or accelerators of lysins has been directed to the nature and variations of the antihemolytic substances in the plasma, and practically no attention has been given to the capacity of the tissues in this activity. This study suggests that inhibitory agents in the cell may play a very important role in the occurrence of hemolysis *in vivo*. It is possible that the slow hemolytic activity produced by slices of normal human tissues (*10, 11*) and the faster activity found in extracts of certain tumor cells (*22, 5*) may be accounted for on the basis of the effective inhibitor concentration.

The precise manner of the mechanism of interaction of these substances remains to be determined, and several possibilities suggest themselves for further study: (1) an inhibitor-lysin complex may normally exist, which may be acted on by a heat-labile enzyme to release a heat-stable lytic agent (*13, 16*); (2) by enzyme action a lytic agent is produced which, in turn, can be inactivated by the inhibitor; (3) the lytic agent is an enzyme which can act directly on the red cell membrane, but the inhibitor behaves as a preferred competitive substrate; (4) the inhibitor may, in some manner, increase the resistance of the red cells against hemolysis.

Ponder (*11*) has suggested that, under conditions where the production of a lysin may remain constant, the effective concentration of the lysin may be altered by the addition of accelerators or inhibitors, "... the net effect of which is an inhibitory one." The demonstration here of lysins and inhibitors within the cell and of the consequences of differences in the effective concentration of the latter lends support to that idea.

References

1. BERGENHEM, B., and FAHRAEUS, R. *Z. ges. exp. Med.*, 1936, **97**, 555.
2. BORSOOK, H., *et al.* *J. biol. Chem.*, 1950, **184**, 529.
3. CLAUDE, A. *J. exp. Med.*, 1946, **84**, 61.
4. FLEXNER, L. B., and FLEXNER, J. B. *J. cellular comp. Physiol.*, 1946, **27**, 35.
5. GROSS, L. *Proc. Soc. Exp. Biol. Med.*, 1947, **65**, 292.
6. HIMWICH, H. E., and FAZEKAS, J. F. *Am. J. Physiol.*, 1941, **132**, 293.
7. HOGEBOOM, G. H., SCHNEIDER, W. C., and PALLADE, G. E. *J. biol. Chem.*, 1948, **172**, 619.
8. LEE, J. S., and TSAI, C. *Quart. J. exp. Physiol.*, 1942, **31**, 271, 281.
9. LE PAGE, G. A., and SCHNEIDER, W. C. *J. biol. Chem.*, 1948, **176**, 1021.
10. MAEGRAITH, B. G., MARTIN, N. H., and FINDLAY, G. M. *Brit. J. exp. Path.*, 1942, **24**, 58.
11. PONDER, E. *J. gen. Physiol.*, 1944, **27**, 483.
12. ———. *Hemolysis and related phenomena.* New York: Grune & Stratton, 1948.
13. ———. Personal communication.
14. POTTER, V. R., and ELVEHJEM, G. A. *J. biol. Chem.*, 1936, **114**, 495.
15. POTTER, V. R., SCHNEIDER, W. C., and LIEBL, G. J. *Cancer Research*, 1945, **5**, 21.
16. TYLER, D. B. *Proc. Soc. Exp. Biol. Med.*, 1949, **72**, 491.
17. ———. *Arch. Biochem.*, 1950, **25**, 221.
18. ———. *J. biol. Chem.*, 1950, **184**, 711.
19. ———. Unpublished observations.
20. TYLER, D. B., and VAN HARREVELD, A. *Am. J. Physiol.*, 1942, **136**, 600.
21. TSAI, C., and LEE, J. S. *Chinese J. Physiol.*, 1941, **16**, 165.
22. WEIL, R. *J. med. Research*, 1907, **16**, 287.

Methanol Precipitation of Influenza Virus

Arden W. Moyer, George R. Sharpless, Maurice C. Davies, Kenneth Winfield, and Herald R. Cox

Section for Viral and Rickettsial Research, Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

Sometime subsequent to the report by Cox, Van der Scheer, Aiston, and Bohnel (*1*) on purification and concentration of influenza virus in fresh chorioallantoic (CA) fluid by methanol precipitation, difficulties were encountered in obtaining good yields. As indicated by chicken red cell agglutination (CCA) titers (*2*), the yields obtained from various lots of infected fluids ranged from 25 to 79% instead of the 95–110% recorded in the original publication (*1*). The cause of this decreased yield is still unknown, but it may have been associated with variations in the components of allantoic fluid induced by seasonal, dietary, or other factors inherent in the laying flocks from which the egg supply was drawn.

No improvement in the yield was produced by varying the pH, alcohol concentration, time, and temperature of elution. Tests performed at various steps of the process indicated that the virus was being precipitated but was not being eluted.

Excellent recoveries of virus again were obtained when the concentration of the eluting phosphate buffer solution, pH 7.0, was changed from 0.1 *M* to 0.5 *M*. Further trials led to the adoption of 0.3 *M* phosphate buffer solution as the preferred eluant for influenza virus. Shown in Table 1 are some representative data obtained with two strains of influenza virus.

