has to conclude that folic acid is necessary for the maintenance of early fetal life in mice and rats. This conclusion is also advanced by Nelson and Evans(4).

Summary. 4-amino-pteroylglutamic acid was found to be lethal to the early embryo of mice and rats. Fetal death was observed with doses which temporarily caused depletion of the bone marrow but no other significant signs of intoxication in mothers.

Received April 19, 1950. P.S.E.B.M., 1950, v74.

Partial Purification of Viruses with an Anion Exchange Resin.* (17856)

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A variety of techniques have been developed for the purification of viruses. Methods which have been applied to poliomyelitis viruses include: adsorption to, and elution from alumina gel(1) or aluminum hydroxide(2); half saturation with ammonium sulfate(3); filtration(4); procedures involving ether extraction, protein precipitation and centrifugation or ultracentrifugation (5-9); methanol precipitation(10); and protein precipitation with protamine sulfate(11). A relatively simple and rapid procedure for the partial purification of the Lansing strain of poliomyelitis virus has been developed in this laboratory. It utilizes a strong-base anion exchange resin. The method is also applicable to the extraction of poliomyelitis virus from human feces and Theiler virus (TO) from mouse feces and presumably may be applied to other viruses as well.

With this method, it is possible to eliminate rapidly the bulk of extraneous material and to obtain a preparation that has not lost a significant amount of virus. Advantages of the method include a considerable saving of time, the requirement of only the simplest equipment, and the elimination of lengthy chemical procedures.

Materials and methods. The Lansing strain of poliomyelitis virus from nervous tissue of mice, poliomyelitis virus from human feces, and Theiler virus obtained from the feces of normal mice were used in these experiments. The Lansing virus and Theiler virus were assayed in 4-5 and 3-4-week-old mice respectively (CFW-albino agouti strain, Carworth Farms), using the intracerebral route of infection. Poliomyelitis virus extracted from human stools was tested intracerebrally in monkeys (*Macaca mulatta*). Of the 3 strong-base anion exchange resins tested (Amberlite XE-56, Amberlite XE-75, and Amberlite XE-67)† only Amberlite XE-67 proved satisfactory for virus purification.

* Aided by a grant from the National Foundation for Infantile Paralysis, Inc.

† Supplied by the courtesy of Rohm & Haas Co., Philadephia, Pa.

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The resin is used in the chloride exchange form as it is supplied by the manufacturer. It is a very fine powder, the particles passing through a 325 mesh screen. The following procedure with Amberlite XE-67, carried out at room temperature, proved to be the simplest and most rapid. It is adaptable to the handling of large quantities of infected material and can, if necessary, be carried out without the use of the centrifuge.

(A) Ten grams of dry Amberlite XE-67‡ are added to each 100 ml of a 1% crude suspension of central nervous system tissue (CNS) of mice infected with Lansing virus. The mixture is gently shaken by hand in a stoppered bottle for 20 minutes and then passed through a Buchner funnel using a No. 1 quantitative filter paper. The *effluent* is clear, colorless and non-infective.

(B) The sediment, consisting of a resin-tissue-virus mixture, is washed with sterile distilled water in an amount equal to the original volume; the mixture being stirred in the Buchner cup as it is filtered. This removes the larger particles which have not been adsorbed to the resin. There may or may not be virus in the *wash*.

(C) The resin-tissue-virus sediment is again shaken for 20 minutes in a stoppered bottle with an amount of 10% disodium acid phosphate solution equal to the original volume. This is then passed through a Buchner funnel. The *eluate* is clear, colorless and contains virus. When the eluate is to be inoculated intracerebrally, the excess disodium acid phosphate is first removed by dialysis.

When this procedure is employed to extract human poliomyelitis virus or Theiler virus from feces, the supernatant fluid from a centrifuged 10% fecal suspension is used. Approximately 20% resin by weight is necessary to adsorb the virus from feces. To prevent bacterial infection, 1 mg of Streptomycin and 1000 units of penicillin are added for each milliliter of the final eluate. The procedure is otherwise identical to that described for Lansing virus. The final solution is clear, colorless and relatively non-toxic. It has lost little, if any, of the infectivity of the original suspension. It should be noted that Amberlite XE-67, because of its microscopic particle size, will pass through filter paper if it remains in suspension. It is, therefore, necessary to have a sufficient amount of lipoid or colloidal material in the starting suspension to aggregate the fine Amberlite particles and to retain them in the sediment. For this reason, a 1% crude suspension of nervous tissue prepared in a Waring Blendor is more satisfactory than the supernatant fluid from a highly centrifuged 10% suspension. When fecal material is used, a 10% fecal suspension centrifuged between 2000 and 4000 rpm for 60 minutes at 40°F has been found satisfactory. If however, the effluent is still not clear, centrifugation at 18,000 rpm for 1 hour at 40°F (New International Centrifuge, angle head) is necessary after each step in the procedure. If centrifugation is necessary in the last step, it should be done after the excess disodium acid phosphate has been dialyzed. A modification of the Conway procedure was used to determine total nitrogen content(12,13).

Results. This report is based on 7 experiments with Lansing virus in CNS tissue and 5 experiments with Theiler virus in mouse feces. The examples shown in Table I are representative of each group.

That a considerable amount of extraneous material is removed by the procedure is indicated by the clear, colorless solutions obtained and by the marked decrease in nitrogenous material shown in Table I. The total nitrogen in experiments (b) and (c) is reduced from 288 and 322 γ per ml in the original CNS suspension to 4.2 and 11.2 γ respectively in the final eluate. This represents only 1.5% and 3.5% of the original nitrogen. Similarly, the total nitrogen content of the 10% fecal suspension is reduced

‡ Although column operations are most often used in exchange-resin work, this procedure is not suitable for Amberlite XE-67 due to its small particle size.

