Mammalian Viruses and Rickettsiae

Their purification and recovery by cellulose anion exchange columns has significant implications.

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Peterson and Sober (*1*) and Sober *et al.* (*2*) described the preparation of the cellulose ion exchangers, DEAE-SF and ECTEOLA-SF, and their use in column chromatography of proteins. The observations of Commoner *et al.* (*3*) on the chromatography with ECTEOLA-SF of natural tobacco mosaic virus and reconstituted virus nucleoprotein, as well as those of Taussig and Creaser (*4*) and Creaser and Taussig (*5*) on chromatography and purification of T1, T2r, and T2r+ bacteriophages with this cellulose ion exchanger, suggested its use in chromatography of animal viruses. This article reports the results of exploratory chromatographic studies on the purification of several representative animal viruses and rickettsiae. It demonstrates that the chromatographic procedure provides high yields of purified infectious agents and discusses some of the implications of the findings for further fundamental and applied research.

Methods

ECTEOLA-SF and DEAE-SF cellulose anion exchangers were used for column chromatography and purification of poliomyelitis (Mahoney, type 1, Lansing, type 2, and Saukett, type 3), ECHO (type 13), and Coxsackie (type A9) viruses from ultracentrifuged concentrates of tissue-culture fluids from infected monkey kidney or Detroit-98 (D-98) cells (*6*). The behavior of concentrates of Q fever and of epidemic typhus fever rickettsiae from chick-embryo yolk sacs and of Colorado tick fever virus in homogenates of brains from infected suckling mice was also examined.

Preparations of ECHO, Coxsackie, and polio, types 1 and 3, were made from infected monkey kidney cell monolayers in MS solution (*7*) containing 5 percent calf serum. Polio, type 2, was prepared from D-98 cell monolayers in Eagle's HeLa cell medium (*8*) with 5 percent horse serum added. Virus concentrates were prepared from tissue-culture fluids clarified by centrifugation at 3500 *g* for 30 to 60 minutes, followed by centrifugation at 100,000 *g* for 1 to 2 hours. The sediments were suspended in 0.01 or 0.02*M* phosphate buffer, *p*H 7.1, then recentrifuged at 100,000 *g* for 1 to 2 hours, and finally resuspended in phosphate buffer. Partially purified concentrates of epidemic typhus and Q fever rickettsiae were made from infected chick-embryo yolk sacs by a modification of Bovarnick and Snyder's method (*9*). The preparations of Colorado tick fever virus were homogenates of 20 percent (wt./vol.) suspensions, in 0.02*M* phosphate buffer, of brains from infected, moribund, suckling mice.

Several virus preparations were made from appropriately infected tissue cultures grown in the presence of phosphorus-32 in order to label the virus particles with a radioactive tracer. In such cases, MK or D-98 cells were overlaid with Eagle's medium modified to contain 0.001*M* citrate buffer instead of phosphate buffer. To this medium was added 2.5 μg of KH_2PO_4 per milliliter, 0.01 to 0.07 mc/ml of carrier-free P^{32} as orthophosphate, and dialyzed calf or horse serum (2 or 5 percent).

Columns of cellulose ion exchangers 1 cm in diameter and 7 to 13 cm in length were employed. Such columns contained 1.3 to 2.4 g of ECTEOLA-SF (0.2 milliequivalent/g) or 0.7 to 1.4 g of DEAE-SF (1.0 milliequivalent/g). Preparations of viruses and rickettsiae were loaded onto the columns in 1- to 5-ml quantities and eluted with successive 1-ml quantities of phosphate buffer, or phosphate buffer containing increasing concentrations of NaCl; volumes of 10 to 20 ml of each eluent were used. The usual elution schedule was as follows: 0.01*M* phosphate buffer, 0.02*M* phosphate buffer, 0.1*M* NaCl in 0.02*M* phosphate buffer, 0.25*M* NaCl in 0.02*M* phosphate buffer, and 0.5*M* NaCl in 0.02*M* phosphate buffer. Columns were operated under air pressure of about 6 lb/in.^{2} with flow rates of approximately 0.5 ml per minute.

