when the basic polypeptide collides with the surface of the red cell and forms many ionic bonds between basic butyl amino side chains of the peptide and acidic groups at the surface of the cell. Then as these polypeptide-cell complexes which now have a reduced electrical repulsion for other cells collide, more ionic bonds are formed between the polypeptide and the second cells. Thus, the polypeptide serves to link togther the red cells, and by repetition of this process the agglutinated cell masses are formed. Although the bond strength of any one individual ionic bond that is formed in such a process is relatively weak, the formation of many such bonds between the peptide molecule and the red cells stabilizes the complex. Disassociation is greatly reduced compared to that with the free amino acids because the peptide chain holds the positively charged groups of the peptide together and close to the red cell surface to form regions of very high ionic concentration which by mass action favors the combination or recombination with the negatively charged groups at the cell surface.

The 85 unit L-lysine polypeptide was shown to inhibit lysis of sensitized sheep cells upon the addition of complement. It appears likely that at high concentrations, the polypeptide molecules had sufficiently coated the cell surfaces so that complement was unable to cause lysis. Previously it had been shown(6) that the rate of formation of a precipitate of tobacco mosaic virus with its anti-serum was affected by the addition of small amounts of an L-lysine polypeptide. These two phenomena are both believed to result from the ability of the polypeptide molecules to coat acidic surfaces.

Abderhalden(7) had found that proteolytic enzymes were present in the chick embryo after the 7th day of incubation. Evidence was here presented that the synthetic lysine polypeptides were destroyed by enzymes in the allantoic fluid. The L-lysine polypeptides were hydrolyzed *in vitro* by the allantoic fluid at a rapid rate, whereas the DL-polypeptide showed only slight hydrolysis. The enzymes in the fluid could be inactivated by heating the fluids to 100°C.

Summary. Basic, high-molecular weight synthetic lysine polypeptides have been found to agglutinate chicken red cells at concentrations as low as 0.1 μg per ml. The hemagglutination titer of the polypeptides appears to be related to the number and type of amino acid residues per molecule. The polypeptides are enzymatically hydrolyzed by normal allantoic fluid of chicken embryos.

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Inhibitory Effect of Synthetic Lysine Polypeptides on Growth of Influenza Virus in Embryonated Eggs.* (18588)

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Studies on some of the biological properties of a group of basic, high molecular weight, lysine polypeptides recently synthesized in this laboratory(1) revealed them to be capable of combining through ionic linkages with acidic groups at the surface of tobacco mosaic

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TABLE I. Synthetic Lysine Polypeptides.

Polypeptide hydrochloride	Amino acid residues per molecule*	Molecular wt*	Max. tolerated dose in mg/0.1 ml
L-lysine polypeptide	6	980	2.0†
" "	18	3000	1.0
" "	85	14000	.1
" "	158	26000	.2
DL-lysine polypeptide	36	6000	.2
" "	240	40000	.1†
DL-lysine, DL-valine polypeptide (Copolymer)	25	4000	.5

* The number of amino acid residues per molecule and the molecular weights were determined by Van Slyke amino nitrogen analyses of the terminal amino end groups and represent an average figure(1).

† Highest levels tested; the maximum tolerated dose was not determined.

virus. The L-lysine polypeptides formed relatively insoluble complexes with the virus, whose infectivity was thereby markedly reduced(2). Many animal viruses have also been found to have acidic surfaces which allow their combination with basic materials(3). In the present study, a group of basic, synthetic lysine polypeptides were screened for their ability to inhibit the growth of influenza virus in the embryonated egg.

Materials and methods. Stock solutions of the various lysine polypeptides (Table I) were prepared by dissolving their hydrochloride salts in (physiological) saline to a concentration of 10 mg polypeptide per ml. The solutions were adjusted to pH 7.0 to 7.2 and stored at 4°C until needed, when portions were removed and diluted with saline to the desired concentrations.

The influenza virus used was from a stock of frozen allantoic fluids from eggs infected with influenza virus, type A, PR8 strain. Fertile eggs previously incubated for 11 or 12 days were employed throughout in the experiments. The method used for the virus hemagglutination titrations was that of Salk(4,5) employing plastic plates. Serial 2-fold dilutions in saline of virus infected allantoic fluids were made in 0.5 ml amounts using plastic serological plates. One-fourth ml of a 1% suspension of washed chicken red cells was then added to each serial dilution of the fluids. The plates were shaken to mix the fluids and after approximately 2 hours, the end points were read as the highest dilution showing complete or partial hemagglutination.

Experimental. The toxicities of the various polypeptides were determined by injecting graded doses in volumes of 0.1 ml into the allantoic cavities of groups of 10 eggs. After 24 hours incubation, the eggs were candled to determine the number of survivors. The maximum non-lethal doses (MNLD) of the polypeptides are shown in Table I.

Varying doses of the different polypeptides were injected into the allantoic cavities of small groups of eggs. One hundred thousand minimum infective doses (MID_{50}) of the virus were injected either with, before, or after the polypeptides in a volume of 0.1 ml. After incubation for 24 hours, portions of the fluids were removed and titrated individually. Averages of each group were calculated and compared to an equal or larger number of controls inoculated with the virus alone. It is generally recognized that the infectivity of allantoic fluid containing viable virus is roughly proportional to the hemagglutination titer of that fluid. It was believed that the hemagglutination titers measured the amounts of virus with sufficient accuracy for this preliminary work. It was shown(6) that under these experimental conditions the polypeptides are enzymatically destroyed *in vivo* within 24 hours so that they did not interfere with the virus hemagglutination titers. The polypeptides were also tested against smaller virus inocula for longer incubation periods. In some cases the polypeptides were injected initially and again 24 hours later.

