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Received February 10, 1964. P.S.E.B.M., 1964, v116.

Separation of SV40 from Poliovirus by Extraction with l-Butanol. (29234)

H. G. MACMORINE, F. T. SHIMADA, B. KAMOCKI, S. KUNIGELIS AND W. PARISIUS
(Introduced by J. K. W. Ferguson)
Connaught Medical Research Laboratories, University of Toronto, Toronto, Canada

During experimental work on the concentration and purification of poliovirus using the method described by Schwerdt and Schaffer(1), it was observed that the levels of adventitious SV40 were markedly reduced. A detailed study of the level of SV40 at each step in the process revealed that a sharp reduction in infectious SV40 took place when the first aqueous concentrate was washed with 1-butanol.

In early work on the purification of poliovirus of CNS origin, Bachrach and Schwerdt (2) applied Morton's procedure(3) of butanol extraction for removal of non-viral protein. At pH values between 4.5 and 9.0 and ionic strength of 1.0, the virus remained unaltered in the aqueous layer.

The purpose of the present study is to determine whether the observed reduction of infectious SV40 in the presence of poliovirus was due to selective inactivation or to selective removal from the aqueous phase by the 1-butanol.

Three sets of experiments were done. The first was an examination of the effects of butanol on culture fluids high in SV40 but free from poliovirus. The second was to confirm that neither a virulent nor an attenuated strain of poliovirus was inactivated by treatment with butanol. In the third set, pools of attenuated virus vaccine (Sabin) of all 3 poliovirus types, containing adventitious SV40, were extracted with the solvent.

Also, preliminary observations were made on the effect of butanol extraction on a number of other viruses, particularly those commonly associated with tissue cultures of simian origin.

Materials and methods. Pools of SV40 of high titer were prepared by infecting trypsin-dispersed Cercopithecus monkey kidney cells in suspension, planting the infected cells and incubating the cultures for 8 days. After 3 cycles of freezing and thawing the fluids were harvested. The strain of SV40 used was isolated in these laboratories from a culture of Cercopithecus monkey kidney. It was identified by cross-neutralization with immune rabbit serum prepared against Dr. Hilleman's Strain 45-54(4). When grown in monolayers of Cercopithecus monkey kidney cultures it produces the cytopathic effect typical of Hilleman's vacuolating agent.

The poliovirus cultures, free from SV40, were from routine lots prepared for either Salk or Sabin vaccine. Mixed virus pools of poliovirus and SV40 were obtained from lots of Sabin vaccine rejected because of their contamination with SV40. The SV40 found in these pools was identified serologically.

The various strains of simian virus were isolated from routine tissue culture samples taken during the preparation of both Salk and Sabin vaccines. Three of these strains were submitted to Dr. Hull (Eli Lilly & Co.) for identification. The others are serologically different and are designated by our own simian virus numbers. Pools of high titer have been prepared in monolayer cultures.

Titrations of SV40 were carried out by inoculating fully grown roller-tube cultures of trypsin-dispersed kidney cells from Cerco-

pithecus monkeys. Where high titers were expected, 5 tubes per dilution at half or full log intervals were used. Microscopic examinations of the tubes were made over a 14-day period. It should be pointed out that the variability of replicate assays of this virus is much higher than that found for viruses giving strong and definite cytopathic effects. Based on an analysis of 118 titrations on a single virus preparation, the reliability of a single titration at the 95% confidence limits was found to be $\pm$ 0.8 logs. In certain tests, where the log titer was below zero, several hundred tubes were inoculated with undiluted fluid. The poliovirus in mixed virus preparations was neutralized, prior to inoculation, by addition of an equal volume of type specific antipolio rabbit serum and incubation for 2 hours at 37°C.

Polio and other viruses which produce typical effects in cultures of Rhesus monkey kidneys were assayed by the conventional methods using Rhesus monkey kidney cells.

Normal butyl alcohol (l-butanol), certified chemical, was obtained from the Fisher Scientific Co.

To investigate the removal of SV40 from tissue culture fluids, four 100 ml aliquots of crude SV40 virus fluid were shaken vigorously for 15 minutes with equal volumes of l-butanol at approximately 4°C. After 15 minutes' centrifugation at 1200 r.p.m. two distinct phases separated, a lower aqueous layer and an upper butanol layer containing some gel on top of the sharp interface. The aqueous layer was withdrawn and reextracted with butanol as before. The aqueous volume was made up to 100 ml with tissue culture medium. To examine the possibility of inactivation of SV40 by butanol, control samples of the same starting material were brought to near-saturation by addition of 7% solvent. After vigorous shaking, these mixtures gave single phases. They were kept at the same temperature and for the same period as those used for the extraction procedures.

