Effect of Filtration Procedures on Immunogenic Activity and Safety of Formalinized Poliomyelitis Vaccine (Salk Type). (24598)

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Methods for production of formalinized poliomyelitis vaccine on a semi-industrial scale have been described by us(1). Filtration of virus pools before formaldehyde-inactivation through fritted glass filters proved inadequate in production of safe vaccine pools; Seitz filtration of virus suspensions before inactivation was found to be essential for preparation of non-infectious vaccine. In preparation of vaccine pools, we followed Minimum Requirements of U.S. Public Health Service(2) in adding a 2nd Seitz filtration during formaldehyde-inactivation process. We felt, however, that this filtration is deleterious to the antigen and reduces significantly the immunogenic activity of pools treated in this way. To test the effect of filtrations during inactivation process, experiments were carried out to replace or omit the 2nd Seitz filtration. The results of these experiments and their effect on safety and antigenicity of poliomyelitis vaccines are here reported.

Materials and methods. Preparation of tissue cultures and virus suspensions, and safety and potency testing of monovalent pools were carried out according to methods described earlier(1). Changes were introduced in processing and in inactivation of some monovalent pools, as follows: 1. *Filtration of crude live virus suspensions before inactivation.* Two changes were introduced in these filtrations: a) Replacement of Corning fritted glass tubular filters, porosities Medium to Ultrafine, by Seitz clarifying pads, grades K3 and K7 (Seitz Werke, Germany). The filter used was Perfectum filter, model LF 280T (Popper & Sons, U.S.A.) which allows simultaneous filtration through 2 filter pads of 14 cm diameter. The Perfectum filter was adapted for continuous filtration by removal of pressure gauge and addition of stainless steel baffle to avoid rupture of pad by the incoming fluid. Virus suspension was introduced continuously into inlet tube of sterile filter. Sterile air under pressure of 0.3 atm. was applied to the carboy which served as feeding vessel. Filtration through Seitz clarifying pads was preceded by filtration through cotton filter and tubular filter, porosity coarse, and followed by filtration through Seitz sterilizing (STl) pad. b) Washing procedure for Seitz sterilizing pads as well as clarifying pads was altered; instead of Hanks solution, Medium 199 (pH 7.0) was used to wash the pads before passing the virus suspension. 1800 ml of Medium 199 were passed through the pad before filtration of the virus suspension. 2. *Filtration during inactivation process.* a) A number of pools were divided on 4th day of inactivation into 2 approximately equal parts. One part was filtered through washed Seitz sterilizing pad, while the other was passed through Fine-Ultrafine Corning glass tubular filters. Both aliquots were further treated as separate pools; they were inactivated for additional 8 days, and tested for safety and potency separately. b) In several pools filtration on 4th day of inactivation was omitted entirely. 3. *Total time of inactivation* of all monovalent pools was reduced from 14 to 12 days. Large samples, of at least 500 ml, were withdrawn for safety testing on 9th and 12th days of inactivation.

Results. Titers of Seitz filtrates obtained by passing suspensions through Hanks-washed pads are reduced, the loss of virus titer ranging from 0.3 to 0.8 log. Filtrates, passed through Seitz pads washed with M199 prior to filtering, show almost no loss in titer (0.1 to 0.25 log differences being regarded as within experimental error limit of test).

Table I summarizes results in which 4 monovalent pools were each divided, on 4th day of inactivation, into 2 parts, one of which was passed through Seitz filter pad washed with M199, and the other through a Fine-

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TABLE I. Potency of Vaccine Which Had Been Filtered on 4th Day of Inactivation.

Pool No.	Type	Vol (liters) of Seitz filtrate before inactivation	Potency tests*: Guinea pig extinction end point† FUF	Guinea pig extinction end point† Seitz	Guinea pig serum end point‡ FUF	Guinea pig serum end point‡ Seitz	Chick extinction end point† FUF	Chick extinction end point† Seitz	Chick serum end point‡ FUF	Chick serum end point‡ Seitz
201	I	16	2.6	.9	80	5	.9	.0	21	3
202	I	19	1.8	1.8						
206	I	18	3.2	2.6	517	221			291	313
U	III	16	2.5	.0	9	0	1.1	.0	40	2

* FUF = 2nd filtration carried out through Fine-Ultrafine glass filter. Seitz = 2nd filtration carried out through a washed Seitz filter.

† Extinction end point = $\log_{10}$ of 50% titer of antigen.

‡ Serum end point = mean antibody titer of sera from guinea pigs or chicks inoculated with undiluted vaccine.