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TABLE II. Allantoic Fluid Hemagglutination Titers 24 Hours After the Virus Inoculation.

Polypeptide	Dosage in mg./0.1 ml	Survival	Avg titer of	
		Total treated	Treated	Control
A. Polypeptides inj. 1 hr before 100000 MID_{50} of virus.				
6 unit L-lysine	2.0*	4/4	640	1280
6 " "	1.0*	4/4	640	1280
18 " "	1.0	2/4	160	1280
18 " "	.1	4/4	640	1280
85 " "	.1	2/4	160	1280
158 " "	.2	4/6	160	1280
158 " "	.1	8/10	80	1280
36 " DL-lysine	.2	7/16	160	1280
36 " "	.1	4/4	10	1280
240 " "	.1	8/9	640	2560§
240 " "	.05	8/9	640	2560§
25 " " DL-valine	.5	5/6	320	1280
25 " " "	.2	6/6	320	1028
B. Polypeptides inj. together with 100000 MID_{50} of virus.				
85 unit L-lysine	.1	5/6	640	5120†
36 " DL-lysine	.2	10/15	320	1280
240 " "	.1	9/9	640	2560§
240 " "	.05	9/9	80	2560§
25 " " DL-valine	.5	14/18	10	640
C. Polypeptides inj. 3.5 hr after 100000 MID_{50} virus.				
36 unit DL-lysine	.1	7/8	160	640
25 " " DL-valine	.5	6/8	160	640
25 " " "	.5‡	9/12	80	1280

* 15 min. before virus.
† Titer at 30 hr.
‡ 15 min. after virus.
§ The 240 DL-lysine unit titers are of pooled fluids.

Results. The results of representative experiments are shown in Table II. Generally, it was found that the degree of inhibition as measured 24 hours after inoculation increased with the dosage of polypeptide. There was a correlation between the degree of inhibition and the average number of amino acid residues per molecule of the L-lysine polypeptides. With one exception, the greatest inhibition was shown when the polypeptides preceded the virus. The one exception was the 25 unit DL-lysine-DL-valine polypeptide which, when injected with the virus, prevented the development of hemagglutinins within 24 hours in every case.

When the amounts of virus were measured 48 hours or longer after inoculation and treatment with the polypeptides no significant inhibition was obtained even against smaller virus inocula of 1,000 MID_{50}. Repeated doses of the polypeptides initially and 24 hours after the inoculation of virus also failed to prevent the appearance of hemagglutinins by 48 hours.

Discussion. The toxicities of the various lysine polypeptides are seen to be related to the average number and type of amino acid residues per molecule. However, the very long polypeptide chains, the L-158 unit and the DL-240 unit lysine polypeptide, appear to be somewhat less toxic than the L-85 unit lysine polypeptide. It appears likely that the toxic effect of the polypeptides is caused by a combination of the basic peptides with essential acidic groups.

The L-158 unit, DL-36 unit, DL-240 unit lysine polypeptides and the 25 unit DL-lysine-DL-valine polypeptides significantly delayed the production of hemagglutinins even when very large inocula containing 100,000 MID_{50} of virus were employed. The data obtained in these experiments suggest that the basic polypeptides combine directly with the virus particles to inhibit infectivity. Inhibition was evident when the polypeptides were injected before or with the virus. When the polypeptides were injected after the virus, less inhibition was obtained. The 25 unit lysine-valine polypeptide was most effective when it was injected with the virus;

when injected 15 minutes before or after the virus, less inhibition was observed. The fact that no inhibition of virus growth was demonstrable after 48 hours incubation further suggests that the polypeptides combine with the virus but active virus is subsequently released.

The inhibition of tobacco mosaic virus infectivity(2) and this inhibition of the production of influenza virus hemagglutinins by the lysine polypeptides suggest that these or similar synthetic polypeptides might be active against other viruses. The increased activities of the DL-lysine polypeptide and of the DL-lysine-DL-valine polypeptide suggest that the stereochemical structure as well as the amino acid composition of such synthetic polypeptides might be varied in order to produce greater antiviral activity.

Preliminary experiments failed to show a significant chemotherapeutic effect against influenza virus in mice when the polypeptides were administered as an aerosol.

Summary. Various synthetic high molecular weight lysine polypeptides were found to inhibit the growth of influenza virus in the chick embryo.

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Circulating Antibodies in Vitamin-Deficiency States. Pantothenic Acid and Pyridoxine Deficiencies.* (18589)

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(Introduced by Ralph R. Mellon)

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A severe impairment of antibody response has been observed in pyridoxine(1,2) and pantothenic acid-deficient rats(1). Riboflavin, biotin, and thiamine deficiencies had considerably less effect(1,3,4). In our later experiments(5), particular emphasis was placed upon the use of "paired-weighed" in lieu of "paired-fed" rats as controls to the pantothenic acid-deficient animals. The evidence obtained proved conclusively that the absence of pantothenic acid *per se* and not the concomitant inanition was responsible for the decreased serum antibody titers. In addition, the pronounced effects of a relatively mild pantothenic acid deficiency were also demonstrated. It is generally recognized that a decreased antibody titer in serum is not unequivocal proof for the actual impairment of antibody synthesis. Other factors such as the effects of the vitamin deficiency upon the processes of antibody destruction and distribution must be evaluated before a definite relationship between vitamin and antibody synthesis can be established. Thus, a dietary inadequacy of pantothenic acid or pyridoxine may result in: (1) an actual impairment of antibody synthesis; (2) an accelerated rate of antibody destruction; or (3) a failure to release and distribute antibodies from their site of formation into the blood stream. Any one of these three mechanisms could produce a lowered serum antibody concentration.

The present experiments were, therefore, undertaken in an effort to shed some light on

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