Titrations of eluates were made with tenfold serial dilutions placed in tubes containing monkey kidney tissue cultures, and the endpoints, in terms of tissue-culture doses (TCD_{50}), were calculated by the method of Reed and Muench (*10*). Colorado tick fever virus was similarly diluted, although it was necessarily titrated in suckling mice. The radioactivity of dried samples in planchets was assayed with a mica, end-window Geiger-Mueller tube (1.8 mg/cm^2) and a decimal scaler.

Rickettsiae

Figure 1 strikingly illustrates that adsorption from 0.02*M* phosphate onto ECTEOLA-SF and elution with 0.1*M* NaCl in 0.02*M* phosphate buffer and 0.2*M* sucrose removed nearly all contaminating material from partially purified suspensions of Q fever rickettsiae. Preparations suspended in 0.02*M* phosphate buffer containing 0.1*M* NaCl and 0.2*M* sucrose were air-dried on Formvar membranes, fixed with osmic acid vapor, washed with distilled water, and shadowed with a platinum-palladium alloy. Yolk-sac material, still present despite differential centrifugation and celite treatment (Fig. 1, top), was removed by one passage through an ECTEOLA-SF column (Fig. 1, middle and bottom). Similar results were obtained with epidemic typhus rickettsiae.

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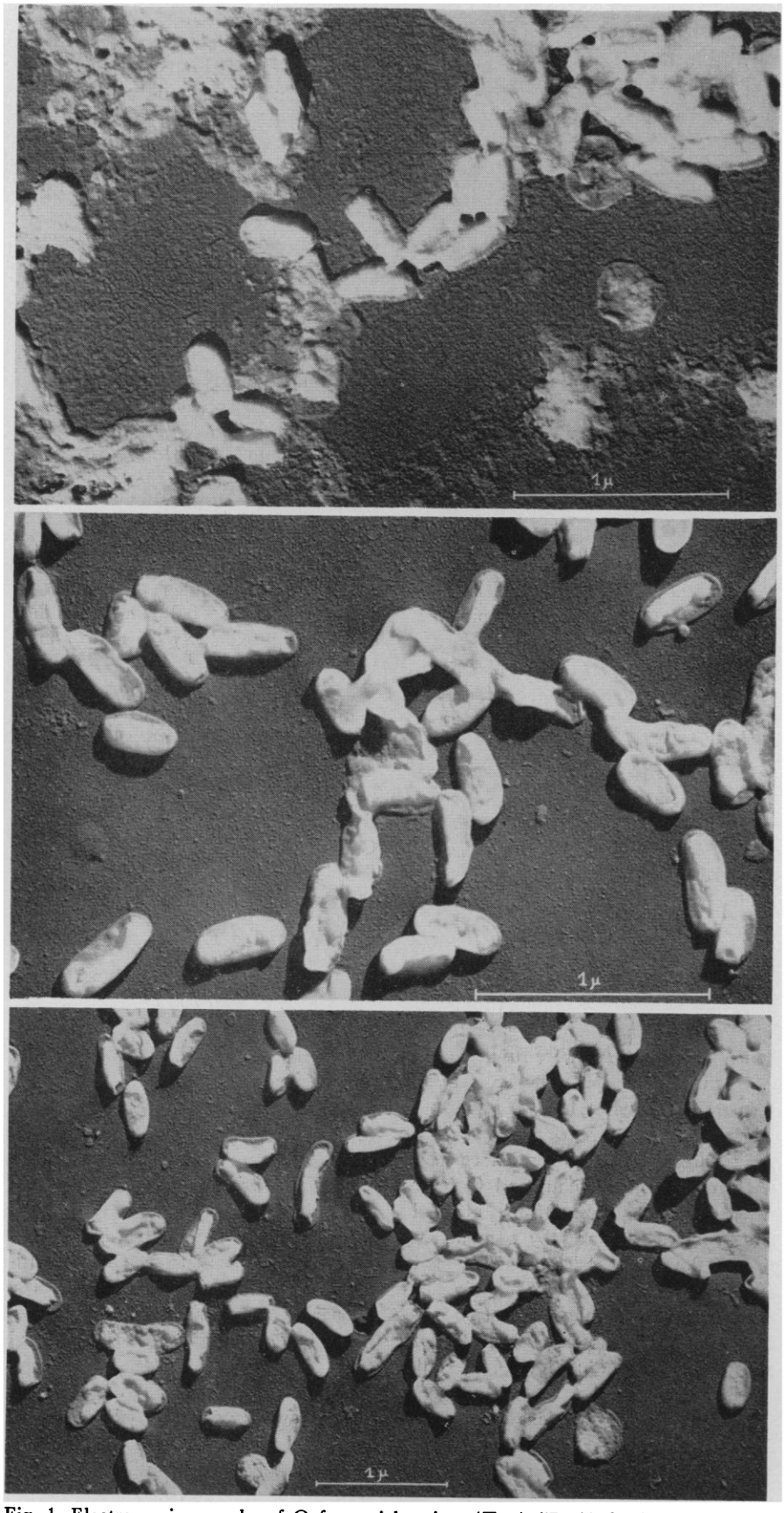