TABLE 1

Lee Strain—fresh allantoic fluid				PR8 Strain—fresh allantoic fluid			
Methanol, %	Phosphate buffer pH 7, *M*	CCA titer	Yield, %	Methanol, %	Phosphate buffer pH 7, *M*	CCA titer	Yield, %
31	0.1	73	60	23	0.1	131	69
31	0.3	114	94	23	0.3	189	100
Original CA fluid		121		Original CA fluid		189	

Satisfactory yields of virus were never obtained when 0.1 *M* phosphate buffer solution, pH 7.0, was used as the eluant for viral precipitates obtained from CA fluids that had been preserved with merthiolate 1: 5,000 and formalin 1: 10,000. As the data in Table 2 indicate, however, the use of 0.3 *M* phosphate buffer solution gave satisfactory yields of virus even from such preserved fluids.

The elution of methanol-precipitated influenza virus with 0.3 *M* phosphate buffer solution, pH 7.0, has been uniformly successful, regardless of uncontrolled variables

TABLE 2

Preserved CA fluid PR8 strain	Methanol, %	Phosphate buffer pH 7, *M*	CCA titer	Yield, %
Batch #1	20	0.1	68	54
	25	0.1	34	27
	30	0.1	0	0
	Original CA fluid		126	
Batch #2	14	0.1	39	41
	17	0.1	40	42
	20	0.1	48	50
	Original CA fluid		96	
Batch #3	23	0.1	96	44
	23	0.2	172	78
	20	0.3	164	74
	23	0.3	178	81
	26	0.3	219	100
	Original CA fluid		220	

or previous treatment of the CA fluids with such preservatives as merthiolate and formalin in concentrations of 1: 5,000 and 1: 10,000, respectively.

References

1. Cox, H. R., *et al. J. Immunol.*, 1947, **56**, 149.
2. NIH directions for titration of chicken red cell agglutination (CCA) activity of influenza virus and vaccines according to Hirst's method, as modified for use in the laboratories of the Rockefeller Institute at Princeton, N. J., Sept. 16, 1946.

A Sex-linked Lethal Gene in the Fowl[1]

K. Goodwin, F. B. Hutt, and R. K. Cole

Department of Poultry Husbandry, Cornell University, Ithaca, New York

This report deals with a sex-linked recessive lethal gene that was found in White Leghorns. The gene, for which the symbol *xl* is suggested, exerts its lethal action during the growing period, with affected females having been observed as early as 23 days of age and as late as 123 days of age. The abnormal physiological mechanism resulting in death has not as yet been determined; nevertheless, the gene induces a set of symptoms which identify it as a specific character.

In the affected chick the symptoms are hyperacute, to the extent that after only a few hours' illness the bird either succumbs, or else it undergoes a remarkable recovery. Sick chicks are invariably discovered early in the morning; the onset of symptoms apparently does not occur during daylight hours. Affected birds display an extreme listlessness, resting quietly on their keels with the tip of the beak touching the floor. In the later stages they may pass into a complete coma. Occasionally dyspnea, or tetanic spasms, or both, are seen. The first attack is not always fatal, some birds having been observed to survive as many as 3 attacks before succumbing to a subsequent one. Among the 64 affected females observed in 1947 and 1948, 2 apparently made a complete recovery (in one instance after 1 attack, and in the other instance after 2), and the birds lived to reproduce. Deaths appear to be concentrated on certain days, suggesting that some environmental factor, as yet unrecognized, precipitates the condition. The dead birds are well-fleshed, and findings at autopsy are essentially negative.

The condition came to the attention of this department when a breeder of White Leghorns experienced a number of losses for which no specific cause could be found. Since these chicks were all from one sire, the influence of heredity was suspected by the owner and by Grayson B. Mitchell, who made the autopsies. The suspected sire (K-1) and 4 dams from the mating that had produced affected chicks on the breeder's farm were then given to us for study.

During a 2-year period, sire K-1 was mated to these 4 females and to unrelated females from the college breeding flock. The results of these matings, given in Table 1, show that: (*a*) approximately one-half the daughters succumbed to the *xl* lethal, (*b*) no sons were affected, and (*c*) the frequency of the lethal condition in the progeny was independent of any relation of the dams to the sire, K-1.

TABLE 1

GENETIC TESTS WITH SIRE K-1 AND WITH 13 OF HIS SONS

Matings	Male progeny			Female progeny		
	No.	Died, other causes	Died, *xl* lethal	No.	Died, other causes	Died, *xl* lethal
K-1 × 4 related ♀♀	23	2	0	13	1	7
K-1 × 14 unrelated ♀♀	32	5	0	42	4	21*
Totals for Sire K-1	55	7	0	55	5	28
Three carrier sons of K-1†	89	4	0	77	5	36
Ten noncarrier sons of K-1†	244	8	0	287	5	0

* Includes 2 birds that showed symptoms but did not die.
† Mated with unrelated females.

These preliminary matings provided evidence that the lethal effect was caused by a single sex-linked recessive gene, and that sire K-1 was heterozygous. One-half the daughters received the lethal gene from sire K-1 and died, whereas the one-half receiving the normal allele survived. No affected sons could be produced in this type of mating, since each received the dominant normal allele from his dam.

By chance segregation, one-half the sons of sire K-1 should have received the recessive allele from him and should therefore have been heterozygous (*Xl xl*) for the character. When 13 of these were tested, 3 were found to be carriers, as was proved by the fact that approxi-

[1] Number 23 in the series by F.B.H. entitled "Genetics of the Fowl."