throughout the cytoplasm of cells in certain areas, and in other areas extra-cellular virus was found in large numbers. 2. Small or medium sized inclusions were always located at the nuclear membrane and in contact with it. The inclusions consisted of masses of virus particles imbedded in a matrix which was dense in small inclusions and less dense in larger inclusions. The virus particles were ovoid and varied in length from 0.23 μ to 0.28 μ. Sectioned virus particles indicate that all have a dense outer wall, and some could be observed to have an internal structure. 3. The possible relationship of these observations to the growth cycle of vaccinia virus has been considered.

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Concentration of Influenza Virus (PR8 Strain) by a Cation Exchange Resin.* (19510)

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The application of ion exchange resins to the separation of closely related chemical substances by adsorption or exchange of ions has proven in the past few years to be a valuable adjunct in the purification and concentration of biological products. Ion exchange resins have been used previously to purify partially a number of neurotropic viruses(1). The resins in this case were so adjusted that when suspensions of infected chick embryos and mouse brains were passed through columns of resin, inert nitrogenous material was removed and the virus appeared in the percolate fluid. Poliomyelitis and Theiler viruses have also been partially purified by adsorption of the viruses and inert nitrogenous material on anion exchange resins followed by selective elution of the viruses(2).

In the present experiments a method has been developed to concentrate rapidly and to purify partially the PR8 strain of influenza virus. It was observed that the virus was selectively adsorbed on columns of a specially prepared cation exchange resin. At the same time a large proportion of nitrogenous impurities in the starting material passed through the resin columns into the percolates. The virus could be subsequently eluted from the resin in a fraction of the original volume of

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starting material.

Methods. An Amberlite cation exchange resin of the carboxylic acid type, XE-64,† was used. The resin was passed through graded screens and particles between 60 and 100 mesh were placed in a beaker fitted with a mechanical stirrer and converted from the acid to the sodium form with 1-normal sodium hydroxide. The resin was then thoroughly washed with several changes of distilled water. Pyrex glass columns (19 mm I.D.) fitted at the lower end with 1-hole rubber stoppers were charged with 20 ml of the wet resin which was supported on layers of coarse sand and glass wool. The columns were connected to vacuum flasks with rubber tubing. The charged columns were rinsed with distilled water and the resin was then converted to a mixed salt-acid form by treatment with 3 volumes of 1-normal acetate buffer at pH 5.6, the last volume of buffer remaining in contact with the resin for 15 minutes. The columns were again rinsed with 3 volumes of distilled water and drained. Chorioallantoic fluid was collected from 12-day-old chick embryos infected with the PR8 strain of influenza virus, pooled, centrifuged 5 min. at 2000 rpm and stored at —70°C until used. Normal chorioallantoic fluid from chick embryos of the same age was similarly collected and stored. Fifty to 100 ml of fluid was passed through single columns of resin with the aid of negative pressure in 20 to 40 minutes at room temperature and the percolates were collected. The columns were then eluted with four 5 ml portions of 10% sodium chloride and the eluates were collected separately. The percolates and eluates together with the original infected fluids, referred to as control fluids, were saved for various tests. Hemagglutinin titers were determined by a modification of the Salk technic. One-half ml of infected chorioallantoic fluid was serially diluted with saline in 2-fold steps and 0.5 ml of a 1% saline suspension of washed chicken erythrocytes was added to each tube. The endpoint was taken as the last tube in which a pattern was observed after standing at room temperature for 30 minutes. The infectivity of preparations was determined by the chorioallantoic inoculation of 0.2 ml of decimal dilutions of virus into 11- or 12-day-old chick embryos, using groups of 5 embryos for each dilution. Samples of chorioallantoic fluid collected 48 hours later from each chick embryo were tested for the presence of viral hemagglutinin and the results were expressed as the log ID_{50} of the inoculum. Analyses for total nitrogen were performed by the micro-Kjeldahl method. Values for non-protein

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TABLE I. pH, Total Nitrogen Content and Virus Content of Uninfected and Infected Chorioallantoic Fluids and Resin Percolates and Eluates.