Fig. 1. Electron micrographs of Q fever rickettsiae. (Top) "Purified" (*9*) rickettsiae. (Middle) Rickettsiae from the same preparation after adsorption onto an ECTEOLA-SF column and elution by 0.1*M* NaCl at comparable magnification. (Bottom) Typical low-magnification field of a purified preparation. Rickettsiae were prepared for observation and electron micrographs were made by Edgar Ribi and William R. Brown of the Rocky Mountain Laboratory.

Viruses

Table 1 summarizes the chromatographic behavior of the viruses studied. The viruses, as determined by titration, emerged from the columns in well-defined regions of the eluate and invariably in excellent yield.

ECHO. Preparations of ECHO-13 virus, concentrated from tissue-culture fluids from monkey kidney cells infected in the presence of P^{32}, were adsorbed onto ECTEOLA-SF and eluted. Eluates containing viable virus were pooled, concentrated by centrifugation, and readsorbed onto an ECTEOLA-SF column from 0.01*M* phosphate buffer at *p*H 7.1. The virus could again be eluted with 0.1*M* NaCl (Fig. 2). Results which relate the amount of virus recovered and the radioactivity eluted are illustrated in Fig. 2. This figure shows that 88 percent of the virus recovered was in 1 ml of eluate. This fraction contained most of the radioactivity (0.3 count/min per 2000 tissue-culture doses). ECHO-13 could also be eluted from DEAE-SF by 0.1*M* NaCl, as determined by titration, although the elution diagram showed appreciable tailing.

Coxsackie. Coxsackie A9 virus, from a P^{32}-labeled tissue-culture fluid concentrate, suspended in 0.02*M* phosphate buffer, readily passed through an ECTEOLA-SF column, and 98 percent of the added virus appeared in the first few milliliters of effluent (Fig. 3). This effluent contained only 5 percent of the radioactivity initially present in the preparation.

Poliovirus. Polio, type 2, derived from infected, P^{32}-labeled D-98 cells and suspended in 0.02*M* phosphate buffer was passed through an ECTEOLA-SF column. Titratable virus and radioactivity showed definite correspondence (Fig. 4) although less than 4 percent of the radioactivity added to the column appeared in effluents containing 99 percent of the detectable virus. Most of the titratable virus appeared before the fifth milliliter of 0.02*M* phosphate buffer had passed through the column (Fig. 4*A*). The elution of this virus from DEAE-SF was similar to that from ECTEOLA-SF, but less sharp. Effluents which contained virus were pooled and reexamined chromatographically as follows: Without further treatment, one portion was again chromatographed. The elution pattern was identical with that of the virus-containing portions of the original tissue-culture fluid concentrate (Fig. 4*B*).

About 0.4 counts/min per 2000 tissue-culture-doses were found in the 1-ml fraction which contained most of the detectable virus. Another portion was dialyzed, first against running tap water and then against 0.02*M* phosphate buffer. Eighty-five percent of the radioactivity was retained within the dialysis bag despite prolonged and repeated dialysis. In addition, 78 percent of the radioactivity of another aliquot was found in the sediment after centrifugation at 80,000 *g* for 100 minutes. The elution diagram of a portion of this non-dialyzable material was identical with that of polio 2 virus derived from the crude concentrate or from column eluates. These findings demonstrated that the chromatographic behavior of the virus remained constant during these treatments.