Ultrafine Corning tubular glass filter. Safety tests carried out on large samples, ranging 400-700 ml, taken 3 days prior and at end of inactivation period, from Seitz filtered as well as from Fine-Ultrafine glass filtered aliquots, were unequivocal: no live virus was isolated from any of these samples nor from subcultures carried out at one, 2, and 3 week intervals. Results of potency tests showed in 2 instances (pools 201 and U) significant differences in antigenicity. The guinea pig extinction point was 50 and 300 times higher, respectively, for aliquots filtered through Fine-Ultrafine filters than for those filtered through Seitz filters. This difference is also evident from guinea pig serum end point titers, as well as from chick extinction and serum end point titers. In the monovalent Type I pool, 202, no loss of potency seemed to have resulted from filtration through the Seitz filter. The 2 aliquots of pool 206 also showed no marked difference, whether filtered through Seitz or Fine-Ultrafine filter. Thus it appears that filtration through a Seitz filter on 4th day may sometimes affect immunogenic activity of the vaccine. Filtration through a Fine-Ultrafine filter seems to do no harm to the antigenicity of the material.

A number of monovalent pools were left unfiltered during inactivation period and the results of safety and potency tests carried out on such pools are presented in Table II. For comparison, results are given also on safety and potency tests carried out on pools which have undergone Seitz filtration on 4th day of inactivation. Samples of 300-800 ml, withdrawn from all these pools 3 days prior and at the end of inactivation period, contained no live poliovirus. Guinea pig extinction points (expressed in $\log_{10}$ units) were very high for pools in which the second filtration was omitted; they ranged from 2.2 to 3.2, and in most instances were higher than 2.5. Guinea pig serum end point titers ranged from 60 to over 1000. Antigenicity of monovalent pools, which had been filtered through Seitz filter on 4th day of inactivation was much lower, especially for Type I pools.

To strengthen confidence in the absolute safety of pools in which the 2nd Seitz filtration was omitted, additional samples were withdrawn from several pools on 7th day of inactivation (5 days prior to end of inactivation period) and tested for safety in the usual manner. Four such 7th day samples of 300-500 ml each were tested and found free of live poliovirus. These findings, in addition to consistent negative safety tests on the 9 monovalent pools included in Table II, show that omission of 2nd Seitz filtration was not at the expense of safety. After incorporation of these monovalent pools into trivalent vaccines, absence of live poliovirus was further proved by safety tests carried out in tissue culture on large amounts of trivalent vaccines (over 1500 ml), by safety tests in cortisone-treated monkeys, and by use of such vaccines in large numbers of infants and children, many of whom were triply negative for poliomyelitis antibodies at time of vaccination.

Discussion. To ensure absolute safety of formalinized poliomyelitis vaccine, the U. S. Public Health Service Technical Committee on Poliomyelitis Vaccine recommended dou-

TABLE II. Results of Safety and Potency Tests on Monovalent Pools in Which Filtration during Formaldehyde Inactivation Process Was Omitted, as Compared with Lots Filtered through Washed Seitz Pads.

Pool No.	Type	Vol (liters) of Seitz filtrate before inactivation	Filtration on 4th day of inactivation	Results of safety tests* a	b	Guinea pig extinction point	Guinea pig serum end point
200	I	4.3	Omitted	300	750	2.2	70
209	I	18.0		400	500	3.1	60
212	I	13.5			800	>2.5	160
215	I	15.5		300	450	>2.0	
216	I	15.0		500	350	>2.0	
210	II	12.0		600	600	3.2	≥1000
213	II	12.0		400	650	3.1	
208	III	6.0		650	550	>2.5	
211	III	13.0		450	350	2.9	
190	I	19.0	Seitz	350	500	.6	
193	I	17.0		600	700	.9	
195	I	16.0		450	600	1.3	
191	II	16.0		500	500	1.7	
194	II	13.0		550	600	1.4	
197	II	10.0		450	700	2.6	
192	III	14.0		500	550	1.3	
198	III	15.0		450	350	1.8	

* Milliliters tested up to 14 days' subculture and found negative. a = sample taken 3 days before end of inactivation period; b = sample taken at end of inactivation period.

ble Seitz filtration, the first before addition of formalin, and the second during the inactivation process(3). This recommendation was based on experience gained in various laboratories in United States, which showed that the second filtration through Seitz filter pads is much superior to filtration through glass filters in yielding a product free of detectable poliovirus(4). Experience in the field on use of such doubly-filtered vaccines (commercial U.S.A. vaccines) in children proved recently to give poor antibody responses after basic 2-dose immunization especially for Type I and III components(5,6 and personal communications). Immunogenic activity of such vaccines in triply negative children proved to be much inferior to that of the experimental vaccines used in the past by Salk(7).

