100 the original volume in 0.9% NaCl. Cells remaining in the culture flasks were scraped from the glass into saline, washed once, resuspended in a volume of 5-10 ml and subjected to ultrasonic vibration for 10 minutes in a Raytheon Sonic Oscillator, model DF 101. The sonicate, combined with virus concentrated from culture fluid, was clarified by centrifugation at 3,000 rpm. The final preparation gave a hemagglutinin titer of 1/512 to 1/2560 and contained 10^5 to 10^6 $TCID_{50}$ per ml. Hemagglutination tests were performed at 4°C using 0.4 ml of virus dilution and 0.1 ml of 2% washed chicken erythrocytes in 0.9% NaCl buffered to pH 7.2 with M/15 phosphate. Hemagglutination inhibition tests were performed at 4°C in buffered saline using 5 hemagglutinating units of virus for 0.1 ml of 2% chicken erythrocytes in a final volume of 0.7 ml. End points were read by pattern after 30 minutes. For infectivity titrations, washed monolayers of stable human amnion cells in screw cap tubes were inoculated in duplicate with 0.2 ml of serial tenfold dilutions of virus, and fresh growth medium was added after 45 minutes' incubation at 37°C. Three days later the cultures were examined for hemadsorption with guinea pig erythrocytes(6). The virus titer was taken as the highest dilution that produced 2 or 3 areas of hemadsorption in one or both duplicate tubes. The neuraminidase activity in concentrated virus preparations was estimated by measuring the rate at which N-acetylneuraminic acid was liberated from a standard substrate, Collocalia mucoid(7), and expressed as a percentage of the total N-acetylneuraminic acid obtainable by hydrolysis at 80°C for 20 minutes in 0.1 N H_2SO_4. In various experiments, Collocalia mucoid and virus sonicate preparations were mixed in the proportion of 2 to 20 mg of mucoid per mg of viral sonicate nitrogen measured by the microKjeldahl procedure (8). 0.2 ml aliquots of the mixture were withdrawn at zero time, and subsequently at appropriate intervals, heated to 75°C for 10 minutes to destroy enzyme activity, and stored at 4°C until analyzed for free N-acetylneuraminic acid by the thiobarbituric acid method of Warren(9). A sample of the standard preparation of mucoid was hydrolyzed with each set of determinations.

Experimental results. The effect of type 2 parainfluenza virus on chicken erythrocyte receptors was determined. 3 ml of virus (hemagglutinin titer of 1/1024) was mixed with 12 ml of 3% chicken erythrocytes, representing a ratio of virus to erythrocytes approximately 400 times that of the hemagglutinating unit. Virus absorption and elution were promoted by holding the mixture for 15 minute periods alternately at 4°C and 37°C, with agitation, through a total of 6 cycles. After storage overnight at 4°C, the cells were centrifuged, washed 5 times in buffered saline, and used in hemagglutination titrations with various myxoviruses. Erythrocytes treated with type 2 parainfluenza virus were stable, and no longer agglutinable by the same virus. Receptors for the other myxoviruses tested, however, were unaffected (Table I).

The pH optimum for type 2 parainfluenza virus neuraminidase activity in M/15 phosphate or acetate buffers was between 4.5 and 5.5 with Collocalia mucoid as substrate (Fig. 1). Under these conditions, the most active virus preparations released 40-50% of the total N-acetylneuraminic acid present in the

† Kindly supplied by Dr. W. R. Dowdle, Respirovirus Unit, Communicable Disease Center, Atlanta, Ga.

TABLE I. Effect of Type 2 Parainfluenza Virus on Chicken Erythrocyte Receptors.

