TABLE VI. Effect of CO_2 Gas and Type of Diluent upon EEE Virus in 10% Mouse Brain Suspensions Stored 4 Months on Dry Ice.

Type of seal	Titers after storage in various diluents			
	Distilled water	Saline	0.1% bovine albumin	30% horse serum
Unstored control	7.8, 8.2	7.8, 8.8	8.7, 8.6	8.2, 8.7
Flame-sealed ampules	8.5, 8.2, 8.3	9.0, 8.7, 8.0	9.0, 8.5, 9.3	7.8, 9.3, 8.2
Open ampules	8.2, 7.7, 8.0	7.0, 8.0, 8.5	8.0, 8.5, 8.0	8.3, 8.5, 8.2

Summary. A 4-month study on the influence of certain factors upon eastern equine encephalitis virus in stored mosquitoes and brain tissue revealed the following: (1) Killing of EEE-infected mosquitoes by brief exposure to ether or chloroform fumes had no ill effects upon the virus they contained. (2) A constant temperature of −70°C or −20°C was found best for preserving EEE virus in intact, infected mosquitoes. Several hours at room temperature caused loss of viability of most of the virus present, as did fluctuations 2 to 5 times weekly from 0°C to −20°C. Fluctuations in temperature below the freezing point also caused some drop in virus titer. (3) Carbon dioxide gas in an open type dry ice chest had no apparent deleterious effect upon EEE virus in infected, intact mosquitoes, intact mouse brain tissue, or mosquito and mouse brain suspensions in distilled water, buffered saline, 0.1% bovine albumin, or 30% normal horse serum. (4) Distilled water was an unsuitable diluent for suspensions of EEE-infected mosquitoes. Buffered saline and 0.1% bovine albumin both gave better, but still rather poor results. Thirty per cent normal horse serum proved superior, since with it no drop in virus titer was observed. (5) All the above diluents gave satisfactory results in 10% EEE-infected mouse brain suspensions during the 4-month test period.

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Use of a Cation Exchange Resin for Isolation of Influenza Virus. (20988)

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The adsorption on and elution from ion exchange resins of a number of different viruses has been demonstrated by several workers. The partial purification of several neurotropic viruses was achieved by Muller (1) by the use of a cationic resin. An anionic resin was used by Lo Grippo(2) for the purification of the Lansing strain of poliomyelitis, and the extraction of poliomyelitis virus from human fecal material. That influenza virus can be adsorbed and eluted from cation resins has been shown by Muller and Rose(3), and Puck and Sagik(4). The studies presented here were undertaken to determine whether unadapted influenza virus from human sources behaves in a similar manner, and whether ion exchange resins can be used for the isolation of influenza virus.

Materials and methods. A cation exchange resin, Nalcite HCR-X12, 50-100 mesh,† was converted from the acid to the sodium form by several washings of 10% NaCl until the pH of the supernate was the same as that of the salt solution before addition to the resin. It was then washed with distilled water until washings no longer produced a precipitate when $AgNO_3$ was added. Resin which had been used was washed 3 to 5 times with distilled water then kept in a 56°C water bath for several days in 10% NaCl. This served to elute and inactivate any virus adsorbed

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to the resin.

A preliminary study was made to determine whether an early passage, egg-adapted strain of influenza A virus (A/Va./11/53) isolated during the winter epidemic of 1953 could be adsorbed on and eluted from the cation resin, and whether the recovered virus remained in an active form.