Ultraviolet absorption spectra of polio 2 preparations (10^{10} TCD_{50}/ml) purified by passage through an ECTEOLA-SF column possessed a characteristic nucleoprotein absorption peak at 260 mμ which was not apparent in the spectrum of the input material (Fig. 5).

Poliovirus, type 1, concentrated by centrifugation, washed, and resuspended in 0.01*M* phosphate buffer, *p*H 7.1, was added to an ECTEOLA-SF column. The major portion was recovered in the first eleven milliliters of 0.01*M* phosphate buffer eluent. Type 3 poliovirus in tissue-culture fluid, diluted 1:10 with 0.02*M* phosphate buffer, *p*H 7.1, and added to an ECTEOLA-SF column, was recovered in the first five milliliters of the initial eluent, 0.02*M* phosphate buffer. These findings do not necessarily imply different elution characteristics for these polioviruses, since the initial conditions were not comparable.

Colorado tick fever. Five milliliters of Colorado tick fever virus, in the form of 20 percent mouse-brain homogenate, were adsorbed onto a 10-cm ECTEOLA-SF column. Elution results are given in Table 2. Most of the titratable virus (67 percent) was eluted by 0.50*M* NaCl in phosphate buffer, whereas only 13 percent of the total protein added to the column was recovered in this fraction. Thus, partial virus purification was achieved, even from very crude starting material, and virus recovery was satisfactory.

In the experiments described, material loaded onto the columns (except for the Colorado tick fever suspension described above) generally contained from 0.2 to 1.0 mg of protein [0.7 to 3.5 absorbance units determined by the procedure of Lowry *et al.* (*11*); protein values are expressed in terms of a standard solution of crystallized bovine albumin, Armour and Co., Chicago, Ill.]. Eluates with the major portion of titratable virus usually contained less than 20 μg of protein per milliliter. When these eluates were again adsorbed and eluted, protein was generally not detectable (less than 1 μg) in the virus-containing fractions, although most of the virus was recovered.

Discussion

The foregoing data indicate that rickettsiae and some mammalian viruses can be adsorbed onto and selectively eluted from ECTEOLA-SF and DEAE-SF columns. The conditions under which this can be accomplished also permit the recovery of essentially all the virus added to the columns and allow the removal of much extraneous substance.

Coxsackie A9, and poliovirus, types 1, 2, and 3, did not appear to be ad-

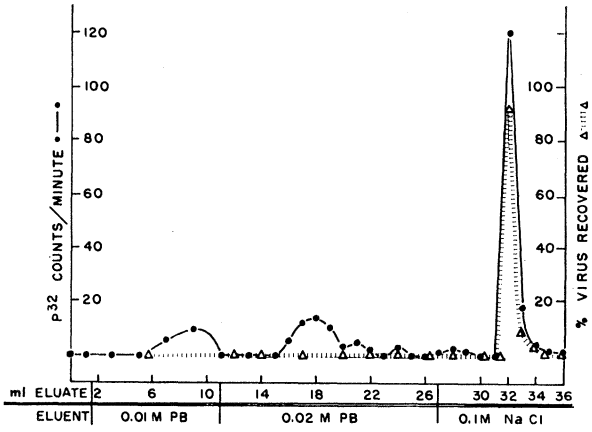

Fig. 2. Relationship between virus recovery and radioactivity (P^{32}) from a preparation of ECHO-13 virus grown on P^{32}-labeled monkey kidney cells, adsorbed onto an ECTEOLA-SF column, and eluted by 0.1*M* NaCl.

