Protection of Embryonated Eggs Infected with Infectious Bronchitis or Newcastle Disease Virus by Polypeptides.* (20444)

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Studies on the antiviral properties of synthetic lysine polypeptides have revealed them to inhibit tobacco mosaic virus infectivity(1), to inhibit the growth of influenza virus in the chick embryo(2), and to possess antiviral activity against a bacteriophage(3). A report on the protective effect of lysine polypeptides on embryonated eggs infected with several strains of infectious bronchitis virus and also Newcastle disease virus is presented here.

Materials and methods. The L-lysine polypeptide hydrochloride of molecular weight 4900 was supplied by Dr. R. R. Becker(4). This was dissolved in physiological saline and adjusted to pH 6.3. Solutions of protamine sulfate, lysozyme and L-lysine were prepared in a similar manner. The glutamic acid polypeptides(5) were dissolved in an equivalent amount of alkali and adjusted to pH 7.3. All solutions were passed through a Seitz filter and tested for bacterial sterility. The viruses‡ employed in this study included 4 strains of infectious bronchitis virus of chickens (IBV) as follows: (a) a recently isolated Wisconsin strain, (b) the Jungherr strain, (c) the egg adapted strain of Beaudette and Hudson, and (d) the egg adapted Van Roekel strain; as well as the Newcastle disease virus, New Jersey K. D. strain. Inoculum pools of the various viruses were prepared by allantoic sac passage. Infectious bronchitis virus was stored in inactivated normal horse serum at −70°C to maintain its titer. The infectivity titers of the viral inocula were determined by inoculation of serial decimal dilutions of the virus pools into groups of 6 eggs each. One-tenth or 0.2 ml amounts of the materials to be tested for their protective effect were injected into the allantoic sac of eggs 1 hour before or after challenge with virus by the same route. Infectious bronchitis virus and Newcastle disease virus were inoculated into the allantoic sac of eggs in the 9th and 11th day of embryonic development respectively. All eggs were incubated at 37°C and candled

TABLE I. Effect of Polypeptides in Allantoic Sac of Embryonated Eggs Infected with Infectious Bronchitis Virus (IBV).

1st inj.	2nd inj. (1 hr later)	Dead/ total‡	Avg day of death
A. Wisconsin Strain—early egg passage§			
mg			
10 PGA*	10 LD_{50} IBV	9/9	2.7
0.2 PL†	" " "	4/9	5.3
Saline	" " "	8/8	2.7
10 PGA	Saline	0/5	—
0.2 PL	"	0/8	—
0.04 PL	10 LD_{50} IBV	4/10	3.5
10 LD_{50} IBV	0.2 mg PL	2/9	—
Saline	10 LD_{50} IBV	7/9	2.4
0.2 PL	100 LD_{50} IBV	2/10	—
Saline	" " "	9/9	2.4
0.2 PL	1000 LD_{50} IBV	2/9	—
Saline	" " "	10/10	2.4
B. Wisconsin Strain—15-17th egg passage			
0.2 PL	100 LD_{50} IBV	11/20	3.1
0.2 Protamine	" " "	16/19	4.6
Saline	" " "	16/18	2.8
0.2 L-Lysine	100 LD_{50} IBV	9/10	2.3
0.5 Lysozyme	" " "	6/8	2.7
Saline	" " "	8/8	2.6
C. Jungherr Strain—7th egg passage			
0.2 PL	100 LD_{50} IBV	7/11	4.1
Saline	" " "	11/11	3.4
0.2 PL	10 LD_{50} IBV	2/11	—
0.2 Protamine	" " "	6/11	4.1
Saline	" " "	9/12	3.6

* PGA—Polyglutamic acid of avg molecular wt 4800.

† PL—Polylysine of avg molecular wt 4900.

‡ Computed for deaths 6 days after inoculation.

§ The exact passage not known.

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TABLE II. Effect of Polypeptides in Allantoic Sac of Embryonated Eggs Infected with Egg-Adapted Infectious Bronchitis Virus (IBV).

1st inj.	2nd inj. (1 hr later)	Dead/ total	Avg day of death
	A. Beaudette and Hudson Strain		
mg			
0.4 PL*	10 LD_{50}	3/8	—
1.8 PGA†	" "	5/5	2.3
Saline	" "	7/9	1.5
0.4 PL	100 LD_{50}	9/9	1.5
5 Lysozyme	" "	8/8	1.5
Saline	" "	8/8	1.7
	B. Van Roekel Strain		
0.4 PL	10 LD_{50}	13/13	1.6
Saline	" "	13/14	1.5

* PL—Polylysine of avg molecular wt 4900.
† PGA—Polyglutamic acid of avg molecular wt 4800.

TABLE III. Activity of Polypeptides in Allantoic Sac of Embryonated Eggs Infected with Newcastle Disease Virus (NDV).