Methods. Five ml of a 1/5 dilution of this egg-passage virus were added to 80 g wet resin in 250-ml flasks, shaken and kept at 4°C for 2 hours with gentle shaking every 20 minutes. The supernates were then withdrawn and tested for infectivity by intra-allantoic inoculation of 11-day-old embryos and the allantoic fluids from the embryos tested for hemagglutinins after 48 hour incubation. Elution of the virus was carried out by adding 5 ml of 10% NaCl to the resin, and keeping the flasks at 4°C for 2 hours with shaking every 20 minutes. The eluates were removed at the end of this period and infectivity titers determined by inoculating 11-day-old chick embryos intra-allantoically with serial 10-fold dilutions of the eluates, 6 embryos per dilution. Fifty per cent end points were calculated by the Reed and Muench method(5). Infectivity titers were also determined on the original 1/5 suspension which was further diluted to compensate for water contained within the wet resin. The water content of wet resin was found to be approximately 0.5 ml/g. Throat washings and swabs were obtained in sterile skim milk or beef heart infusion broth during the epidemic period in January and February 1953, in connection with epidemiological studies(6). They were preserved at −50°C to −70°C for 8-10 months before testing with resin. The routine procedure for influenza virus isolation as carried on in this laboratory consisted of intra-amniotic inoculation of 0.2 ml of the sample in 11-day-old chick embryos, incubation for 5 days at 37°C, after which the lungs, allantoic and amniotic fluids were harvested. The lungs were ground in alundum and suspended in the allantoic and amniotic fluids, and a second passage carried out by inoculating 0.2 ml of the suspension intra-amniotically into 11-day-old chick embryos. After 48 hours incubation at 37°C, living eggs were chilled overnight at 4°C. Allantoic and amniotic fluids were then collected and tested for hemagglutination with 0.4% chicken and guinea pig cells. Virus isolations involving the use of resin were conducted by adding 5 ml of throat sample to 30-40 g of wet resin in flasks. After gentle shaking, the flasks were kept at 4°C for 2 hours and shaken every 15-20 minutes. The supernates were then withdrawn and 2.5 ml of 10% NaCl added to the flasks. After 2 hours elution at 4°C the eluates were removed and 2.5 mg of streptomycin and 500 units of penicillin were added per ml of eluate. Eleven-day-old embryos were inoculated intra-amniotically with the eluates, each embryo receiving 0.2 ml. The procedure thereafter was the same as that described above.

Results. Table I presents the data of an experiment on the determination of infective virus recovered after adsorption and elution from the cation resin of early passage, egg-adapted influenza virus. Five aliquots of the same diluted allantoic fluid were treated separately and titers determined simultaneously. Virus adsorption to the cation resin is usually complete and cannot be detected in the supernate either by hemagglutination or infectivity tests. The ID_{50} of the treated aliquots varied from $10^{-2.60}$ to $10^{-3.50}$ compared to $10^{-3.65}$ for the untreated, indicating a loss not exceeding 10-fold. Puck and Sagik(4) had previously shown that they could recover virus from cation resins with hemagglutination titers the same as that of the original suspension.

Table II shows the results of virus isolations attempted on 106 throat samples selected by 2 separate categories. The 33 samples in Group A were untested samples taken

TABLE I. Hemagglutination and Infectivity Titers of Influenza Virus Treated with Cationic Resin. 5 treated aliquots.

	Hemagglutination titers		50% egg infectivity dose	
	Before adsorption	After adsorption	Supernate	Eluate
	1/16	0	0	$10^{-2.74}$
	"	0	0	$10^{-3.48}$
	"	0	0	$10^{-2.60}$
	"	0	0	$10^{-3.50}$
	"	0	0	$10^{-3.25}$
Control	"			$10^{-3.65}$

TABLE II. Comparison of Influenza Virus Isolations by Routine and Cation Resin Methods.

Group	No. samples	No. isolations: Routine	Resin	Both methods	Routine + Resin −	Routine − Resin +
A	33	13	17	19	2	6
B	73	4	10	10	0	6

from individuals from whom virus had been isolated in another sample by the routine method. Thirteen of these proved to be positive by the routine method as compared to 17 by treatment with resin, with 19 different isolations by both methods. There were 2 samples from which virus could not be recovered by the resin, as compared to 6 failures by the routine procedures. Virus could not be recovered on a second attempt on these 6 samples by the routine method. In a second group, 73 samples which had previously been found negative by the routine method were retested. Of these, there were 4 virus isolations by the routine method and 10 by the resin method. In both experiments, there were a total of 12 samples from which virus was isolated by resin treatment but not by the routine method on 2 different tests.

Recently Kelly(7) has reported that resin treatment improved detection of Coxsackie virus in sewage. The evidence presented in Table II shows that the number of virus isolations was increased by the use of a cation resin. Although the volume of eluate used was one-half the original amount of sample added to the resin, the degree of virus concentration is slight, if any. A possible explanation for the increased number of virus recoveries by resin treatment may be that by such a procedure, the throat sample is cleared of virus inhibitors, either specific antibodies or non-specific substances present in the respiratory tract. The technics involved in the use of ion exchange resins are simple and may be useful in improving the efficiency of virus isolation.

Summary. Early passage, egg-adapted Type A influenza virus is completely adsorbed on a cation exchange resin, and nearly all of the adsorbed virus can be eluted as infective particles. Influenza A virus was recovered from human throat washings and swabs more frequently with the use of resin than without it.

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