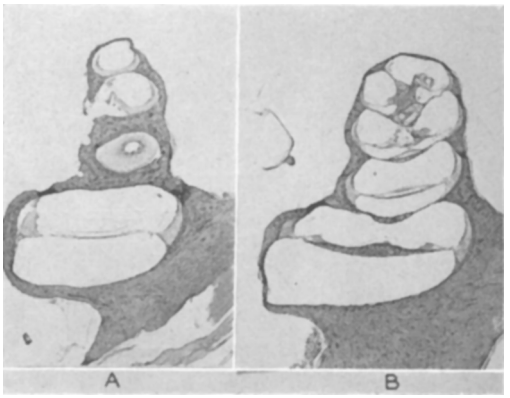

FIG. 1.
A. Exp. 60. Large hole and deliberate injury in Turn 3. Reissner's membrane and basilar membrane torn. Beneath the large hole is the outer end of a small hole that entered Turn 2, scala media.
B. Exp. 67. Hole entering modiolus in Turn 3. Axis of hole is perpendicular to plane of section.

nervous structures in labyrinth and modiolus.

Summary. The aural microphonic of guinea pigs was recorded from the round window and from copper wires applied to or passing through tiny holes drilled through the bony shell of the cochlea. When the guinea pig's respiration fails gradually, the microphonic falls to a few percent of its original strength before the heart stops. The failure is partly reversible if respiration improves.

Frequently near the postmortem level the microphonic undergoes partial or complete half-wave rectification. The phase corresponding to condensation in the external canal is more depressed than the phase corresponding to rarefaction. This effect also is temporarily reversible.

Extensive surgical injury including amputation of the two apical turns may cause little or no change in the microphonic (1000 cycles per second) at the round window over several hours.

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17117. Differences in Hemagglutination by Strains of Influenza Virus in Presence of Egg White and Normal Serum.*

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Substances of various origin have been shown[1-10] to inhibit the hemagglutination reaction of influenza virus. Evidence has also been presented[5,11,12] suggesting that some of these inhibitors are identical with, or closely related to, the receptor substance of red cells. When it was shown[13] that serum inhibits hemagglutination by heated virus to a much higher degree than hemagglutination by fresh

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1 Hirst, G. K., *J. Exp. Med.*, 1942, **75**, 49.

2 Hirst, G. K., *J. Exp. Med.*, 1943, **78**, 99.

3 Burnet, F. M., *Austral. J. Sc.*, 1947, **10**, 21.

4 Green, R. H., and Woolley, D. W., *J. Exp. Med.*, 1947, **86**, 55.

5 Friedewald, W. F., Miller, E. S., and Whatley, L. R., *J. Exp. Med.*, 1947, **86**, 65.

6 Lanni, F., and Beard, J. W., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 312, 442.

7 Bovarnick, M., and deBurgh, P. M., *J. Exp. Med.*, 1948, **87**, 1.

8 Ginsberg, H. S., Goebel, W. F., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1948, **87**, 411.

9 Hardy, P. H., Jr., and Horsfall, F. L., Jr., *J. Exp. Med.*, 1948, **88**, 63.

10 Francis, T., Jr., and Minuse, E., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 291.

11 Burnet, F. M., McCrea, J. F., and Anderson, S. G., *Nature*, 1947, **160**, 404.

TABLE I.
Comparison of Inhibition Titer of Two Samples of Rabbit Serum and Egg White on Hemagglutination by PR8 and Lee Strains.

Strain	Treatment	Inhibition titer* of Rabbit serum No. 1	Rabbit serum No. 2	Egg white No. 1	Egg white No. 2
PR8	unheated	<10	<10	10	<10
	30′ 56°	<10	<10	40	<10
Lee	unheated	40	40	10	<10
	30′ 56°	160	160	2560	640

* Numbers give highest dilutions of inhibitor which are effective when added to virus.

virus, it was suggested that[12,14] the inhibition phenomenon could be explained on the basis of an enzymatic reaction as had previously been proposed[1] for the action of virus on red cells. According to the enzymatic theory, heating inactivates an enzyme of the virus which in the fresh state is able to destroy the inhibitor. The question arises whether all inhibitors are of the same chemical nature or whether different substances are involved. In terms of the enzymatic theory a plurality of inhibitors might also necessitate the assumption of different enzymatic factors in the virus. To obtain some evidence which could be used for answering this question, the behavior of different influenza strains toward the inhibitors contained in normal rabbit serum and egg white was compared.