1st inj.	2nd inj. (1 hr later)	Dead/ total	Avg day of death
mg			
2 PGA*	100 LD_{50} NDV	8/8	2.3
0.2 PL†	" " "	7/7	2.4
0.2 Protamine	" " "	7/7	2.4
Saline	" " "	8/8	2.3
0.4 PL	6 LD_{50}	8/15	3.1
5 Lysozyme	" "	10/10	2.5
Saline	" "	13/14	2.7

* PGA—Polyglutamic acid of avg molecular wt 15600.
† PL—Polylysine of avg molecular wt 4900.

daily or twice daily for 6 days after infection (6). The criterion of antiviral activity was the survival of the embryo.

Experimental and results. The maximum non-lethal dose of the lysine polypeptide and protamine sulfate per embryo was 1 and 0.5 mg respectively. Lysozyme and glutamic acid polypeptides were not toxic at 5 mg and 15 mg per embryo respectively.

Some typical results of screening experiments carried out with several strains of infectious bronchitis virus are presented in Tables I and II. Table I-A summarizes the results obtained with an early egg passage of a strain of infectious bronchitis virus isolated in Wisconsin which was still pathogenic to chickens. As little as 0.2 mg of polylysine when injected 1 hour prior to inoculation with from 100 to 1000 LD_{50} of virus allowed 80% of the embryos to survive whereas all the control embryos were dead within 6 days. Injection of polylysine 1 hour after inoculation with 10 LD_{50} of virus also showed a marked protective effect. Four hundredths mg of polylysine was sufficient to protect against 10 LD_{50} of virus. Polyglutamic acid of the same molecular weight showed no protection. Table I-B reveals that inhibition by polylysine of infectious bronchitis virus is diminished with increased egg passage. Thus in several experiments only 30-50% of the embryos survived 100 LD_{50} of virus passed 15 to 17 times when treated with 0.2 mg of polylysine as compared with 80% survival with the earlier passage of the virus.

Three other strains of infectious bronchitis virus were screened for inhibition by the polypeptides to further test the suggestion that with increased egg passage the virus becomes more resistant to inhibition by polylysine. They were the Jungherr strain which had been through 7 egg passages, the Beaudette and Hudson strain which had been through several hundred egg passages and was non-pathogenic to birds, and the Van Roekel strain which had been through at least a hundred egg passages. Table I-C reveals that significant protection of embryos infected with 10 and 100 LD_{50} of the Jungherr strain was obtained. Table II-A shows that the Beaudette and Hudson strain was inhibited at the 10 LD_{50} level but not at 100 LD_{50}. No protection by polylysine was observed in eggs infected with 10 LD_{50} of the Van Roekel strain as shown in Table II-B.

Polylysine protected eggs against 6 LD_{50} of Newcastle disease virus as shown in Table III. No protection was observed against 100 LD_{50} of the virus. As much as 5 mg of lysozyme had no protective action.

Discussion. Polylysine was found to protect embryonated eggs infected with infectious bronchitis virus. Protamine also afforded a small amount of protection while lysozyme had little or no effect. The data on the 4 strains of infectious bronchitis investigated suggest that with increased egg passage the virus becomes more resistant to polylysine ininhibition. The virus also loses its antigenicity and becomes less pathogenic to birds with increased egg passage(7). Polylysine has

been shown to inhibit tobacco mosaic virus infectivity by direct combination with the virus(8). It is therefore suggested that polylysine inhibits infectious bronchitis virus by a similar combination of the basic polypeptide with acidic groups on the surface of the virus protein. With egg passage, the virus becomes less antigenic, the surface pattern of the virus protein is modified perhaps in such a manner as to render it less available for combination with polylysine. It is also possible that the resistance to polylysine inhibition with increased egg passage reflects only an increase in the pathogenicity of the virus.

Conclusion. Polylysine was found to exert a marked protective effect in embryonated eggs subsequently infected with recently isolated infectious bronchitis virus. This protective effect was significantly less after several egg passages and with strains of infectious bronchitis which were completely egg adapted. Embryonated eggs infected with Newcastle disease virus were also protected by polylysine.

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Application of Tissue Clearance Technics in Small Animals.* (20445)

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Careful work in many laboratories has established the fact that the disappearance of an isotope from the site of local injection is an exponential function. In his original analysis of the dynamics of tissue clearance, Kety(1) postulated that such an exponential relationship should obtain, if one could assume that the arterial concentration of the tracer were small enough to be neglected. That this assumption is substantially true in the case of clearance from the tissue of man has been amply confirmed in many laboratories(2-7). A most stringent test of the validity of this assumption is the linearity of the disappearance curve over long periods of time; thus, McGirr(3) and Rapaport *et al.*(4) find no significant deviation from linearity in disappearance curves followed for more than 60 minutes. However, data obtained in similar experiments with small animals frequently yield curves which are not strictly exponential. This is especially evident where the disappearance is followed for periods greater than 20 or 30 minutes. Thus, in short term investigations (8-10), *i.e.*, 5 to 10 minutes, the clearance of radioisotopes from various sites of injection in rats, rabbits and dogs was quite linear. When the period of observation was extended beyond 30 minutes deviations from the simple exponential relationship were always found(9, 10).

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