In our own experience, immunogenic activity of doubly-filtered vaccines was low, especially for Types I and III. Guinea pig extinction points (expressed in $\log_{10}$ units) of such doubly filtered pools ranged from 0.6 to 1.3 for Type I, 1.4 to 2.6 for Type II, and 1.3 to 1.8 for Type III poliovirus. Infants and children, receiving 2 basic inoculations of 0.5 to 1.0 ml each of such vaccines, showed conversion in only 10 to 20%. The evidence suggested that Seitz filtration during inactivation process removed large amounts of antigen (especially Types I and III). The present report confirms this assumption. Monovalent pools in which the 2nd Seitz filtration was either omitted or replaced by filtration through a Fine-Ultrafine filter, proved to be of high antigenicity in guinea pigs and also in triply negative infants and children (our unpublished results). The safety of such treated pools was shown in our data as unequivocal, all pools tested were free of live poliovirus. However, whether omission of a 2nd Seitz filtration can consistently result in safe vaccines needs further confirmation on a larger number of pools, and possibly on larger volumes of vaccine.

Summary. Seitz filtration of poliomyelitis vaccine pools during formaldehyde-inactivation process was found to lower their immunogenic activity. Monovalent vaccine pools in which filtration through Seitz filter pads during inactivation process was either omitted or replaced by filtration through fritted glass filters, proved to be of high antigenicity both in guinea pigs and in triply negative children. Routine safety tests in tissue culture carried out on such pools were consistently negative.

Additional proof of the safety of such pools was provided by absence of live poliovirus from large samples withdrawn as much as 5 days prior to end of inactivation period, and also by use of these vaccines in the field.

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Inhibition of Pepsin and Lysozyme by Acidic Polymers (24599)

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A series of acidic macromolecules arising from polymerization of phenols, or phenolic carboxylic or sulfonic acids have been shown to inhibit ribonuclease and other hydrolytic enzymes(1). Certain members of the series likewise showed activity in suppressing formation of gastric ulcers in Shay rats.* We were interested to learn if this biological activity could be correlated with inhibitory power against pepsin and/or lysozyme. Levey and Sheinfeld(2) and Marini and Levey(3) reported that chondroitin sulfuric acid inhibits pepsin *in vitro*, and that this polymeric acid also reduces the number of ulcers formed in the Shay rat stomach. Whereas pepsin has long been implicated in the etiology of gastric ulcers(4) the participation of lysozyme was suggested by K. Meyer *et al.*(5) and has been doubted by Reifenstein *et al.*(6), who feel that the increased tissue lysozyme found in the margins of active, but not of chronic, gastric ulcers represents a tissue response to the lesions rather than an etiological factor. Reports on inhibition of lysozyme action by acidic polymers and related compounds have appeared from several laboratories; the substances include hyaluronic acid(7), treburon and heparin(8,9) alkyl sulfates, fatty acids and alcohols(5,10,11). *Polymers.* The 4 polymers discussed in this paper were prepared as described(1) by condensation of I. Formaldehyde and hydroquinone sulfonic acid. II. Formaldehyde and 3,4-dihydroxybenzene sulfonic acid. III. Furfural and hydroquinone sulfonic acid. IV. Acetaldehyde and hydroquinone sulfonic acid.

Methods. Measurement of lysozyme inhibition. The criterion most widely used to evaluate lysozyme activity, *viz.*, clearing of a suspension of *M. lysodeicticus*(12,13,14,15) probably is not reliable(16) when the reaction is allowed to proceed at pH2 followed by alkalinization(17). Optical methods appeared unattractive for study of inhibition by colored substances. Enzyme activity was measured by viscosimetry according to Meyer and Hahnel(18). The substrate (80 mg) was dissolved in 30 ml of McIlvaine's buffer (pH 2.15) containing 0.2 M sodium chloride. A small amount of gummy residue was removed by centrifugation after the solution had been agitated for one hour. The solution may be kept for at least 8 hours at room temperature without change in viscosity. Twice recrystallized lysozyme (Worthington Biochemical Corp.) was dissolved in 0.9% sodium chloride to give 500 μg/ml. The polyphenolsulfonic acids used as inhibitors were dissolved in distilled water. Viscosimetry was patterned after the procedure used by Northrop for measurement of peptic activity(19). Four-ml aliquots of substrate solution were placed into 50-ml test tubes. To each tube, 0.5 ml

* Dr. A. Plummer of this Department observed the anti-ulcer effect in Shay rats. We thank Dr. Plummer for permission to refer to his results.