blood pressure and concentration of arterial muscle potassium. It is suggested therefore that the reversal of the anti-hypertensive effect of quinethazone by prednisolone may be related to the induction of a higher concentration of arterial muscle potassium through mobilization of intracellular water. This concept presupposes that the data obtained from analyses of aorta tissue may be extrapolated to smaller arteries and that alterations in peripheral resistance are mainly responsible for blood pressure changes.

With these considerations in mind, sufficient data are available to postulate that arterial muscle potassium has a regulatory effect on peripheral resistance. It is well known, for instance, that potassium is a vital component in the contractile mechanism of smooth muscle of arteries as well as other organs. In addition, recent reports indicate that this cation may influence basal arterial tone. Thus, Bevan and Osher reported that while potassium sensitized the contraction of artery strips to norepinephrine, it shortened the resting length of the strips through direct action on smooth muscle cells(6). Barr *et al* also showed that shortening of artery strips was a positive function of intra-cellular potassium(7). Furthermore, Laszt and Hamoir maintain that vascular muscle contains "tonactomyosin," a protein which regulates tone under the direct influence of cellular potassium(8).

It follows that the anti-hypertensive effect of quinethazone may, in part, result from loss of arterial tone through dilution of arterial muscle potassium. On the other hand, prednisolone could reverse this anti-hypertensive effect by restoring tone through mobilizing cellular water and concentrating muscle potassium. This effect may shed some light on the mechanism by which anti-inflammatory steroids are known to improve circulation in a variety of hypodynamic states.

Summary. Presnisolone, but not desoxycorticosterone, administration reverses the anti-hypertensive effect of quinethazone. The elevation of blood pressure is associated with an increase in concentration of arterial muscle potassium through the mobilization of cellular water. The direct influence of potassium on arterial tone may be involved in this relationship.

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Received July 14, 1964. P.S.E.B.M., 1965, v118.

Mechanism of Dextran Sulfate Inhibition of Attenuated Poliovirus.* (29752)

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In recent years reports on the effect of sulfated polysaccharides on several viruses have been published. The viruses studied include picornaviruses such as polio(1-3), encephalomyocarditis virus (EMC)(4) and foot-and-mouth disease virus (FMDV)(5,6), herpes simplex virus(7) and myxoviruses such as influenza A2 and B(8). The effect of sulfated polysaccharides on plaque size has primarily been investigated. With poliovirus and FMDV the effect of agar extracts and/or

* This investigation was supported by grants from U. S. Public Health Service (AI-04193-03) and from Swedish Medical Research Council.

dextran sulfate has also been used as a marker characteristic(1-3,5,6). It has also been convincingly demonstrated that one of the most widely used poliomarkers, the d-marker(9), depends on the presence of sulfated polysaccharides in the agar(10).

The mechanism of action of the sulfated polysaccharides has been studied by Liebhaber and Takemoto(4) who found an impaired adsorption of dextran sulfate sensitive strains of EMC virus in the presence of dextran sulfate. Since it is well known that polyions may inhibit several enzymes(11) it has also been suggested(10) that the sulfated polysaccharides may act by interfering with a later stage of the infectious process, *i.e.*, when the nucleic acid is liberated from the protein.

This study describes the effect of dextran sulfate on various stages in the multiplication of attenuated poliovirus type 1.

Material and methods. Cell cultures. Primary cultures of calf kidney cells were prepared as described earlier(6). Monolayer cultures of cynomolgus monkey kidney were grown in plastic petri dishes in Hanks' salt solution with 0.5% lactalbumin hydrolysate and 4% calf serum. After outgrowth the plates were changed to Eagle's minimal essential medium and used for plaque assays.

Viruses. The following 2 strains of poliovirus type 1 were used: strain E206 isolated from a case of paralytic polio and obtained from the National Bacteriological Laboratory in Stockholm, Sweden, and the Sabin vaccine strain L Sc 2ab kindly provided by Dr. A. Sabin, Cincinnati, Ohio.