Viruses*	Reciprocal of hemagglutinin titer: Normal erythrocytes	Reciprocal of hemagglutinin titer: Erythrocytes treated with parainfluenza 2 virus
Influenza		
PR_8 (A)	64	64
Lee (B)	256	256
Great Lakes (B)	16	16
Swine	512	512
Newcastle disease	64	64
Mumps	512	512
Parainfluenza 2	512	<8

* All viruses except parainfluenza were in the form of infective chorioallantoic fluid.

mucoid in 8-10 hours (Fig. 2). Sonicates of uninfected cells displayed negligible neuraminidase activity. The rate of neuraminidase activity at 4°C over this period of time was half that at 37°C. Heating the virus for 10 minutes at 50°C did not reduce neuraminidase activity. However, heating at 75°C for 10 minutes abolished all detectable activity. Divalent cations (10^{-3} M concentrations of Co^{++}, Cu^{++}, Fe^{++}, Mg^{++}, Mn^{++}, Ni^{++}, and Zn^{++}) had no effect on enzyme activity at pH 5.5 at 37°.

Attempts were made to convert type 2 parainfluenza virus in cell sonicate at pH 6.8-7.2 to the indicator state by heating at 56°C for appropriate periods of time. In preparations having a hemagglutinin titer of 1/2048 and an infectivity titer of 10^5 $TCID_{50}$ per ml, hemagglutinin and infectivity disappeared after one hour, and enzyme activity only after 2 hours of heating at 56°C (Table II). Under these conditions, it was not possible to destroy infectivity without also losing all hemagglutinin. Thus the virus could not be changed to the indicator state as originally defined(10,11).

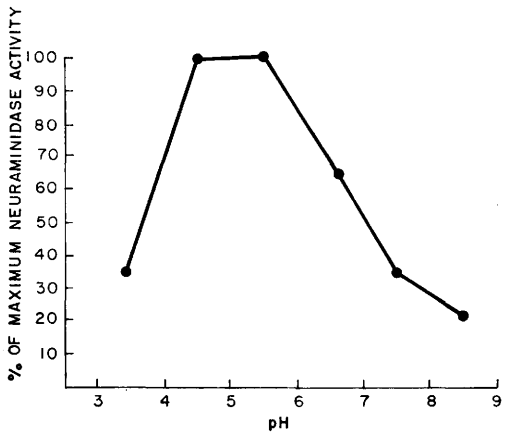

FIG. 1. pH optimum for neuraminidase activity of type 2 parainfluenza virus. Virus incubated with Collocalia mucoid in M/15 acetate or phosphate buffers (pH 3.5 to 7.5) for 19-23 hr. Summary of 5 experiments.

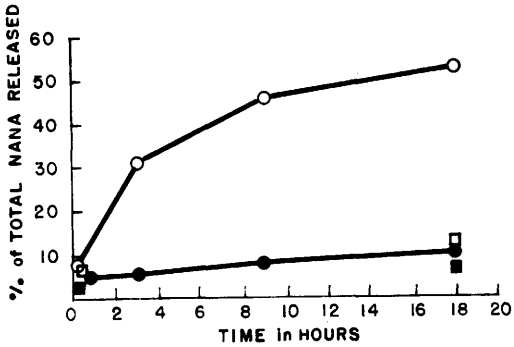

FIG. 2. Neuraminidase activity in sonicates of infected and uninfected cells. ○, Infected cell sonicate and Collocalia mucoid; □, uninfected cell sonicate and Collocalia mucoid; ●, infected cell sonicate and buffer; ■, uninfected cell sonicate and buffer. (All at 37°C, pH 5.5.)

TABLE II. Effect of Heat on Infectivity, Hemagglutinin and Neuramidase Activity.

Duration of heating to 56°C	Reciprocal of hemagglutinin titer	Infectivity titer	Neuraminidase activity*
0 min	2048	10^{-5}	45
10 "	128	10^{-4}	45
30 "	8	10^{-3}	34
1 hr	<4	$<10^{-1}$	15
2 "	<4	$<10^{-1}$	10
5 "	<4	$<10^{-1}$	0

* % of total hydrolyzable NANA liberated in 19 hr from Collocalia mucoid at pH 5.5 and 37°C.

