SFV did not multiply in cultures of normal adult rabbit tissue and caused no deaths or apparent illnesses in rabbits bearing fibromas.

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Factors Interfering with the Hemolytic Property of Mumps Virus.*† (18748)

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In earlier experiments(1), it was observed that amniotic fluids from embryonated eggs containing mumps virus exerted a more powerful and constant hemolytic activity than did virus-containing allantoic fluids from the same eggs(1-3). Allantoic fluids from uninfected eggs were shown to inhibit the hemolytic action of mumps virus and this inhibitory property was not destroyed by incubation with fresh mumps virus for 3 hours at 37°C(3). However, when infected allantoic fluids were dialyzed with phosphate-buffered saline in the cold, their hemolytic activity increased markedly to levels comparable to those obtained with amniotic fluids of similar virus content(3). Furthermore, in the course of earlier experiments(1), it had been observed that an isotonic sodium citrate buffer used as a diluent with infected allantoic fluids resulted in more active hemolysis than when an isotonic phosphate-buffered saline of similar pH was employed. These observations suggested that an inhibitory constituent for the mumps virus hemolysin was present in infected allantoic fluids and that this component was removed by dialysis or sodium citrate. Further studies on the properties and possible nature of this inhibitory factor are reported here.

Materials and methods. An egg-adapted strain of mumps virus was employed for the inoculation of 6- to 8-day-old embryonated eggs by the amniotic or allantoic routes(1). Following incubation at 35°C for 5 to 6 days, the extra-embryonic fluids were harvested separately, placed in glass ampoules, sealed and stored at —70°C. Where dialysis was used, these fluids were placed in cellophane tubing in a large volume of isotonic phosphate-buffered saline (pH 7.2) at 4°C for 24 hours. Hemagglutination and hemolysis tests with chicken erythrocytes were carried out as previously described(2).

Effect of dialysis and diluent. Dialysis of mumps virus-infected amniotic or allantoic fluids with phosphate-buffered saline at 4°C for 24 hours resulted in an increase in hemolytic activity, which was more marked with the allantoic fluids (Table I). Furthermore, the paradoxical increase in hemolysis observed in the first few serial dilutions of untreated allantoic fluids disappeared. An isotonic sodium citrate buffer used as diluent resulted in an increased hemolytic activity with undialyzed allantoic fluid, but had no effect on the dialyzed preparation (Table II). Neither of these procedures had any significant effect on the hemagglutinating activity of the virus.

Effect of an ion exchange resin. The effects observed following dialysis or use of the sodium citrate buffer suggested that some ionic constituent of the allantoic fluid might be re-

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1. Morgan, H. R., Enders, J. F., and Wagley, P. F., *J. Exp. Med.*, 1948, v88, 503.

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TABLE I. Effect of Dialysis on Hemagglutinative and Hemolytic Activity of Egg Fluids Infected with Mumps Virus.

Test fluid		Test	Dilution of fluid 2	4	8	16	32	64	128	256	512	1024	2048	Buffer control
Amniotic	Original	HA*	+	+	+	+	+	+	+	+	+	±	0	0
		HL†	30	27	26	22	10	4	3	0	0	0	0	0
	Dialyzed	HA	+	+	+	+	+	+	+	+	+	±	0	0
		HL	67	57	46	34	16	8	5	4	0	0	0	0
Allantoic	Original	HA	+	+	+	+	+	+	+	+	+	+	±	0
		HL	11	19	20	19	15	4	0	0	0	0	0	0
	Dialyzed	HA	+	+	+	+	+	+	+	+	+	+	±	0
		HL	56	42	30	23	15	8	0	0	0	0	0	0

* Hemagglutination. † Hemolysis expressed as %.

TABLE II. Effect of Phosphate and Citrate Buffers on Hemagglutinative and Hemolytic Activities of Allantoic Fluid Infected with Mumps Virus.

Test fluid	Test	Buffer	Dilution of fluid 4	8	16	32	64	128	256	512	1024	2048	4096	Buffer control
Allantoic fluid #1	HL*	Phosphate	7	8	10	1	0	0	0	0	0	0	0	0
		Citrate	37	29	24	13	9	8	4	0	0	0	0	0
	HA†	Phosphate	+	+	+	+	+	+	+	+	+	+	±	0
		Citrate	+	+	+	+	+	+	+	+	+	+	±	0
Allantoic fluid #1 dialyzed	HL	Phosphate	39	28	21	15	9	6	1	0	0	0	0	0
		Citrate	38	30	24	16	7	6	0	0	0	0	0	0
	HA	Phosphate	+	+	+	+	+	+	+	+	+	+	±	0
		Citrate	+	+	+	+	+	+	+	+	+	+	±	0

* Hemolysis expressed as %. † Hemagglutination.

TABLE III. Comparison of Effect of Dialysis and Treatment with Ion Exchange Resin on Hemagglutinative and Hemolytic Activity of Mumps Virus.