Virus assays. Serial 10-fold dilutions were inoculated in 0.1 ml amounts on cell monolayers in plastic petri dishes previously washed twice with phosphate-buffered saline (PBS). After adsorption for 30 minutes monkey kidney plates were overlaid with 4 ml of an overlay containing Earle's solution with 0.22% $NaHCO_3$, 0.05% lactalbumin hydrolysate, 2% calf serum and 0.95% special agar Noble (Difco) in final concentrations. The plates were kept in a humidified atmosphere of approximately 5% CO_2 in air and were stained after 2 days with an overlay containing neutral red in a concentration of 1/20,000. Plaques were read on the following day.

Dextran sulfate was kindly provided by AB Pharmacia, Uppsala, Sweden. Four preparations with different molecular weights were used: sodium dextran sulfate 2,000 (batch no. To 264, molecular weight 2,000,000), sodium dextran sulfate 500 (batch no. To 259, molecular weight 500,000), sodium dextran sulfate with molecular weight 55,000 (lot no. pH 878/4) and sodium dextran sulfate with molecular weight 3,300 (Op. no. 104). In addition a ^{35}S-labeled preparation of sodium dextran sulfate 500 (batch no. Phi DX 02A 14723) was used in some experiments.

Sephadex sulfate. This cation exchanger was kindly prepared by AB Pharmacia from Sephadex G-25 fine by forming half esters between hydroxyls of the glucose backbone and sulfuric acid. The final product (Op. no. 7360) contained 13.6% sulfur and had a capacity of approximately 3.5 meq/g. The ion exchanger was suspended in deionized water, washed with 0.1 N HCl and water and finally equilibrated with 0.05 M tris-HCl buffer pH 7.2. Columns approximately 5 × 1 cm were then prepared as described elsewhere (12) and used for chromatography experiments.

Purification of virus. Virus was grown in bottle cultures of cynomolgus monkey kidney with phosphate-free Eagle's medium. It was labeled with ^{32}P by addition of 0.25 mc of carrier-free sodium orthophosphate after adsorption. When cell destruction was complete, the bottles were frozen and thawed, cell debris removed by centrifugation at low speed and the supernate concentrated in a polymer 2-phase system(13). This was carried out by adding dextran sulfate 2,000, polyethylene glycol 6,000 (Carbowax, Carbon & Carbide Chem. Co., New York) and NaCl to a final concentration of 0.20% (w/w) dextran sulfate, 6.45% (w/w) polyethylene glycol and 0.3 M NaCl. The mixture was shaken, poured into a separatory funnel and left in the cold overnight to separate. The small bottom phase was then drained off together with the interface and 4 M NaCl added to it to a final concentra-

tion of 1 M. The mixture was thoroughly shaken and again left to separate overnight. When the phases were separated, the top phase was recovered and the dextran sulfate precipitated by addition of 1 M KCl. The precipitate was spun down and the supernate dialyzed against 0.05 M sodium phosphate buffer pH 7.6 with 0.02 M NaCl. This material was filtered through a DEAE-Sephadex (AB Pharmacia) column (2 × 30 cm) with 0.05 M sodium phosphate buffer pH 7.6 with 0.02 M NaCl as eluting buffer. The first peak of radioactivity eluting from the column contained more than 95% of the infectivity of the input virus. The eluate was mixed with one-tenth volume 1 M tris-HCl buffer pH 7.6 with 0.1 M $MgCl_2$ after which CsCl (2.35 g/5 ml) was added. The resulting precipitate, which contained no radioactivity, was spun down and the supernate transferred to lusteroid tubes and spun for 16 hours at 39,000 rpm in a Spinco SW 39 L rotor. The gradients were then emptied through a pinhole in the bottom, 3 drops/fraction being collected. Tests were made for radioactivity and infectivity and as shown in Fig. 1 the 2 peaks coincide. The active fractions were then pooled, dialyzed against PBS diluted 10-fold overnight and used for experiments. Virus prepared in this way contained about 5% RNAase digestible acid-soluble material.

Results. Effect of varying amounts of dextran sulfate. The effect of varying amounts of dextran sulfate was first studied by plaquing the L Sc 2ab strain under agar overlays containing different concentrations of dextran sulfate 2,000. Only plaques of the same size as the control were counted. The results (Fig. 2), show that there is a sharp decrease in number of normal sized plaques between a concentration of approximately 3 and 10 μg/ml and that higher amounts of dextran sulfate do not significantly increase the inhibition. This is regarded as due to dextran sulfate resistant mutants, which are regularly present to about 0.1% of the progeny.