An attempt was made to recover SV40 from the collected butanol and interphase material. To the pooled solvent washes, 30 ml of tissue culture medium and 75 ml of amyl acetate were added. The mixture was shaken for 10 minutes and centrifuged for 15 minutes at 1200 r.p.m. This yielded an aqueous phase of approximately 35 ml. A second addition of medium and amyl acetate was made and extraction carried out as before. The pooled aqueous phases were made up to 100 ml with medium.

When all parts of the experiment were completed, samples were diluted for assay and added to roller-tube cultures of Cercopithecus monkey kidneys. The titer of the starting fluid was sufficiently high that dilution to the end-point of infectivity gave nontoxic samples without removal of the butanol.

To determine whether the effect of saturation with butanol differed from that of emulsification with excess solvent the following experiment was carried out. To 20 ml of high-titer SV40 tissue culture fluid 20 ml of l-butanol were added. The mixture was kept under continuous agitation so that the phases did not separate. Samples were withdrawn at intervals and diluted in a sufficient volume of tissue culture medium to give one-phase aqueous solutions.

Two experiments on the effect of butanol on poliovirus were carried out as follows. In the first, 250 ml of a Type 2 attenuated virus pool, diluted 1:10 in tissue culture medium, were extracted with an equal volume of butanol and sampled at intervals. In the second, a pool of Type 2 virulent virus (M.E.F.1) was extracted with an equal volume of solvent. Both of these extractions were done on unfiltered fluids.

For selective removal of SV40 from attenuated poliovirus vaccines, pools of each of the 3 poliovirus types of Sabin vaccine, high in adventitious SV40, were used. One liter of each fluid was stirred for one hour with an equal volume of l-butanol at 4°C. After centrifugation for 30 minutes at 1800 r.p.m., the aqueous layer was withdrawn. As aqueous solutions saturated with l-butanol are somewhat toxic to tissue cultures, the butanol was removed by dialyzing for 24 hours against distilled water and for 16 hours against tissue culture medium.

The dialyzed fluids were passed through

TABLE I. Extraction of SV40 Tissue Culture Fluids with l-Butanol.

	Titer ($-\log_{10}$/0.2 ml) Experiment No.			
	1	2	3	4
Starting fluid	5.0	—	5.3	5.3
After 2 extractions with equal vol of l-butanol	1.0	1.0	1.5	1.3
Recovered from butanol layer	—	2.5	2.7	2.5
Saturated with l-butanol (no extraction)	4.6	4.5	5.5	5.1

ST1 Seitz filter pads prewashed with a solution of gelatin.

The effect of l-butanol on 11 viruses other than polio and SV40 was studied as follows: Ten or 20 ml samples of the viruses listed in Table IV were stirred for 30 minutes with equal volumes of l-butanol. After centrifugation for 30 minutes at 1400 r.p.m. the aqueous layers were withdrawn and dialyzed against distilled water for 24 hours and against Hanks' balanced salt solution for a further 24 hours.

Results and discussion. The results in Table I show that 2 extractions with equal volumes of l-butanol lowered the titer of SV40 in the aqueous layer by approximately 4 logs. That the action of the solvent on SV40 was one of extraction into the solvent phase rather than inactivation was demonstrated in 2 ways. First, an addition of 7% butanol did not reduce the infectious titer significantly. Second, an attempted recovery of SV40 from the solvent layer, although yielding much less than the theoretical amount of virus, showed that live SV40 had been transferred to and survived in the butanol phase. The method used removed only a fraction of the dissolved water. In addition, samples removed from an emulsion of equal parts of virus fluid and butanol were found to be unaltered in virus concentration.

TABLE II. Extractions of Poliovirus Tissue Culture Fluids with l-Butanol.

