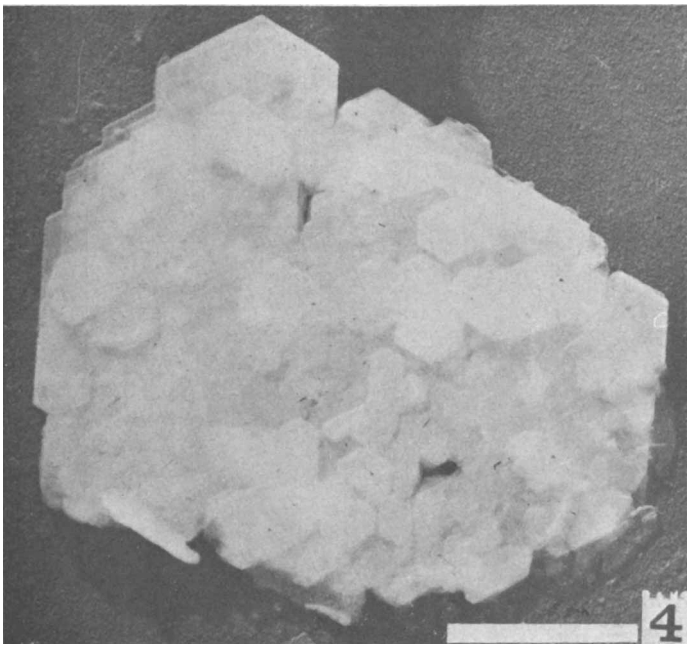

FIG. 4.
Large crystalline aggregate showing the laminated structure and many adherent smaller crystals. Note the tendency for the orientation of the crystals to be affected by their neighbors. Cr shadowed.

ference with the blood supply because of the lateral pressure with resultant drying of the tops of the papillae. Desquamation does not occur so that the growth becomes capped with a firm, dessicated cutaneous horn(3). If the conditions of drying and internal pressure are sufficient then crystallization may occur. The formation of such discrete crystals means that there must be available in a local region and in some concentration, a substance which can undergo crystallization. Whether these crystals are formed by a lytic action during degeneration or whether it is a normal substance produced during the growth process is not known.

Summary. The finding of crystalline platelets in the water extracts of papillomatous lesions of the wild and domestic rabbit is noted. A few of the elementary properties of the crystals are discussed.

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Agglutination of Red Cells by Synthetic Lysine Polypeptides.* (18587)

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It has been shown that basic high molecular weight lysine polypeptides(1), are capable of aggregating tobacco mosaic virus(2), and ag-

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1. Becker, R. R., and Stahmann, M. A., *J. Am. Chem. Soc.*, in press.

TABLE I. Hemagglutination by Synthetic Lysine Polypeptides.

Polypeptide	Amino acid residues per molecule*	Molecular wt*	Hemagglutination activity,† μg/ml
L-lysine polypeptide	6	980	7.8
,, ,,	18	3000	0.48
,, ,,	85	14000	0.24
,, ,,	158	26000	0.12
DL-lysine, DL-valine polypeptide	25	4000	3.9
DL-lysine polypeptide	36	6000	1.9

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

† Smallest amount causing hemagglutination.

glutinating certain bacteria(3). In this investigation, the ability of a number of the synthetic lysine polypeptides to agglutinate red blood cells was studied. The polypeptides were found to agglutinate erythrocytes at concentrations as low as 0.1 μg of polypeptide per ml. When the peptides were injected into embryonated eggs, it was found that the allantoic fluid contained enzymes capable of destroying the compounds. The agglutination of erythrocytes and this enzymatic destruction of the polypeptide by allantoic fluid are reported here.

Materials and methods. The various synthetic lysine polypeptide hydrochlorides (Table I) were dissolved in (physiological) saline to concentrations of 10 mg per ml; the solutions were adjusted to pH 7.0-7.2 and kept at 4°C. Aliquots were removed and diluted with saline to the desired concentrations as needed. The hemagglutination titrations of the polypeptides were carried out in plastic plates using the pattern end point(4,5). To 0.5 ml of serial 2-fold dilutions of the polypeptide solution was added 0.25 ml of a 1% suspension of washed chicken red cells. The plates were shaken to mix the contents; and after standing approximately 2 hours, the endpoints were read as the highest dilution showing partial or complete hemagglutination. The endpoints were found to be reproducible to within one dilution. The allantoic fluids were obtained from fertile eggs on the 12th day of incubation. The sterile fluids were pooled and cleared by centrifugation at low speeds before use.

Experimental. Various polypeptides were titrated for their hemagglutination endpoints. Table I shows the hemagglutination titers. It is seen that the titers of the L-lysine polypeptides are related to their chain lengths; the 36 unit DL-lysine and the 25 unit DL-lysine-DL-valine polypeptides were less active when compared to the L-lysine series.

Solutions of the amino acids L-lysine, DL-lysine, DL-valine, L-histidine, and L-glutamic acid were tested for hemagglutination. No agglutination was produced by any of the amino acids. The highest level contained 80,000 μg amino acid per ml. This was almost 1 million times the concentration of the 158 unit L-lysine polypeptide at its endpoint.