Effect of dextran sulfate with different molecular weight. This was investigated by plaquing the L Sc 2ab strain under overlays containing 100 μg/ml of dextran sulfates with molecular weights between 3,300 and 2,000,000. The inhibitory effect of dextran sulfate is slight with low molecular weight, but increases markedly with molecular weights above 100,000 (Fig. 3). However, when the molecular weight increases above 1,000,000 the increase in effect on virus inhibition is slight.

Effect of dextran sulfate on adsorption of strain L Sc 2ab. It has been demonstrated (14,15) that the attachment of virus to sensitive cells has 2 stages: a temperature independent reversible attachment followed by a temperature dependent irreversible attachment, *i.e.*, eclipse. The effect of dextran sulfate on these 2 steps was therefore studied separately.

The effect of dextran sulfate on the reversible attachment was first investigated. Thirty monkey kidney plates were washed twice with Hanks' medium with 0.5% lactalbumin hydrolysate (Hanks' + LAH). Four ml Hanks' + LAH were then added to half the plates, the other half receiving Hanks' + LAH with 0.05% dextran sulfate 2,000. After incubation for 30 minutes at 37° these media were sucked off and 0.1 ml of a dilution of L Sc 2ab in Hanks' + LAH with or without sulfate, previously incubated 30 minutes at 37° and containing approximately 50 PFU, was added. The plates were again incubated at 37° and at various times 3 plates of each kind were withdrawn, washed 4 times with Hank's + LAH and agar overlay added. As shown in Fig. 4 dextran sulfate inhibits the attachment of L Sc 2ab.

Next the effect of dextran sulfate on both steps, *i.e.*, both reversible attachment and eclipse, was studied in a similar way. To do this an identical experiment was carried out, but after the last wash 0.5 ml rabbit polio antiserum diluted 1/10 was added and the plates again incubated for 30 minutes at 37°. The plates were then washed 4 times with Hanks' + LAH and agar overlay added. The results (Fig. 5) show a greater difference between the dextran sulfate-treated and the control plates than in experiments described in Fig. 4, probably because the antiserum treatment inactivates the virus which has only attached reversibly.

