The Difference in Elutability of Poliovirus and SV_{40} from a DEAE Column. (27488)

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Recent reports(1,2,3) have shown that virus vaccines grown on monkey kidney cells are contaminated with the SV_{40} virus. These reports also describe infections in human volunteers given the SV_{40} virus by the respiratory route. Virus was recovered from the throat washings of these volunteers, and specific antibodies were demonstrated in their sera. Furthermore, SV_{40} antibodies have been found in the sera of people who have received formalin-treated poliovirus and adenovirus vaccines which were contaminated with the SV_{40} virus. So far, human beings have shown no ill effects from SV_{40} virus. However, Eddy(4) has shown that newborn hamsters develop tumors when they are injected with a supernate of a medium in which rhesus monkey kidney cells had been grown. She has identified the causative agent as the SV_{40} virus. This virus is found in the kidneys of approximately 70% of "normal" monkeys. A vaccine made from a specific virus which has been grown on these contaminated monkey kidney cells will contain not only the specific virus but SV_{40} virus as well. Therefore, to insure the safety of vaccines made from viruses grown on monkey kidney cells, it is important to develop a method which will eliminate the SV_{40} virus.

This report describes a method which frees poliovirus from detectable quantities of SV_{40} virus. The method depends upon the differential elution of poliovirus and SV_{40} virus from a cellulose N,N-diethylaminoethyl ether (DEAE) ion exchange column.

Materials and methods. Tissue culture. Grivet monkey kidney cells, obtained from Microbiological Associates, were maintained in medium 199 containing 2% inactivated chicken serum.

Viruses. LSc,2ab strain of Type 1 poliovirus was obtained from Dr. A. B. Sabin. This strain is the Type 1 virus in Sabin's live oral poliovaccine. SV_{40} virus was obtained from Dr. M. R. Hilleman. Both strains were grown in medium 199 free of serum.

Serum. Human serum which had a high titer against Type 1 poliovirus and no demonstrable antibodies against the SV_{40} virus was used for neutralization of the poliovirus.

Ion exchange column. DEAE was washed 3 times with distilled water, 3 times with 0.25M NaOH in 0.5M NaCl, and 3 times with 0.2M KH_2PO_4. Finally it was washed 2 times with 0.02M phosphate buffer pH 7.1. A thin slurry was packed into a chromatographic tube (30 cm by 1 cm) with an air pressure of 10 lb. The final DEAE column was 10 cm in height and was equilibrated with repeated washings with the phosphate buffer.

Preparation of virus for chromatography. Grivet monkey kidney cells were infected with SV_{40} virus. Four days later, they were inoculated with the LSc,2ab poliovirus. When the doubly infected cells were completely destroyed, they were frozen and thawed 3 times in a mixture of 95% ethyl alcohol and CO_2. The material was centrifuged 30 minutes at 7500 RPM. The supernate resulting from this centrifugation was then centrifuged for 2½ hours at 30,000 RPM. (A Spinco Model L ultracentrifuge was used for both centrifugations.) The pellet resulting from the second centrifugation was suspended in 2.1 ml of the phosphate buffer. Two ml of the resuspended pellet were loaded onto the DEAE column and eluted.

Virus titration. All titrations for the poliovirus were carried out using serial 10-fold dilutions of the eluates and of the material loaded onto the column. One-tenth ml aliquots of each dilution were inoculated into 5 Grivet monkey kidney cell tubes. The tubes were observed for 1 week for cytopathogenic changes. To assay the SV_{40} virus, it was necessary to neutralize the poliovirus present in the inputs and eluates. This was accomplished by mixing equal parts of high-titer human anti-poliovirus serum and dilutions of

TABLE I. Elution from DEAE of SV_{40} Virus Grown on Grivet Monkey Kidney Cells.

Material titrated	Titer	Titer 1st subpassage	Titer 2nd subpassage
Input	$10^{5.4}$		
.02M PO_4 eluate	10^{0}	10^{0}	10^{0}
.05M NaCl* eluate	10^{0}	10^{0}	10^{0}
.10M " * "	10^{0}	10^{0}	10^{0}
.15M " * "	10^{0}	10^{0}	10^{0}
.20M " * "	$10^{2.0}$		
.50M " * "	$10^{4.1}$		

* In .02M PO_4 buffer.

the input and eluates. After incubation at room temperature for 1 hour, 0.2 ml of each mixture was inoculated into each of 5 Grivet monkey kidney cell tubes. The tubes were observed for 2 weeks for cytopathogenic changes. Those tubes which showed no cytopathogenic changes were subpassaged; 0.2 ml of the undiluted culture fluid were inoculated into each of 2 Grivet monkey kidney cell tubes and observed for 2 weeks. All titers were calculated by the Reed-Muench method.

Results. Table I shows the chromatographic pattern of SV_{40} virus and the eluant used to develop the column. It will be seen that no SV_{40} virus was recovered from the column unless the concentration of NaCl in the eluant was 0.2M or greater. We have previously shown that all strains of poliovirus are eluted from a DEAE column when 0.075M NaCl is used as the eluant. Thus one would expect that elution with 0.075M NaCl in phosphate buffer would separate poliovirus from an SV_{40} virus contaminant.

TABLE II. Elution from DEAE of Poliovirus (LSc,2ab) and SV_{40} Virus Grown Jointly on Grivet Monkey Kidney Cells.