Test fluid	Treatment	Test	Dilution of fluid 2	4	8	16	32	64	128	256	512	1024	2048	Buffer control
Allantoic	Control	HA*	+	+	+	+	+	+	+	+	+	0	0	0
		HL†	6	5	9	7	4	3	0	0	0	0	0	0
	Dialysis	HA	+	+	+	+	+	+	+	+	+	+	0	0
		HL	46	35	24	18	13	8	6	0	0	0	0	0
	Ion exchange resin	HA	+	+	+	+	+	+	+	+	±	0	0	0
		HL	32	26	22	15	12	5	4	0	0	0	0	0

* Hemagglutination. † Hemolysis expressed as %.

sponsible for the inhibition of the mumps virus hemolysin. Infected allantoic fluid was allowed to flow slowly through a column of a phosphate-buffer washed ion exchange resin (Amberlite I R-100§), which was known to remove a number of cations. Comparative effects of dialysis and treatment with this ion exchange resin are presented in Table III. These data indicate that treatment with an ion exchange resin, known to remove such cations as calcium, magnesium and manganese, will eliminate an inhibitor for the mumps virus hemolysin from infected allantoic fluids. Treatment with the ion exchange resin may also result in the loss of small quantities of virus.

Effects of various metallic ions. Previous experiments with dialysis and sodium citrate buffers, as well as a report by Burnet(4) on hemolysis by Newcastle Disease virus, suggested that calcium ions might be responsible for the inhibitory action observed with allantoic fluid virus preparations. Results obtained with the ion exchange resin indicated that calcium, magnesium or manganese ions might

§ Obtained from Rohm and Haas Co., Philadelphia.

4. Burnet, F. M., and Lind, P. E., *Aust. J. Exp. Biol. and Med.*, 1950, v38, 129.

TABLE IV. Effect of Calcium and Magnesium and Manganese Ions on the Hemolytic and Hemagglutinative Activity of Mumps Virus

Test fluid	Ion added to PO_4 buffer, mg %	Test	Dilution of fluid 2	4	8	16	32	64	128	256	512	1024	2048	4096	Buffer control
Allantoic	0	HL*	23	26	26	24	17	8	5	0	0	0	0	0	0
	Ca-1	"	11	10	6	5	0	0	0	0	0	0	0	0	0
	Mg-1	"	23	32	29	24	17	12	7	0	0	0	0	0	0
	Mn-3	"	21	26	23	16	10	7	4	0	0	0	0	0	0
Allantoic	0	HA†	+	+	+	+	+	+	+	+	+	+	+	±	0
		HL	39	25	21	15	9	8	1	0	0	0	0	0	0
dialyzed	Ca-5	HA	+	+	+	+	+	+	+	+	+	+	+	±	0
		HL	6	0	0	0	0	0	0	0	0	0	0	0	0
	Mg-5	HA	+	+	+	+	+	+	+	+	+	+	+	±	0
		HL	25	20	12	10	6	3	0	0	0	0	0	0	0

* Hemolysis expressed as %. † Hemagglutination.

TABLE V. Effect on Hemolytic Activity of Mumps Virus of the Addition of Calcium to Virus-Infected Allantoic Fluids.

Test fluid	Ca conc added to fluid, mg %	Hemolysin test,* dilutions of fluids 2	4	8	16	32	64	128	256	512	Buffer control
Allantoic dialyzed	0	41	33	23	17	10	7	5	3	0	0
	.5	31	30	23	15	12	7	3	0	0	0
	1	24	20	17	17	11	6	3	0	0	0
	2.5	15	16	13	13	9	7	3	0	0	0
	5	14	14	20	18	13	11	4	0	0	0
	10	10	9	10	7	6	0	0	0	0	0

* Hemolysis expressed as %.

be responsible. To determine the relative effects of these various ions, salts of each metal were added to the phosphate-buffer diluent in a series of tests in the concentrations indicated in Tables IV and V.

Calcium in concentrations of 1 to 10 mg % exerted an increasing inhibitory action on mumps virus hemolysis, while manganese at a level of 3 mg % appeared to exert no inhibition and 5 mg % of magnesium had some inhibitory effect. Concentrations of 5 mg % of calcium and magnesium had no influence on the hemagglutinating capacity of mumps virus.

Discussion. Removal of the inhibitory factor for the mumps virus hemolysin from allantoic fluid by dialysis suggests a small particle size, and neutralization or removal by use of sodium citrate buffer suggests calcium as the active agent. The action of the ion exchange resin gives further evidence for the possible role of calcium or other cations. The experiments on the inhibitory effect of various cations indicates that calcium has a potent inhibitory effect on the mumps virus hemolysin. It may be that calcium is the inhibitory substance present naturally in allantoic fluids, but its specific identity with this substance must be further established.

Conclusions. An inhibitor for the mumps virus hemolysin occurring in allantoic fluids of embryonated eggs infected with mumps virus can be removed by dialysis, treatment with a cation exchange resin or use of a sodium citrate buffer as diluent. These results suggest that it is a cation, probably calcium, magnesium or manganese. Of these 3, only calcium has demonstrated any significant inhibitory action on the mumps virus hemolysin.

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