glucose to glycogen(8). In any case, the observed changes are in harmony with the hypothesis that in mammalian as well as in avian malaria, disturbance in Co A metabolism is significant in the pathogenic effects of the infection.

Summary. Co A concentration and total content in liver, spleen, and kidney of normal rats and rats with infections of *Plasmodium berghei* have been described. During the course of infection the concentration of Co A in the liver dropped but the total amount present was unchanged. In the spleen the concentration remained static, whereas the total was proportional to the increased size of the organ. The kidney showed no appreciable changes in Co A, but a decreased concentration of free pantothenate was apparent in young infected animals.

1. Trager, W., *J. Exp. Med.*, 1952, v96, 465.
2. ———, *J. Protozool.*, 1954, v1, 231.
3. Singer, I., Hadfield, R., and Lakonen, M., *J. Infect. Dis.*, 1955, v97, 15.
4. Kaplan, N. O., and Lipmann, F., *J. Biol. Chem.*, 1948, v174, 37.
5. Klein, H. P., and Lipmann, F., *ibid.*, 1953, v203, 101.
6. Olson, R. E., and Kaplan, N. O., *ibid.*, 1948, v175, 515.
7. Moulder, J., and Taliaferro, W. H., *J. Infect. Dis.*, 1955, v97, 137.
8. Mercado, T. I., and von Brand, T., *Exp. Parasitol.*, 1954, v3, 259.

Received January 4, 1956. P.S.E.B.M., 1956, v91.

Inhibition of Mumps and Influenza B Virus Multiplication by Synthetic Poly-D-Lysine.* (22249)

EMEE TSUYUKI, H. TSUYUKI, AND MARK A. STAHMANN.
Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

It has been previously reported that the synthetic basic polypeptide, poly-L-lysine, protects the chick embryo for a period following inoculation with a number of animal viruses such as mumps virus(1), infectious bronchitis, Newcastle disease viruses(2), and influenza B virus(3). A comparative study using a synthetic basic polyelectrolyte, as polyvinylamine, showed that this polymer was not as effective a virus inhibitor as poly-L-lysine and was almost 5 times more toxic to the chick embryo(3). Proteolytic enzymes (4) in the chick embryo have been demonstrated to destroy poly-L-lysine injected into the allantoic fluid(5). Since this enzymatic destruction of the L-polypeptide might be a factor that limits its protective action against viruses and as D-polypeptides are usually less susceptible to enzymatic hydrolysis, we have studied the effectiveness of poly-D-lysine against mumps and influenza B viruses in the chick embryo.

Materials and methods. The mumps and influenza B Lee strain viruses† were cultivated in chick embryos according to a previously described procedure(1,3). The virus cultures and the procedures for inoculation, harvesting and estimation of the extent of virus multiplication by hemagglutination titrations, were similar to those used by Green and Stahmann(1,3). In all experiments fertile White Leghorn eggs were employed. Poly-D- and poly-L-lysine hydrochlorides of molecular weights of about 3,900 and 3,600 respectively (determined by end group analysis) were prepared by methods published elsewhere(6-8). The maximum non-lethal dose of poly-D-lysine was approximately 0.1 mg and that of poly-L-lysine was 1.0 mg per chick embryo.

* Published with approval of Director of Wisconsin Agri. Exp. Station. Supported in part by research grant (No. E-101) from National Microbiological Institute of National Institutes of Health, U. S. Public Health Service and from the Herman Frasch Foundation.

† The authors are indebted to Dr. N. S. Koobyarian for these virus cultures.

TABLE I. Reduction of Hemagglutination Titer of Mumps Virus in Allantoic Fluid of Chick Embryo Injected with Polylysine.

1st inj., 0.1 cc intra-allantoic	2nd inj., 0.1 cc intra-allantoic (1 hr later), E.I.D.†	Hemagglutination titer* — Individual allantoic fluids					Geom. mean	Log mean	Difference from control log
Saline	10	160	80	160	32	80	88	1.94	
.04 mg DPL‡	"	0	0	0	0	0	0		1.94
" LPL§	"	0	0	0	0	0	0		1.94
Saline	100	256	128	64	256	256	169	2.23	
.04 mg DPL	"	32	0	0	2	8	8	.83	1.40
" LPL	"	0	16	16	2	4	7	.90	1.33
Saline	1000	256	512	256	128	256	256	2.41	
.04 mg DPL	"	32	8	0	0	16	16	1.20	1.21
" LPL	"	64	0	4	8	0	13	1.40	1.31
Saline	10000	256	256	256	256	128	223	2.35	
.04 mg DPL	"	64	16	32	0	16	27	1.43	.92
" LPL	"	64	32	16	64	16	32	1.51	.84

* Expressed as the reciprocal. † E.I.D. = Embryo infectious dose. ‡ Poly-D-lysine of avg molecular wt 3900. § Poly-L-lysine of avg molecular wt 3600.

Results. (a) *Effect of poly-D- and poly-L-lysine on inhibition of mumps virus multiplication in chick embryo.* Data of Table I show comparative effectiveness of poly-D- and poly-L-lysine in their inhibition of mumps virus multiplication. Groups of 5 embryos were injected intraallantoically with 0.04 mg of each polypeptide followed one hour later with 10 to 10000 embryo infectious doses of mumps virus. Controls were injected with saline. After 6 days of incubation at 37°C the eggs were harvested and hemagglutination titers of the individual allantoic fluids determined. It has previously been shown that poly-L-lysine cannot be demonstrated in the allantoic fluid by hemagglutination technic longer than about 24 hours after injection into the allantoic sac of the chick embryo(5), however, poly-D-lysine was demonstrated by the same technic to persist in the embryo up to 36 hours after injection. The data of Table I show that poly-D-lysine is equally as effective in inhibiting mumps virus multiplication as poly-L-lysine. It was thought that a difference in the rate of destruction of poly-D-lysine and poly-L-lysine as it influences the antiviral activity might be shown at lower concentrations of the polypeptides. Table II summarizes the results obtained when 0.001 to 0.04 mg levels of poly-D- and poly-L-lysine were administered to embryos injected with 1000 embryo infectious doses of mumps virus. Although the effectiveness of polylysine had decreased considerably at the 0.001 mg level, the comparative effectiveness of both types of polypeptides remained essentially the same.