Exp.	Material titrated	Titer Sabin LSc,2ab	Titer SV_{40}	Titer SV_{40} subpassage
1	Input	$10^{8.2}$	$10^{4.5}$	
	.075M NaCl eluate	$10^{8.1}$	10^{0}	10^{0}
2	Input	$10^{8.5}$	$10^{4.2}$	
	.075M NaCl* eluate	$10^{8.3}$	10^{0}	10^{0}
3	Input	10^{8}	$10^{3.8}$	
	.075M NaCl* eluate	10^{8}	10^{0}	10^{0}

* In .02M PO_4 buffer.

Table II records results obtained when LSc,2ab poliovirus and SV_{40} virus, grown together, were eluted from a DEAE column with 0.075M NaCl in phosphate buffer. All of the poliovirus loaded onto the column was found in the eluate. In contrast, no SV_{40} virus was demonstrated in the various preparations despite subculture and blind passage.

Discussion. The results described here show that SV_{40} virus has a much greater avidity for a DEAE column than does poliovirus. The polioviruses are weakly held by the DEAE, which is an anion exchanger; they appear in high titer early in the elution. SV_{40} virus, however, acts as a stronger anion and is held firmly by the column until a high concentration of salt is used as the eluant. This avidity for the DEAE column is a property of other viruses such as polyoma, measles and adenovirus-2. The results shown in Tables I and II might be taken as indicating that the separation of SV_{40} virus and poliovirus had been achieved. However, we cannot entirely exclude the possibility that traces of the SV_{40} virus were eluted from the column and were not detected by the tissue culture technic employed. In our experiments, the Grivet monkey kidney cell tissue culture tubes were observed for 2 weeks. It is possible that if these cultures had been observed for a longer period, the presence of traces of SV_{40} virus might have been detected.

Summary. The differences in avidity for a DEAE cellulose ion exchange column exhibited by polioviruses and SV_{40} virus make it possible to reduce the content of SV_{40} virus in a mixture of the 2 viruses to a trace at most. SV_{40} virus has a much greater avidity for a DEAE cellulose ion exchange column than do polioviruses. This fact permits the freeing of poliovirus from detectable SV_{40} virus.

Addendum: In a subsequent experiment, those Grivet monkey tubes inoculated with the .075M NaCl eluate were held for 5 weeks. Then they were subpassaged into Grivet monkey cells and held for a further 3 weeks. No cytopathogenic changes were seen in any of the tubes. Therefore, the possibility of SV_{40} virus being in the eluate would seem to be extremely doubtful.

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Intestinal Absorption of Ca-45 and Sr-85 as Affected by the Alkaline Earths and pH.* (27489)

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Strontium nuclides are absorbed *via* the digestive tract and are deposited and retained to a high degree by the skeleton(1). Investigators have reported a clear cut reduction of the Sr-90 burden with increased ingestion of calcium(2,3). Harrison *et al.*(4), however, reported that, when dietary strontium increased, absorption of an oral dose of radioactive strontium increased. Wasserman(5) ascertained from everted intestinal sacs that Ca-Sr discrimination under given conditions was dependent upon metabolically active membranes. Dumont *et al.*(6) using an *in vivo* preparation of rat small intestine found that in the concentration range of 0 to 25 mM, strontium flux appears to be passive, though restricted. Calcium transport may not, however, be ascribed to passive independent movement of calcium ions since at higher concentrations (12.5 and 25 mM), the efflux of calcium from blood to intestinal lumen increases more than expected. Earlier studies from this laboratory indicated that the absorption of Sr-85 and Ca-45 from the small intestine was related inversely to the concentration of calcium in the perfusate and that the effect on Ca-45 absorption was greater than the effect on Sr-85 absorption(7). The present study is an extension of this work aimed at determining the effect of other alkaline earths and pH on intestinal absorption of Sr-85 and Ca-45.

Methods. A total of 180 Sprague-Dawley female rats with an average weight of 200 g was perfused and a minimum of 6 rats was used for each treatment. The rats were fed a commercial pelleted diet containing 1.3% Ca, 0.9% P and 5 USP units of vit. D per gram of diet for at least 20 days before being fasted overnight prior to the perfusion. Each rat was anaesthetized with sodium pentobarbital (3.2 mg/100 g body weight), a laparotomy performed, and the small intestine cannulated by insertion of the proximal polyethylene cannula into the mouth, through the stomach and tying it off in the upper 2 cm of the duodenum. The distal cannula was inserted through an incision in the intestinal wall about 2 cm proximal to the ileocecal junction. During the perfusion, the intestine remained inside the body cavity and was kept moist with saline-soaked gauze at a temperature of approximately 37°C. The small intestine of the anaesthetized rat was perfused *in situ,* using the technic described by Schanker *et al.*(8). The perfusate was 5 mM in KCl, 20 mM in dextrose, and 0, 1, 5 or 25 mM in the alkaline earths, calcium, strontium, magnesium, barium or beryllium, all in the chloride form. The total osmolarity of all solutions was adjusted to 300 mOsm. by addition of NaCl. In the alkaline earth series of experiments, pH was maintained at 7, except for the beryllium groups. In the pH series, the zero level of the alkaline earths was used at each pH level from 1 through 12. The total length of the small intestine was

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