Materials and methods. Virus Preparation. Allantoic fluid from eggs infected with the different strains of virus was used either unheated or after heating for 30 minutes at 56° or 61°C, during the first 14 days after harvesting. The following strains were examined: Swine, Oti, PR8, Weiss, Marton, Baum, FMl, Lee and Warner. The passage history of these strains is given in Table II.

Inhibitors. (a) Rabbit serum. Blood was drawn from normal, fully grown rabbits by cardiac puncture. After clotting and centrifugation, the serum (NRS) was pipetted off and heated for 30 minutes at 56°C.

(b) Egg white. The egg white (EW) of several eggs was separated from the yolk and filtered twice through a single layer of gauze. All fluids were stored at +4°C in rubber stoppered test tubes.

Inhibition tests. A titration of virus using Salk's method[15] was made before every test. A virus concentration equal to eight times the highest hemagglutinating dilution was used in the inhibition test. Two-fold dilutions of inhibitor in saline starting with a 10-fold dilution were prepared in 0.25 cc amounts, and to all dilutions 0.25 cc of the indicated dilution of virus was added. After thorough mixing the tubes stood at room temperature for 30 minutes. Then 0.5 cc of 0.25 per cent suspension of chicken blood cells was added. Results were read after 75 minutes. A parallel titration of the virus dilution used in the inhibition test was made in every experiment to insure that the right amount of virus had been used.

Results. Comparison of different batches of inhibitors. In a preliminary experiment the inhibiting action of 2 different batches of rabbit serum and egg white on the hemagglutination of PR8 and Lee strains was examined. It can be seen in Table I that the 2 rabbit sera had the same inhibition titer, but the 2 batches of egg white gave different results. The inhibition titers with Batch No. 1 of egg white were higher than with Batch No. 2. In comparing the effect of an inhibitor on different influenza strains, it is, therefore, important to use the same sample of inhibitor for the whole series. The experiments reported below were all done with the same samples of rabbit serum and egg white.

Susceptibility of unheated virus to NRS and EW. Table II shows that the examined

[12] Hirst, G. K., *J. Exp. Med.*, 1948, **87**, 301, 315.
[13] Francis, T., Jr., *J. Exp. Med.*, 1947, **85**, 1.
[14] Burnet, F. M., *Lancet*, 1948, **254**, 7.
[15] Salk, J. E., *J. Immunol.*, 1944, **49**, 87.

TABLE II.
Inhibiting Action of Rabbit's Serum and Egg White on Hemagglutination of Nine Different Strains of Influenza Virus.

Strain	Passage	Treatment	Inhibition titer† of rabbit serum	Inhibition titer of egg white
Swine	1976 M38 E25*	—	<10	10
		30′ 56°	<10	2560
Oti	M179 E37	—	10	40
		30′ 56°	40	5120
PR8	198 M593 E86	—	<10	20
		30′ 56°	<10	80
Weiss	F3 M32 E41	—	10	40
		30′ 56°	20	5120
Marton	E10	—	160	40
		30′ 56°	320	5120
Baum	F19 M10 E15	—	40	160
		30′ 56°	1280	5120
FM1	Lilly M37 E17	—	40	80
		30′ 56°	2560	5120
Lee	F8 M137 E122	—	40	10
		30′ 56°	160	2560
Warner	E6 M17 E2	—	640	20
		30′ 56°	2560	5120

* Number after F = number of ferret passages; number after M = number of mouse passages; number after E = number of egg passages.
† Numbers give highest dilutions of inhibitor which are effective when added to virus.

TABLE III.
Inhibition of Rabbit Serum on Influenza Strains Heated to 56°C and 61°C for 30 Min.

		Dilution of rabbit serum									
Strain	Treatment	10	20	40	80	160	320	640	1280	2560	5120
PR8	56°	+	+	+	+	+	+	+	+	+	+
	61°	—	—	—	—	—	—	—	+	+	+
Weiss	56°	—	—	+	+	+	+	+	+	+	+
	61°	—	—	—	—	—	—	—	—	+	+
Oti	56°	—	—	—	+	+	+	+	+	+	+
	61°	—	—	—	—	—	—	—	—	+	+
Lee	56°	—	—	—	—	—	+	+	+	+	+
	61°	—	—	—	—	—	—	—	+	+	+

+ = hemagglutination.
— = no hemagglutination.

strains differ in their behavior toward the 2 inhibitors. Six strains are more susceptible to inhibition by EW, three strains are more strongly inhibited by NRS. The greatest difference in behavior toward the 2 inhibitors was observed in the case of the Warner strain which was inhibited in a 32-fold higher dilution of NRS than of EW.