To test the possibility that the amino acids would inhibit polypeptide hemagglutination, solutions containing 1% red cells and various concentrations of the amino acids were used to titer the 85 unit L-lysine and 36 unit DL-lysine polypeptides. No hemagglutination inhibition was detected although amino acid concentrations as high as 40,000 μg per ml were tested.

It was of interest to determine whether red cells, agglutinated by the polypeptides, would lyse in the presence of complement. Experiments were carried out in which the 85 unit L-lysine polypeptide was tested for lytic properties. It was found to be unable to lyse red cells in the presence of complement. Hemolysin-sensitized sheep cells were agglutinated by concentrations of 100, 10, 1 and 0.1 μg per ml of the 85 unit polypeptide, and sufficient complement was added to lyse the controls. Within an hour, complete lysis occurred in mixtures containing no added polypeptide and 0.1, and 1.0 μg polypeptide per ml; after approximately 8 hours, the cells agglutinated by

2. Burger, W. C., Master of Science Thesis, Biochemistry Department, University of Wisconsin, 1950.

3. Stahmann, M. A., Burger, W. C., Sill, W., and Walker, J. C., unpublished results.

4. Hirst, G. K., *J. Exp. Med.*, 1948, v87, 301.

5. Hirst, S. A., *Science*, 1948, v108, 479.

TABLE II. Destruction of the 85 Unit L-Lysine and 36 Unit DL-Lysine Polypeptides by Normal Allantoic Fluid *in Vitro*.

85 unit L-lysine polypeptide			36 unit DL-lysine polypeptide	
	Hemag. activity			
Temp. of incubation, °C	Time, hr*	μg/ml	Temp. of incubation, °C	Hemagglutination activity after 22 hr,* μg/ml
4	30	0.12	4	1.6
25	30	1000†	25	1.6
37	6	1000	37	3.1
51	2	1000	51	12.5
65	4	1000	70	25
100	30	0.10		
4‡	30	0.12	4‡	1.6
65‡	30	0.12	70‡	1.6

* Portions of the solutions were removed and titrated after ½, 2, 4, 6, 8, 12, 14, 24, and 30 hours incubation.

† The undiluted test solutions, containing 1000 μg polypeptide per ml, did not agglutinate red cells.

‡ Control, in saline.

10 μg per ml of the polypeptide lysed; but 100 μg of polypeptide per ml prevented lysis and the cells remained strongly agglutinated.

Hirst(4) has shown that treating fowl red cells with periodate destroys their ability to adsorb influenza and other related viruses. Using Hirst's method, periodate treated cells were prepared. These cells which were unable to take up influenza virus, readily adsorbed the 85 unit L-lysine polypeptide in amounts equal to untreated red cell controls. This indicates that virus receptors are not necessary for agglutination by the polypeptide.

When allantoic fluid from eggs which had survived injection of large amounts of lysine polypeptides were titrated for residual polypeptide, no hemagglutination was observed. However, when the 85 unit L-lysine polypeptide was serially diluted in allantoic fluid and titrated immediately, the endpoint was the same as that obtained by serially diluting the polypeptide in saline. This showed that there was no polypeptide hemagglutination inhibitor in the allantoic fluid. Rather these observations suggested a destruction or removal of the polypeptide *in vivo* in the egg. *In vitro* experiments in which the polypeptide was incubated with pooled allantoic fluid at various temperatures indicated destruction of the L-polypeptide. That this destruction was an enzymatic destruction of the polypeptide is suggested by the data shown in Table II. These data show that at 4° there is no destruction of the L-85 unit polypeptide, but rapid destruction occurs above 25°. Similar experiments in which the 36 unit DL-lysine polypeptide replaced the L-isomer showed only very slight destruction. When the L-polypeptide was incubated for 24 hours with allantoic fluid which had previously been heated to 100° there was no loss of activity while fluids which had previously been heated to 65° retained the capacity to inactivate completely the hemagglutinating activity of the polypeptide.

Discussion. The hemagglutination titers of the various synthetic lysine polypeptides appear to be correlated with the average number and type of amino acid residues per molecule. It is seen here that the longer L-lysine polypeptides are more effective in hemagglutination in low concentration than the shorter L-lysine polypeptides. The reduced endpoint of the DL-polypeptide as compared to the L-polypeptides shows that the L-lysine configuration is most effective. The DL-valine-DL-lysine polypeptide also showed a reduced activity.

The complete absence of agglutination by high concentration of lysine and other amino acids and the increase in activity as the molecular size of the lysine polypeptides is increased indicate that the lysine residues must be joined together to cause hemagglutination. It is postulated that hemagglutination occurs

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.

6. Stahmann, M. A., and Mathews, R. E. F., unpublished results.

7. Abderhalden, E., and Steinbeck, E., *Z. Physiol. Chem.*, 1910, v68, 312.

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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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1. Becker, R. R., and Stahmann, M. A., *J. Am. Chem. Soc.*, in press.