Various mucoids were tested for their ability to inhibit unheated type 2 parainfluenza virus hemagglutinin. For comparison, a standard A_1 strain of influenza virus (FM_1), which had been heated at 56°C sufficiently long to destroy infectivity without appreciable loss of hemagglutinin, was included as an indicator virus. Hemagglutination by this indicator was inhibited by 1 μg each of Collocalia mucoid, sputum inhibitor(12-15), and purified viral receptor substance from human

erythrocytes(16). All of these latter substances, at levels of 50 μg, as well as horse serum in a dilution of 1/10, were inactive against type 2 parainfluenza virus hemagglutinin. The same horse serum at a dilution of 1/500 was active against an inhibitor-sensitive strain of A_2 influenza virus (RI/5+).‡

Type 2 parainfluenza virus hemagglutinin could be dissociated from neuraminidase in sonicates of infected cells by centrifugation 100,000 *g* for 1 hour, the hemagglutinin being found only in the sediment and most of the enzyme in the supernatant. Separation of soluble enzyme from other sonicate components by electrophoresis in agar was unsuccessful.

Comment. Type 2 parainfluenza virus agglutinates and elutes from erythrocytes in a manner analogous to that of influenza virus, with consequent loss of receptors for the homologous virus. Partial destruction of these receptors by 32 HA units of fresh type 2 parainfluenza virus preparations has been reported(5). In the present experiments, complete inactivation of homologous receptors was achieved by using approximately 10 times this concentration of virus ($\pm$400 HA units). Receptors for other myxoviruses tested, however, were not affected under these conditions.

Type 2 parainfluenza virus enzyme liberates N-acetylneuraminic acid from Collocalia mucoid at temperature and pH optima similar to those reported for the influenza virus neuraminidase (37°C and pH 5.5)(4). In contrast to most influenza viruses, however, type 2 parainfluenza virus cannot be converted by heating at 56°C into the "indicator state." In this respect, type 2 parainfluenza virus resembles the A_2 (Asian) strains of influenza virus, in which the hemagglutinin is destroyed before the neuraminidase by heating at 56°C(7).

On the other hand, the hemagglutinin of infective type 2 parainfluenza virus is not inhibited by sialomucoids active against numerous strains of influenza virus, including A_2 strains, or by the gamma inhibitor of horse serum, suggesting that the groupings necessary for combination with type 2 parainfluenza virus may differ significantly from those which bind influenza virus.

‡ Kindly provided by Dr. P. W. Choppin.

1. Gottschalk, A., *Biochim. Biophys. Acta,* 1957, v23, 645.
2. Howe, C., Rose, H. M., Schneider, L., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 88.
3. Gottschalk, A., *Nature,* (Lond.), 1958, v181, 377.
4. Howe, C., Lee, L. T., Rose, H. M., *Nature,* 1960, v188, 251.
5. Chanock, R. M., *J. Exp. Med.,* 1956, v104, 555.
6. Shelokov, A., Vogel, J. E., Chi, L., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 802.
7. Howe, C., Lee, L. T., Rose, H. M., *Arch. Biochem. Biophys.,* 1961, v95, 512.
8. Markham, R., *Biochem. J.,* 1942, v36, 790.
9. Warren, L., *J. Biol. Chem.,* 1959, v234, 1971.
10. Francis, T., *J. Exp. Med.,* 1947, v85, 1.
11. Stone, J. D., *Aust. J. Exp. Biol. Med. Sci.,* 1949, v27, 337.
12. Rose, H. M., Erlanger, B. F., Raymond, S., Howe, C., *Atti del VI Cong. Intern. di Microbiol.,* 1953, v3, 113.
13. Marmion, B. P., Curtain, C. C., Pye, J., *Aust. J. Exp. Biol. Med. Sci.,* 1953, v31, 505.
14. Howe, C., MacLennan, J. D., Mandl, I., Kabat, E. A., *J. Bacteriol.,* 1957, v74, 365.
15. Howe, C., Rose, H., Lee, L. T., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 420.
16. Howe, C., Avrameas, S., St. Cyr, C. deV., Grabar, P., Lee, L. T., *J. Immunol.,* 1963, v91, 683.

Received May 26, 1964. P.S.E.B.M., 1964, v116.