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TABLE I.
Partial Purification of Viruses with Anion Exchange Resin (Amberlite XE-67).

Suspensions tested	Exp.	Total N, γ/ml	Infectivity: Dilutions 10-1	10-2	10-3	10-4	LD_{50} Log 10
	A. Lansing poliomyelitis virus from mouse CNS.						
Original 1% suspension	a	—		4/7*	0/8	0/8	2.0
	b	288		7/7	5/7	0/8	3.3
	c	322		7/7	4/8	2/8	3.2
Effluent	a	—		0/8	—	—	—
	b	9.5		0/9	—	—	—
	c	8.4		0/10	—	—	—
Water wash	a	—		0/8	—	—	—
	b	<1.5		3/8	—	—	—
	c	5.6		4/10	—	—	—
Dialyzed eluate	a	—		4/8	0/7	0/8	2.0
	b	4.2		5/7	3/7	0/8	2.6
	c	11.2		10/10	—	—	—
	B. Theiler virus (TO) from the feces of normal mice.						
Supernatant fluid of a 10% fecal suspension		189	7/8	4/8	0/8	—	1.9
Effluent		68.9	0/8	—	—	—	—
Water wash		35	0/8	—	—	—	—
Dialyzed eluate		18.2	4/6	1/7	0/8	—	1.3

* Numerators indicate the No. of mice paralyzed and denominators No. of mice injected. Mice dying without symptoms of paralysis are omitted.

TABLE II.
Selective Elution of Lansing Poliomyelitis Virus from Amberlite XE-67 with High Concentration of Disodium Acid Phosphate.

Material tested	pH eluate	Infectivity of suspensions
Original 1% CNS suspension	6.6	8/8*
Effluent	5.2	0/8
Eluate with 5% NaCl†	4.9	0/8
" " 10% NaCl	4.8	0/10
" " 5% $NaHCO_3$	9.0	0/10
" " 10% "	9.1	0/10
" " 5% Na_2HPO_4	6.8	0/10
" " 10% "	7.2	6/9

* Numerators indicate the No. of mice paralyzed and denominators No. of mice injected. Mice dying without symptoms of paralysis are omitted.

† Excess salt removed by dialysis before intracerebral inoculation.

from 189 to 18.2 γ of nitrogen per ml. This represents 9.6% of the original nitrogen. It may also be seen in Table I that the final preparations are almost as infective as the original suspensions. The titer in the eluate for the Lansing virus is 2.6 (LD_{50} log of dilution) as compared with a titer of 3.3 in the starting suspension of experiment (b), and the titer in the eluate for the TO virus is 1.3 (LD_{50} log of dilution) as compared with a titer of 1.9 in the starting suspension.

That the virus is adsorbed to the resin is

indicated by the infectivity of the various suspensions shown in Table II. The effluent from the resin-tissue-virus complex is non-infective indicating that the virus is retained with the resin. Before attempting to elute the virus from the resin, the complex was thoroughly washed with distilled water to remove any tissue fragments which might contain virus. The wash fluid was occasionally infective (Table I). Aliquots of the resin-tissue-virus complex were then treated with solutions containing different salts at high concentration and at varying pH. Five and 10% solutions of sodium chloride and sodium bicarbonate, and a 5% solution of disodium acid phosphate did not elute the virus from the resin. However, the virus was eluted with a 10% solution of disodium acid phosphate. It appears that the virus is almost selectively eluted with a high concentration of disodium acid phosphate, leaving the bulk of the nitrogenous material adsorbed to the resin (Table I).

Similar results were obtained in the extraction of poliomyelitis virus from pooled human stools of known infectivity. The final eluate which was clear and colorless contained 9.2 γ of total nitrogen as compared with 236 γ of total nitrogen in the starting suspension. The infectivity of the material was retained as shown by the development of paralysis in 1 of 2 monkeys 7 days after intracerebral injection of 1 ml of the final eluate.

Comment. Muller(14) has recently reported the use of a *cation* exchange resin for partial purification of such neurotropic viruses as the Japanese B encephalitis, Eastern equine encephalomyelitis and Western equine encephalomyelitis. The mode of action of Muller's cation exchange resin in effecting a partial purification of virus differs from that of the anion exchange resin reported in this paper. With the use of the *cation* exchange resin, extraneous nitrogenous material is adsorbed to the resin, leaving the virus in the effluent. On the other hand, with the use of the *anion* exchange resin, it is possible to adsorb viruses to the resin along with the nitrogenous material. The virus is then selectively eluted from the resin.

Summary. A simple and rapid procedure for the partial purification of certain viruses is afforded by the use of a strong-base anion exchange resin (Amberlite XE-67). A clear, colorless solution is obtained which retains its original infectivity but which has lost at least 90% of its nitrogenous material.

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Received April 21, 1950. P.S.E.B.M. 1950, v74.

Anticonvulsant Action of Isopropyl Alcohol.* (17857)

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In previous reports from this department (1,2), it has been shown that isopropyl alcohol can significantly increase the blood acetone and the cortical threshold to electrical stimulation in different species. Since the maximum acetonemia after gastric administration of the isopropyl alcohol did not strictly coincide with the peak of cortical threshold, further examination of the possible mechanism of the anticonvulsant action has been made.

Methods. The cortical threshold was de-

* Supported, in part, by the Stern Fund for Research in Anticonvulsants.

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