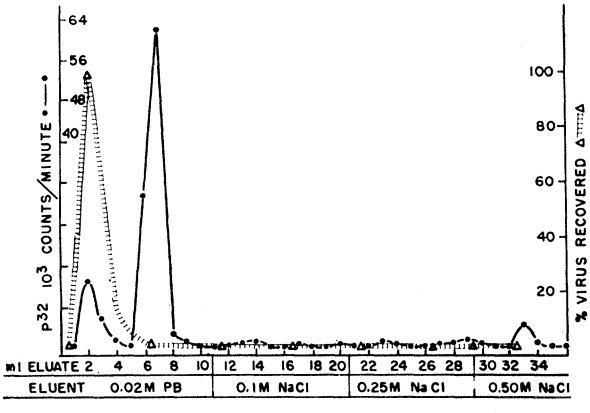

Fig. 3. Relationship between virus recovery and radioactivity (P^{32}) from a preparation of Coxsackie A9 virus grown on P^{32}-labeled monkey kidney cells, adsorbed onto an ECTEOLA-SF column, and eluted by 0.02*M* phosphate buffer.

Table 1. Summary of elution characteristics of viruses and their recovery from ECTEOLA-SF and DEAE-SF columns.

Agent	Source	Eluent*	Titers of virus†	
			Total added	Eluate
Polio 2	Tissue-culture fluid	0.02*M* phosphate buffer	9.0	8.8
Polio 2	Tissue-culture fluid	0.02*M* phosphate buffer*	8.6	8.6
Colorado tick fever	Mouse brain	0.5*M* NaCl	8.9	8.8
ECHO-13	Tissue-culture fluid	0.1*M* NaCl	5.3	5.4
ECHO-13	Tissue-culture fluid	0.1*M* NaCl*	6.8	6.8
Coxsackie A9	Tissue-culture fluid	0.02*M* phosphate buffer	9.6	9.3
Polio 1	Tissue-culture fluid	0.01*M* phosphate buffer	7.8	8.0
Polio 3	Tissue-culture fluid	0.02*M* phosphate buffer	7.5	7.5

* Virus first appeared in large quantity when columns were eluted with phosphate buffer or PB + NaCl in the concentration noted. Those eluents marked by asterisks indicate that results were obtained with DEAE-SF; all others were obtained with ECTEOLA-SF.
† Virus titrations are expressed as $\log_{10}$ of the TCD_{50} or LD_{50}. The titer in the column "Eluate" refers only to the virus released by the eluent noted in column 3.

sorbed under the conditions employed, since they appeared in the very early fractions of the initial eluents. In spite of this behavior, a considerable degree of purification can be achieved. For example, most of the contaminating, phosphorus-containing material can be adsorbed by the ion exchanger and can be separated from the infectious particles. Furthermore, most of the extraneous protein can be adsorbed by the ion exchanger and also separated from the virus. The close correspondence between virus activity and P^{32} distribution also indicates that marked virus purification was accomplished with ECHO-13 and polio 2 viruses. These data indicate the practicality of simply and effectively labeling and purifying useful quantities of infectious mammalian viruses. Such viruses may well provide a much-needed material for studying virus-host relationships and the antigenic properties of animal viruses.