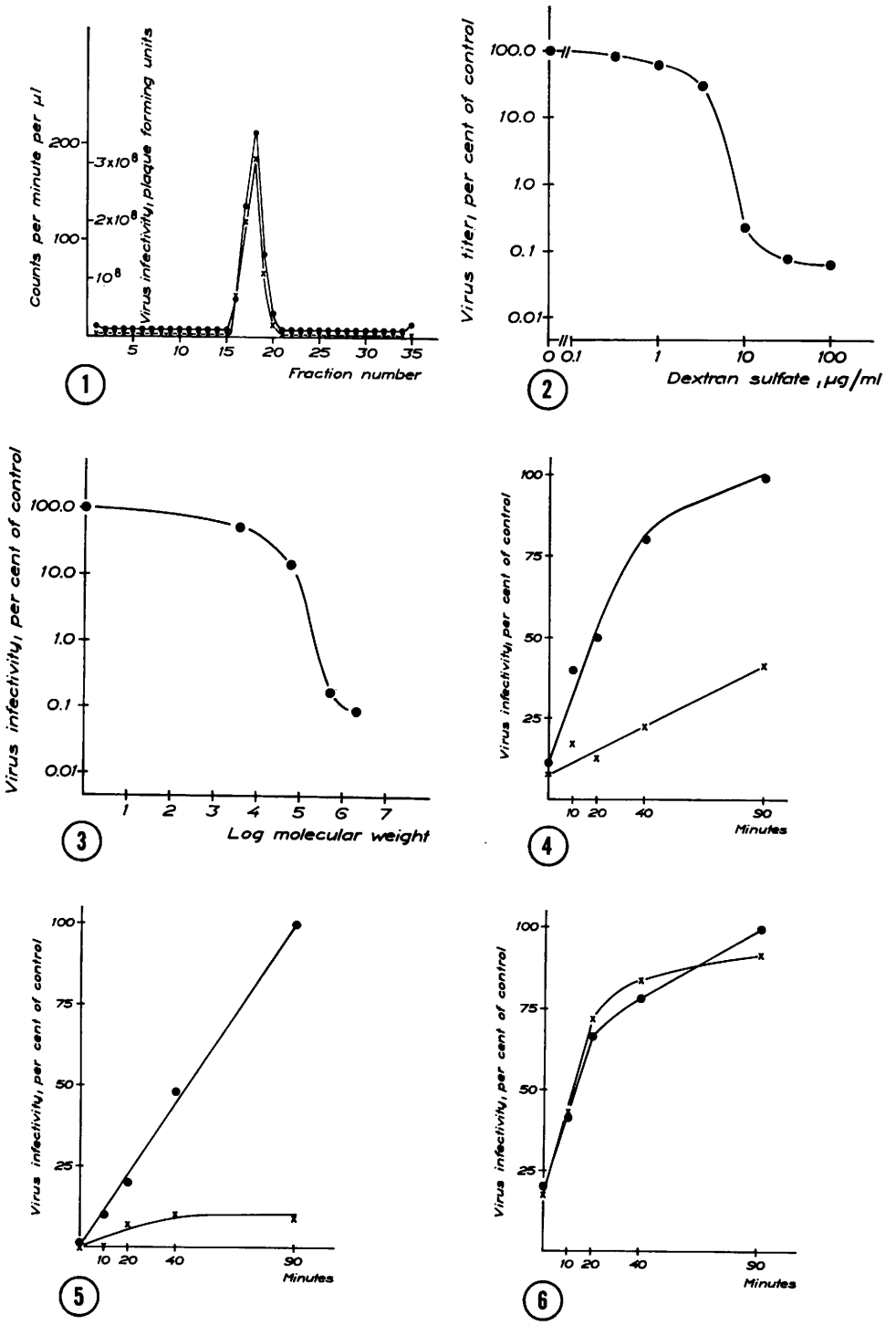

FIG. 1. Sedimentation of ^{32}P-labeled purified poliovirus type 1, strain L Sc 2ab in CsCl gradient. ●——●: Counts/min/μl; ×——×: plaque forming units/0.1 ml.

FIG. 2. Effect of increasing amounts of dextran sulfate 2,000 on plaque-forming capacity of strain L Sc 2ab.

Finally the effect of dextran sulfate on the second step, the eclipse, was studied separately. Thirty monkey plates were washed once with Hanks' + LAH and 3 ml Hanks' + LAH containing approximately 500 PFU was added. The plates were placed in a desiccator, gassed with 5% CO_2 in air and incubated for 2 hours at +4°. The plates were then washed twice with Hanks' + LAH and 3 ml Hanks' + LAH + 0.05% dextran sulfate 2,000 added to half of them and 3 ml Hanks' + LAH to the control plates. After incubation at 37° plates were withdrawn at various intervals, washed twice and 0.5 ml rabbit polio antiserum diluted 1/10 added. The plates were then washed 3 times after 30 minutes incubation at 37° and agar overlay added. The same eclipse rate was observed with or without dextran sulfate present during the irreversible attachment, if virus had been adsorbed at 4° in the absence of dextran sulfate (Fig. 6).

Effect of dextran sulfate on multiplication of infectious nucleic acid. To exclude an effect of dextran sulfate on the steps in virus multiplication following penetration, dextran sulfate inhibition on the multiplication of viral nucleic acid was next investigated. To study this, it was necessary to use a virus-cell system that allows only one cycle of virus multiplication, *i.e.*, a heterologous cell system.

Viral nucleic acid from the strains L Sc 2ab and E206 was prepared by phenol-ether extraction. Calf kidney plates were washed twice with PBS and 0.2 ml of the nucleic acid preparation diluted 10-fold in 2 M $MgSO_4$, was absorbed for 10 minutes at room temperature. The plates were then washed twice with PBS, overlaid with 5 ml Eagle's medium with or without 100 μg/ml of dextran sulfate 2,000 and incubated at 37° for 18 hours. They were then frozen at −60°, thawed and assayed for infectivity on monkey kidney plates. No difference exists between yields with and without dextran sulfate, whether nucleic acid from a virulent or an attenuated strain was used as inoculum (Table I). The yield in this experiment was approximately 27 PFU of virus per PFU of nucleic acid absorbed for the L Sc 2ab strain and approximately 37 PFU of virus/PFU nucleic acid absorbed for the strain E206.

TABLE I. Effect of Dextran Sulfate on Multiplication of Poliovirus Nucleic Acid in Calf Kidney Cells.