TABLE II. Effect of Different Levels of Polylysine on Hemagglutination Titer of Allantoic Fluids Infected with 1000 E.I.D. of Mumps Virus.

1st inj., 0.1 cc intra-allantoic	2nd inj., 0.1 cc intra-allantoic (1 hr later), E.I.D.	Hemagglutination titer of allantoic fluids*	Difference from control log
Saline	1000	256	
.04 mg DPL†	"	16	1.21
" LPL‡	"	13	1.31
Saline	"	256	
.08 mg DPL	"	0	2.41
" LPL	"	4	1.81
Saline	"	256	
.1 mg DPL§	"	0	2.41
" LPL	"	0	2.41
Saline	"	128	
.01 mg DPL	"	8	1.20
" LPL	"	11	1.05
Saline	"	256	
.001 mg DPL	"	38	.83
" LPL	"	32	.90

* Expressed as reciprocal of geometrical mean of hemagglutination titers of allantoic fluids from groups of 5 to 6 embryos obtained 6 days after inoculation.

† Poly-D-lysine of avg molecular wt 3900.

‡ Poly-L-lysine " " " " 3600.

§ Maximum of one survived of a number of experiments done when inj. with 0.1 mg of DPL plus various levels of virus, probably due to the toxicity level of DPL.

TABLE III. Reduction of Influenza B Multiplication in the Chick Embryo by Polylysine.

1st inj., 0.1 ml intra-allantoic	2nd inj., 0.1 ml intra-allantoic (1 hr later), $I.D._{50}$	Hemagglutination titer*	Diff. from control log
Saline	10	320	—
.04 mg DPL†	"	8	1.60
" LPL‡	"	5	1.81
Saline	100	640	—
.04 mg DPL	"	22	1.46
" LPL	"	19	1.53
Saline	10000	640	—
.04 mg DPL	"	78	.91
" LPL	"	80	.90

* Expressed as reciprocal of geometrical mean of hemagglutination titers of allantoic fluids from groups of embryos obtained 2 days after inoculation.

† Poly-D-lysine of avg molecular wt 3900.

‡ Poly-L-lysine " " " " 3600.

(b) *Comparative effect of poly-D- and poly-L-lysine on influenza B virus Lee strain.* The data of Table III show the comparison of poly-D- and poly-L-lysine on influenza B virus. The procedures followed were similar to those used with mumps virus with the exception that the incubation period was only 2 days. The results again show that the two polypeptides have approximately the same antiviral activity.

Discussion. These data demonstrate that poly-D-lysine inhibits mumps and influenza B virus multiplication equally as well as poly-L-lysine. The extent of the inhibition obtained by poly-L-lysine was in agreement with that reported by Green and Stahmann (1-3). Since data have been obtained indicating that poly-L-lysine is metabolized in the allantoic fluid(5), it was thought that the duration of the virus inhibitor activity may be limited by the rate of metabolism of the polypeptide. However, the parallel antiviral activity of both the L and D polypeptides would suggest that poly-D-lysine may also be metabolized or hydrolyzed by the enzymes of the allantoic fluid. This metabolism of the D-polypeptide was further indicated by experiments which demonstrated that poly-D-lysine could not be detected in allantoic fluid by its hemagglutination ability for periods greater than about 36 hours following injection of the D-polypeptide. These observations suggested that there was an enzyme whose specificity was such that it allowed hydrolysis of poly-D-lysine. Experiments were therefore undertaken to demonstrate more clearly the existence and action of this new poly-D-lysyl peptidase. In subsequent experiments of this laboratory, it was found that poly-D-lysine is in fact hydrolyzed by extracts of pancreas powder as rapidly as is poly-L-lysine(6).

Summary. Poly-D-lysine, though ten times more toxic, inhibited mumps and influenza B Lee strain viruses to approximately the same extent as poly-L-lysine in the allantoic sac of the chick embryo. As little as 0.001 mg per embryo of either lysine polypeptide was sufficient to inhibit high levels of viral infection. Evidence was obtained which suggested the existence in allantoic fluid of enzymes which hydrolyze both poly-D-lysine and poly-L-lysine.

1. Green, M., Stahmann, M. A., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 852.

2. Green, M., Stahmann, M. A., and Rasmussen, A. F., Jr., *ibid.*, 1953, v83, 641.

3. Green, M., and Stahmann, M. A., *ibid.*, 1954, v87, 507.

4. Moog, Florence, *Ann. N. Y. Acad. Sci.*, 1952, v55, 57.

5. Rubini, J. R., Stahmann, M. A., and Rasmussen, A. F., Jr., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 659.

6. Tsuyuki, Emee, Tsuyuki, H., and Stahmann, M. A., in press.

7. Becker, R. R., and Stahmann, M. A., *J. Am. Chem. Soc.*, 1952, v74, 38.

8. Ben-Ishai, D., and Berger, A., *J. Org. Chem.*, 1952, v17, 1564.

Received January 9, 1956. P.S.E.B.M., 1956, v91.