	Vol., ml	pH	Total N, mg/ml	Hemagglutinin titer (recip. dilution)	Infective titer (log dilution)
A.	Uninfected chorioallantoic fluid				
C*	70	8	.55	0	
P	70	9.6	.38	0	
E 1	5	9.5	.43	0	
2	5	9.5	.40	0	
3	5	8.9	.20	0	
4	5	8.4	.13	0	
C	80	8	.63	0	
P	80	10	.39	0	
E 1	5	9.8	.55	0	
2	5	9.6	.45	0	
3	5	8.9	.21	0	
4	5	8.6	.12	0	
B.	Infected chorioallantoic fluid				
C	65	7.6	.54	1024	
P	65	9.5	.40	128	
E 1	5	9.2	.47	1024	
2	5	9.1	.35	8192	
3	5	8.4	.17	1024	
4	5	8	.11	512	
C	60	8.2	.68	2048	
P	60	9.2	.56	256	
E 1	5	9.1	.68	8192	
2	5	8.7	.34	16384	
3	5	8.2	.20	2048	
4	5	8.1	.12	512	
C	100	7.4	.94	2048	6.7
P	100	10	.73	256	4.7
E 1	5	9.5	.92	4096	7.3
2	5	9.5	.86	32768	8.7
3	5	8.9	.49	8192	8
4	5	8.4	.30	512	6.8
C	100	6.6	.52	2048	8.1
P	100	9.1	.39	64	6.8
E 1	5	9	.46	1024	7.7
2	5	8.8	.35	32768	9.2
3	5	8	.21	2048	8.4
4	5	7.7	.19	512	7

* C = control; P = percolate; E = eluate.

TABLE II. Hemagglutinin Titer and Total, Non-Protein and Protein Nitrogen Content of Infected Chorioallantoic Fluids and Resin Percolates and Eluates.

	Vol., ml	Hemagglutinin titer (recip. dilution)	N, mg/ml		
			Total	Non-protein	Protein
C*	50	2048	.74	.38	.36
P	50	64	.47	.25	.22
E 1	5	4096	.56	.27	.29
2	5	16384	.48	.22	.26
3	5	512	.34	.11	.23
4	5	128	.08	.05	.03
C	90	2048	.81	.50	.31
P	90	512	.64	.40	.24
E 1	5	8192	.78	.43	.35
2	5	16384	.54	.25	.29
3	5	512	.31	.14	.17
4	5	128	.22	.12	.10

* C = control; P = percolate; E = eluate.

nitrogen were similarly obtained after sodium tungstate precipitation of proteins. Determinations of pH were made by means of the standard glass electrode method.

Results. Table I(A) shows the results of control tests with normal chorioallantoic fluids, indicating that approximately 60 to 70% of the total nitrogen of these fluids appeared in the percolates and that false positive hemagglutinin reactions were not obtained with the eluate fractions. Table I(B) shows that virus could be concentrated at least 8- to 16-fold in the second eluate fractions, as judged by both hemagglutinin and infectivity tests, even though the total nitrogen content was considerably reduced. Table II indicates that the concentration of virus was accompanied by a relative increase of protein over non-protein nitrogen, although the absolute values were less than in the original fluids.

Discussion. In preliminary tests it was found that concentrated solutions of sodium chloride were necessary to elute the virus from the resin. Sodium and chloride determinations, performed in an attempt to ascertain the possible mechanism of virus elution, showed that very little sodium or chloride appeared in the eluates until nearly all of the virus had been recovered. In the fourth eluates they were present in approximately their initial concentration in the eluting solution. In all of the eluates sodium and chloride were present in approximately equivalent amounts. This would appear to indicate that simple ion exchange was not the sole factor involved in the displacement of the virus from the resin.

From the practical standpoint ion exchange resins may prove helpful in the isolation or concentration of viruses. For example, during the outbreak of influenza in New York City in February-March of 1951, the method was successfully employed to concentrate freshly isolated strains of influenza virus from low-titered chorioallantoic fluids in order to permit their use in hemagglutinin-inhibition tests(3).

Summary. A simple method has been described for the rapid concentration and partial purification of the PR8 strain of influenza virus by means of an ion exchange resin.

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