Medium	Yield in PFU/plate	
	Strain L Sc 2ab	Strain E206
Eagle's MEM	4×10^4	1.9×10^5
Idem + 100 μg/ml dextran sulfate 2,000	4.1×10^4	1.8×10^5

Absorption of strain L Sc 2ab by dextran sulfate. Since it was shown that dextran sulfate inhibits the strain L Sc 2ab by interfering with its reversible attachment, the absorption of this strain by dextran sulfate was further studied.

A column of cation exchanger Sephadex sulfate was prepared according to methods and one ml 0.01% ^{35}S-labeled dextran sulfate 500 diluted in 0.05 M tris-HCl buffer pH 7.2 was applied and eluted with the same buffer. About 70% of the material passes unabsorbed through the column and emerges in the first fraction (Fig. 7A). A similar experiment was then carried out using ^{32}P-labeled L Sc 2ab virus purified according to methods and diluted 10-fold in 0.05 M tris-HCl buffer pH 7.2. Fig. 7B shows that most of the virus is absorbed to the column, only about 15% being eluted in the first fraction. When sodium chloride was added to the buffer about 40% was eluted. Finally a mixture of equal parts of 0.01% ^{35}S-labeled dextran sulfate and ^{32}P-labeled virus was ap-

FIG. 3. Effect of increasing molecular weight of dextran sulfate on plaque-forming capacity of strain L Sc 2ab. Concentration used was 100 μg/ml.

FIG. 4. Effect of dextran sulfate on reversible attachment of L Sc 2ab virus on monkey kidney cells. ●——●: Control plates; ×——×: plates with 500 μg/ml dextran sulfate 2,000.

FIG. 5. Effect of dextran sulfate on combined reversible and irreversible attachment of L Sc 2ab. ●——●: Control plates; ×——×: plates with 500 μg/ml dextran sulfate 2,000.

FIG. 6. Effect of dextran sulfate on irreversible attachment of L Sc 2ab previously attached at 4°. ●——●: Control plates; ×——×: plates with 500 μg/ml dextran sulfate 2,000.

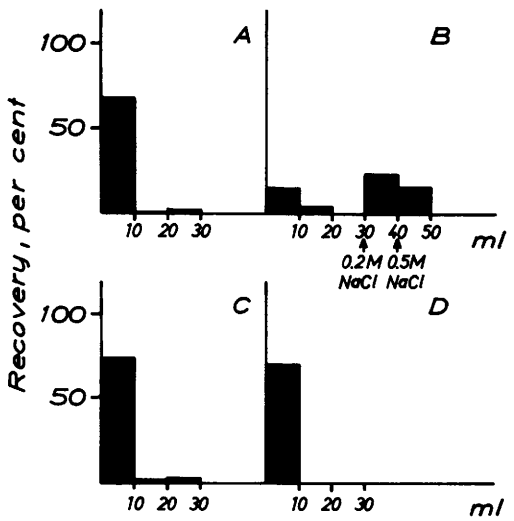

FIG. 7. A: Elution diagram of ^{35}S-labeled dextran sulfate 500 on a column of Sephadex sulfate with 0.05 M tris-HCl buffer pH 7.2. B: Elution diagram of ^{32}P-labeled L Sc 2ab virus on same column. C and D: Elution diagrams on same column of a mixture of equal parts ^{35}S-labeled dextran sulfate (C) and ^{32}P-labeled virus (D) obtained by differential counting.

plied to the column and eluted in the same way. The eluted fractions were counted in the usual way, and also when the Geiger tube was shielded by a piece of paper, which absorbed $>$ 95% of the ^{35}S radiation but only about 25% of the emission from ^{32}P. By means of this differential counting it was found that $\sim$ 70% of both dextran sulfate and virus pass through the column without absorption (Fig. 7C and D). This result indicates that virus is rather adsorbed to the dextran sulfate in the buffer than to the Sephadex sulfate in the column.

Discussion. The results presented on the effect of dextran sulfate on adsorption of L Sc 2ab virus agree with the findings for EMC(4) and herpes simplex(7) and support the view that the effect is due to interference with the first step of virus-cell interaction, *i.e.*, the reversible attachment. This is further strengthened by the demonstration that virus, which is normally adsorbed by the cation exchanger Sephadex sulfate, is not adsorbed if mixed with dextran sulfate. It is also shown that dextran sulfate has little, if any, effect on the later stages of virus multiplication, *e.g.*, the eclipse and the events following the liberation of the viral nucleic acid.