Time of sampling (min)	Titers ($-\log_{10}$/0.5 ml) Type 2 virus (Sabin)	Type 2 virus (M.E.F.1)
0	7.0	6.9
15	6.9	6.7
30	7.0	—
45	6.8	—
60	6.9	6.8

Sweet and Hilleman(5) have reported that SV40 resisted treatment for 18 hours with an equal volume of diethyl ether, an observation repeated by Hsiung and Gaylord(6). We have confirmed this observation on the resistance of SV40 to ether and have found also that the infectivity of this virus is not affected by treatment with chloroform or fluorocarbon.

The results in Table II confirm the earlier observation of Schwerdt and Schaffer. No inactivation of either a virulent or an attenuated strain of poliovirus was detected.

When samples of Sabin polio vaccine, high in adventitious SV40, were extracted with an equal volume of l-butanol and the extracted fluid passed through a Seitz filter, the concentration of the contaminating virus was reduced to a very low level (Table III). The small loss of poliovirus and part of the reduction of SV40 can, perhaps, be attributed to removal on filtration.

Preliminary experiments on the action of l-butanol on 11 viruses, other than polio and SV40, showed that the infectious titer in the aqueous layer was reduced in all cases (Table IV). These results are of an exploratory nature only but they indicate that treatment with butanol either inactivates or removes large amounts of the viruses tested. A few subsequent tests have shown that the action on Herpes strain H_4-16 is one of inactivation.

Summary. Poliovirus and SV40 in tissue culture fluids are neither removed nor inactivated by extraction with diethyl ether, chloroform or fluorocarbon. However, similar treatment with l-butanol removes large amounts of SV40 from aqueous suspensions. The infectious titer of poliovirus is practically unaffected by the same procedure. That the action of l-butanol on SV40 is one of extraction into the solvent phase rather than virus inactivation has been demonstrated in two ways. First, an addition of 1-butanol sufficient to saturate the aqueous suspension did not reduce the infectious titer significantly. Second, the virus was recovered quantitatively from an emulsion of equal volumes of l-butanol and virus fluid. However, when the

TABLE III. l-Butanol Extraction of Attenuated Poliovirus (Sabin).

SV40

Sample	Pre-treatment (titers $-\log_{10}$/0.5 ml)	Post-butanol extraction* Immediately after: Pos tubes /Total	Immediately after: Total vol tested (ml)	6 mo after (1): Pos tubes /Total	6 mo after (2): Pos tubes /Total	6 mo after: Total vol tested (ml)
Type 1	≤1.1	0/99	24.75	0/154	1/100	88.5
" 2	4.9	10/323	80.75	11/185	6/97	94.75
" 3	4.5	9/418	104.5	32/147	5/93	83.25

Poliovirus

(plaque forming units × 10^7/0.5 ml)

Sample	Pre-treatment	Post-butanol extraction
Type 1	4.51	2.35
" 2	4.00	1.93
" 3	6.50	5.17

* All tests done on undiluted virus fluid.

(1) .25 ml of sample per tube. (2) .5 ml of sample per tube.

TABLE IV. Effect of l-Butanol on Viruses Other Than Polio and SV40.

Virus: C.M.R.L. No.	Virus: Hull's No.	Titer ($-\log_{10}$/0.5 ml) pre-treatment	Virus in aqueous phase post-butanol extraction
S.V. 71–56	S.V. 4	5.9–6.3	Titer 2.5
" 31–55	S.V. 15 or 17	7.5–7.9	1.0 ml undil negative
" 55–56	S.V. 12	5.3–5.7	1.0 *idem*
" 114–56	—	6.3–6.5	1.0 "
" 101–58	—	6.1–6.3	1.0 "
" 242–59	—	2.0–2.3	1.0 "
" 35–60	—	6.1–6.3	1.0 "
B virus	—	6.5	1.0 "
B virus	—	6.1	15.0 "
Herpes H_4-16	—	7.45	1.0 "
Herpes H_4-16	—	6.1	15.0 "
Foamy virus	—	1.5	5.0 "
Measles 84F	—	2.9	5.0 "

aqueous and solvent phases were separated, the titer in the aqueous layer was reduced by at least 4 logs (base 10). Extraction of samples of rejected Sabin polio vaccine, high in adventitious SV40, with an equal volume of l-butanol and subsequent Seitz filtration reduced the concentration of the contaminating virus to a very low level. The infectious titer of 11 viruses, other than polio and SV40, was reduced on extraction with l-butanol. It has not been determined whether the effect on these viruses was due to inactivation or removal from the aqueous layer.

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Received February 12, 1964. P.S.E.B.M., 1964, v116.