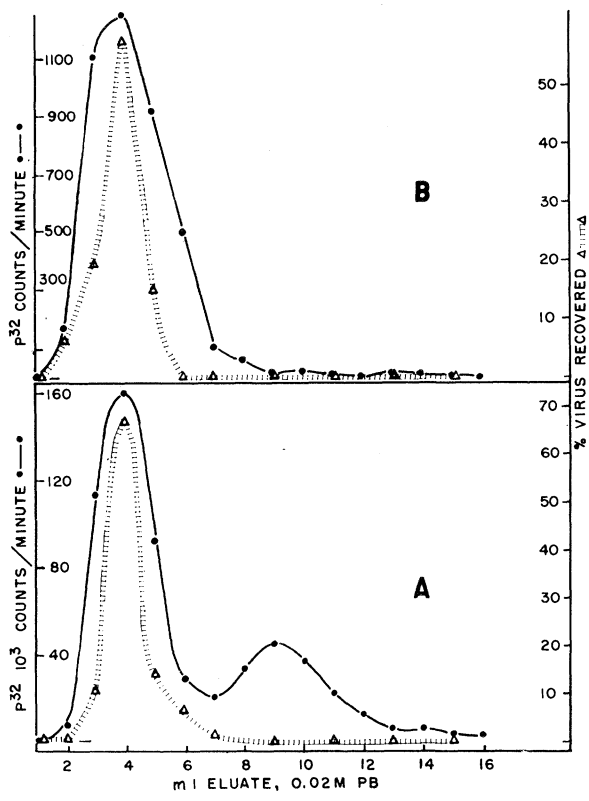

Fig. 4. Relationship between virus recovery and radioactivity (P^{32}) from a preparation of polio 2 virus grown on P^{32}-labeled Detroit-98 cells, adsorbed onto an ECTEOLA-SF column, and eluted by 0.02*M* phosphate buffer. Elution diagrams in *A* were obtained from 2.3 ml of crude concentrate. The third to sixth milliliters of eluates from *A* were pooled and diluted; 2.3 ml of this pool was adsorbed onto and eluted from a duplicate column of ECTEOLA-SF to give the elution diagrams presented in *B*.

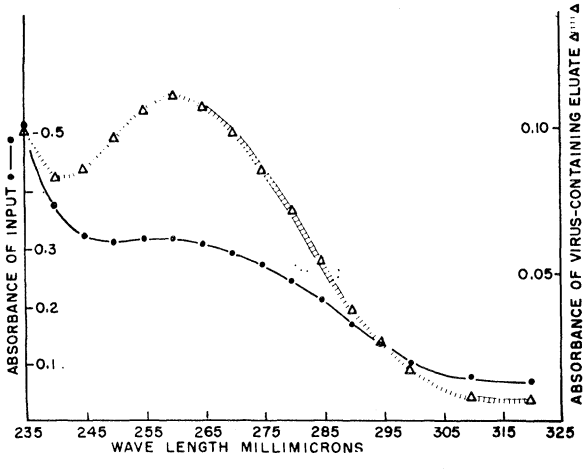

Fig. 5. Absorption spectrum of a crude polio 2 virus preparation compared with the spectrum of the virus-containing eluate obtained after the crude preparation had been adsorbed onto an ECTEOLA-SF column and eluted by 0.02*M* phosphate buffer.

Table 2. Behavior on an ECTEOLA-SF column of Colorado tick fever virus from a mouse-brain homogenate.

Preparation	Number of LD_{50}	Percentage of recovered virus	Protein content of eluates
Original	8×10^8		86 units*
0.02*M* eluate	0	0	8.8 units (10)†
0.10*M* eluate	1×10^8	11	9.2 units (11)
0.25*M* eluate	2×10^8	22	13.1 units (15)
0.50*M* eluate	6×10^8	67	11.1 unit (13)
Total recovered	9×10^8		

* A unit is equivalent to 270 μg of standard crystallized bovine albumin (Armour and Co., Chicago, Ill.) as determined by the procedure of Lowry *et al.* (*11*).
† The figures in parentheses indicate the percentage of original protein recovered in the eluate.

Rickettsiae, as exemplified by the organisms of Q fever and epidemic typhus, can be adsorbed and purified on cellulose exchangers. Rickettsial preparations of the purity indicated by the electron micrographs (see Fig. 1) are of obvious significance for studying rickettsial chemistry, metabolism, and immunology.

It is of particular interest that differences in chromatographic behavior, irrespective of particle size, have been demonstrated. Such different behavior must be presumed to be due to intrinsic differences in the surfaces of these agents. This is strongly suggested by the work of Taussig and Creaser on the coliphages (*4, 5*) in which the virus behavior in a column was shown to be determined by the character of the protein moiety. Among the viruses examined in the present investigation, ECHO-13 and Colorado tick fever are definitely different from each other as well as from the group comprising poliovirus, types 1, 2, and 3, and Coxsackie A9. ECHO-13 was eluted by 0.1*M* NaCl, whereas Colorado tick fever was eluted principally by 0.5*M* NaCl; in contrast, the other species studied passed through the column at very low salt concentrations. The character of the brain suspension may have modified the behavior of the Colorado tick fever virus on the column, but subsequent experiments with preparations containing very small amounts of brain material indicated similar elution behavior.