The results regarding the effect of various amounts of dextran sulfate and dextran sulfate with varying molecular weight agree with other reports concerning the effect of ethylene maleic anhydrid copolymers on ECHO 9 virus(16). The increased inhibitory effect of high-molecular weight dextran sulfate, which occurs with enzymes as well(17) is probably due to the increase in acidity which follows the increase in molecular weight.

The reason for the adsorption of dextran sulfate sensitive virus strains, such as the L Sc 2ab, to this material may be accounted for by a difference in the viral protein between the virulent and the attenuated strains. This would also explain the differences in other respects, such as adsorption to ion exchanger(18). This aspect will be published elsewhere(12).

Summary. The effect of dextran sulfate on the poliovirus strain L Sc 2ab increases up to concentrations of $\sim$ 100 μg/ml. The effect of low-molecular-weight dextran sulfate is slight, but increases rapidly when the molecular weight rises above 100,000, probably reaching a steady state with molecular weights above 2,000,000. Dextran sulfate was shown to inhibit the reversible attachment of virus and had little effect on the subsequent steps of virus-cell interaction. It is also demonstrated that the inhibitory effect of dextran sulfate is probably due to absorption of sensitive virus to the polyanion.

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Received July 14, 1964. P.S.E.B.M., 1965, v118.

Titration of Live Measles and Smallpox Vaccines by Jet Inoculation of Susceptible Children. (29753)

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The results of a series of studies conducted in the United States and in the Republic of Upper Volta indicated that jet-inoculated live measles and smallpox vaccines immunized 97 to 100% of susceptible recipients(1). Most of these children were inoculated with a fixed dose of each attenuated virus, 10^4 tissue culture infectious $dose_{50}$ (ID_{50}) of measles virus and 10^6 chorio-allantoic membrane (CAM) infectious units of vaccinia virus, either singly or in combination.

The present study was designed to determine the effective dose range of these vaccines when given by jet injection as compared to administration by conventional techniques. In addition, an attempt was made to correlate the results of virus titrations in man with those obtained when identical samples of each of the vaccines were tested in the laboratory in conventional potency assay systems.

The clinical work was carried out in Bobo-Dioulasso, Upper Volta, with the assistance of the Upper Volta Health Ministry; laboratory tests were performed at the National Institutes of Health, Bethesda, Md.

Materials and methods. Vaccines. Lyophilized Enders "B" level(2) live attenuated measles vaccine was purchased commercially* in bottles containing fifty 0.5 ml human doses. The Division of Biologics Standards Reference Smallpox Vaccine Lot 2, a lyophilized calf lymph preparation, was used. Vaccines were refrigerated during air shipment to Upper Volta and subsequent storage until used, *i.e.*, a period of about 2 weeks, and were rehydrated with sterile distilled water immediately prior to use. Ten-fold dilutions of freshly reconstituted vaccines were made with 2 ml syringes by serial transfer of 2.0 ml volumes to rubber-stoppered vaccine bottles, each containing 18.0 ml of diluent (medium 199 containing 5% human serum albumin and antibiotics). Rehydrated and diluted vaccines were refrigerated and used within 2 hours.

Vaccinations. The 216 children, age 8 months to 6 years, participating in the study had no history of previous experience with the virus to be administered. Each vaccine dilution was inoculated into 10 or more children. The automatic jet injection apparatus† was regulated to deliver 0.5 ml of vaccine. The operating directions furnished with the apparatus were followed except for smallpox vaccination, where a short length of stiff plastic tubing was fitted over the jet gun nozzle, creating a sleeve that extended 4 mm beyond the nozzle orifice. This simple modification, described in our earlier studies(1), increased the amount of vaccine deposited intradermally. Conventional measles immunization was accomplished by subcutaneous inoculation of 0.5 ml volumes with disposable 2 ml syringes and 21-gauge, 1 inch needles. Percutaneous smallpox vaccination was done with the multiple pressure technique using

* Merck Sharp & Dohme Lot 94216/7064B

† Scientific Instruments Mfg. Corp., New York City.