Susceptibility of heated virus to NRS and EW. Heating for 30 minutes at 56°C made all strains except PR8 highly susceptible to the inhibiting action of EW. Inhibition titers of 2560 to 5120 were obtained. The susceptibility to inhibitor after heating of the virus was one hundred to several hundred fold greater with all strains except PR8 with which the rise was only 4-fold. The effect of heating virus preparations to 56°C on the susceptibility to inhibition by serum was different. In 5 strains the rise in susceptibility was only 2 to 4-fold and in the case of PR8 and swine influenza strain 1976 this temperature had no measurable effect on the susceptibility to NRS. Especially striking was the behavior of the swine influenza strain. Its susceptibility to EW rose several hundred times, while its susceptibility to NRS was not changed. A similar observation with the same virus has already been made by Lanni and Beard.[6] With two strains the rise in titer of NRS was 30 to 60-fold and thus approached the values observed with EW.

With other strains the inhibition of hemagglutination by NRS could be raised to titers similar to those attained by EW if a temperature of 61°C was used for the inactivation of the virus preparations. This is shown in Table III for the PR8, Weiss, Oti and Lee strains. NRS in dilutions of 640-1280 inhibited hemagglutination by heated virus, and thus came close to the values obtained with EW. In the case of the swine influenza strain it was impossible to raise its susceptibility toward NRS as a temperature of 56°C was ineffective and a temperature of 58°C destroyed the hemagglutinating ability of this strain.

Discussion. The observation that an influenza strain may be highly susceptible to one inhibitor and only slightly susceptible to another and the fact that heating will alter the susceptibility of a strain to one inhibitor without changing measurably its susceptibility toward the other is considered evidence for a qualitative difference in the inhibitors involved. If this conclusion is discussed in terms of the enzymatic theory it would have to be assumed that the effect of virus on the two inhibitors is caused by two different enzymatic factors which can exist independently and which can be differentiated by their resistance to heat. The virus factor active against egg white inhibitor is generally more heat labile than the factor against rabbit serum. It is completely or partially destroyed in all strains by a temperature of 56°C whereas, a higher temperature is necessary to inactivate the factor acting against rabbit serum. The independence of the two factors is most clearly demonstrated in the case of the swine influenza strain in which heating to 56°C destroys completely the activity against egg white without measurably altering its activity against serum inhibitor. It is possible that the assumption of still other enzymatic factors for other inhibitors might become necessary, if the enzymatic theory is to be accepted. It seems probable that just as the antigenic composition of strains differs so also will their capacity to react with different chemical substrates be found to vary.

Summary. The hemagglutinating activity of some strains of influenza virus is more susceptible to egg white inhibitor while that of others is more susceptible to inhibition by rabbit serum. Heating to 56°C makes all strains susceptible to inhibition by egg white. When tested with 4 different strains a higher temperature was required to make them as susceptible to inhibition by normal rabbit serum. It is concluded from these results that the inhibitors are not identical. The results are discussed in terms of the enzymatic theory according to which two different enzymatic factors in the virus active against rabbit serum and egg white would have to be assumed.

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17118. Effect of Tagathen* on Histamine-Induced Gastric Lesions in Guinea Pigs.

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The use of synthetic antihistaminics to prevent or diminish the incidence or severity of histamine-induced ulcers in animals has been reported in less than a half dozen papers. Most of these were concerned with the failure of benadryl* (B-dimethylaminoethylbenzhydryl ether HCl) to prevent experimental ulcer formation.[1-4] In all of these reports the "histamine in beeswax" method of Wangensteen and coworkers[5] was used. In addition

* Brand of antihistaminic drug.

1 Crane, J. T., Lindsay, S., and Dailey, M. E., *Am. J. Dig. Diseases*, 1947, **14**, 56.

2 Friesen, S. R., Baronofsky, I. D., and Wangensteen, O. H., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 23.