The experiments reported above are essentially exploratory. Nevertheless, the simplicity of this type of purification and examination of animal viruses encourages the hope that studies of these viruses will be concerned as much with the virus itself as with contaminating

host material. Elimination of contaminating host material from virus vaccines may be very important in reducing "side effects," such as allergic encephalitis, from vaccines which can be obtained only from tissues of whole animals. Chemical inactivations of viruses may also be carried out in systems relatively free of contaminants which would normally react with the inactivating agents and, thus, alter their effects on the viruses themselves. Differences in elution characteristics of the viruses presently investigated strongly suggest that surface properties of animal viruses can be investigated by means of chromatographic methods. In this connection, preliminary experiments (*12*) have shown that formaldehyde-treated polio 2 can be separated from untreated virus.

In general, we feel that procedures such as those described offer the definite hope that studies of animal viruses will no longer be hampered by the past difficulties involved in preparation of purified agents.

Summary

Techniques of column chromatography with cellulose ion exchangers have been successfully applied to mammalian viruses and rickettsiae. Recovery of virus is excellent, and appreciable purification in terms of phosphorus and protein removal has been demonstrated.

Elution characteristics of poliovirus (types 1, 2, and 3), and Coxsackie A9 virus are similar, whereas those of ECHO-13 and Colorado tick fever differ from them as well as from each other.

Elution diagrams of preparations of ECHO-13 and polio 2 viruses grown on P^{32}-labeled tissue cultures show a high degree of correlation between the distribution of titratable virus and the distribution of radioactivity.

A single adsorption and elution of Q fever or epidemic typhus fever rickettsiae results in a striking degree of purification, as demonstrated by electron micrographs.

The chromatographic behavior of the animal viruses and rickettsiae appears to depend more upon the chemical nature of the surfaces of these infectious agents than upon their size.

The chromatographic procedure described may prove useful in the preparation of purified, P^{32}-labeled, fully infectious animal viruses for further fundamental research. It may also prove useful for the removal of unwanted host materials in the preparation of vaccines.

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Charles Franklin Brooks, Meteorologist

Charles Franklin Brooks, professor of meteorology, emeritus, at Harvard University, died suddenly at his home on 8 January 1958, in his 67th year. Although he had retired last year from the directorship of Harvard's Blue Hill Meteorological Observatory, he had remained active in meteorological affairs; on the morning of his death he had made numerous samplings and measurements of the fresh snowfall.

Dr. Brooks's career spanned all the tremendous development in meteorology which has been concentrated in the present century. He was born in St. Paul, Minnesota. The severe summer thunderstorms which occur in that part of the country impressed him during his youth and served to spark his interest in the weather. He applied climatology early in life when he computed his rates for a contract in snow shoveling by the season versus rates per storm.

His university education was pursued at Harvard, where he received the A.B. in 1911, the A.M. in 1912, and the Ph.D. in 1914. His Ph.D. in meteorology was the second to be awarded in this country.

He went to Washington, D.C., to work on the *Atlas of American Agriculture*. Then he became instructor in geography at Yale University (1915–18). Later he served as instructor in meteorology for the U.S. Army Signal Service at College Station, Texas. Following this work he returned to Washington to edit the Weather Bureau's *Monthly Weather Review* (1918–21). For the next decade he taught meteorology and climatology in the Graduate School of Geography at Clark University.

The American Meteorological Society was founded in 1919 largely through the personal energy and enthusiasm of Dr. Brooks. He was elected secretary at the first meeting and served in that office until 1954. He was editor of the society's *Bulletin* during the first 19 years, and more recently he became known as "Mr. American Meteorological Society."

In 1931 he took the post of professor of meteorology at Harvard and director of the Blue Hill Meteorological Observatory. Under his directorship the observatory once more gained international recognition, as the staff and number of projects increased.

In 1940 the Weather Bureau Solar Radiation Field Testing Unit was moved from Washington, D.C., to Blue Hill at Dr. Brooks's suggestion, because the new location provided better exposure and proximity to other laboratories experimenting with the uses of solar energy. The Weather Bureau still maintains a solar observing unit at the Blue Hill Observatory.

In 1932 Dr. Brooks was instrumental in reestablishing a weather observatory at the top of Mount Washington. He became its president and engaged actively in this work until his death. Without his efforts, the Mount Washington Observatory almost certainly would have failed during times of crisis.