PRESERVATION OF THE POLIOMYELITIS VIRUS WHEN DRIED BY THE SUBLIMATION METHOD (PRELIMINARY COMMUNICATION)

L. S. Lozovskaya

From the Moscow Research Institute for Preparations against Poliomyelitis (Scientific Director — Prof. V. D. Solov'ev)

(Recieved September 22, 1958. Presented by Active Member AMN SSSR G. V. Vygodchik)

The most convenient and effective of the methods known at the present time for stabilizing the biological properties of bacterial and viral strains can be considered with justification to be that of storage of the preparations in vacuo in a dried and frozen state. This method has been found to be most valuable when working with relatively unstable viruses.

This is all the more surprising when it is remembered that the first, unsuccessful attempts to dry the poliomyelitis virus led to the widespread belief among virologists that it was impossible to preserve this virus in a dried state. Thus, in a survey of the research on the physical and chemical properties of the poliomyelitis virus, Gard writes: "It has been known for a long time that lyophilization is a bad method of preservation of the virus of poliomyelitis" [1]. Even in the research which led Gard to this conclusion, however, there were findings that in the dried preparations, residual activity of the order of 1% of the original was preserved in the dried preparations [4, 6]. It was later shown that the survival of the poliomyelitis virus after drying could be increased if the sublimation was carried out at −20°, and to the suspension of virus was added sodium thioglycolate, peptone or cystein [3]. In the work referred to, the material dried was a suspension of the brain of mice inoculated with type II and III poliomyelitis viruses; the residual activity of the dried preparations amounted to 32% of that of the original.

Since the maximal yield of poliomyelitis virus is obtained by cultivation in trypsinized monkey tissues, we thought it desirable to study the conditions for drying the virus in culture fluids.

EXPERIMENTAL METHOD

In the experiments we used type II poliomyelitis virus (strain P 712 [5]), sent to the USSR by Prof. A. Sabin. The virus was cultivated in the renal epithelium of monkeys (Macacus rhesus) in Parker 199 medium or in a 0.5% solution of lactalbumin hydrolyzate with 2% calf serum. After complete degeneration of the tissue, which occurred on the 3rd-4th day after inoculation, the culture fluid was collected and allowed to stand for 18-20 hours at 4°. The transparent supernatant fluid was decanted into test tubes which were kept at 4° throughout the experiment for the control investigations. The same material was mixed with a protective medium in different proportions and poured into ampules for drying. For a protective medium we used the yolk of a hen's egg, suspended in 20% saccharose solution. In the choice of medium we were guided by the indications of M. I. Sokolov, who successfully dried influenza virus in a yolk-saccharose medium [2], and also by our own findings in connection with the preservation of measle's virusin dried preparations with the same medium.

In the present paper we give details of virus preparations, vacuum-dried in the laboratory of the Moscow City Bacteriological Institute.* The preparations were dried in a collector apparatus under a vacuum of 25μ

* The author expresses his gratitude to Prof. Blankov for providing the facilities for the work to be done in the vacuum laboratory of the Moscow City Bacteriological Institute.

Preservation of the Biological Activity of the Poliomyelitis Virus in Dried Preparations in Relation to the Concentration of the Protective Medium

Expt. No.	Component concentration medium, volume %		Titer of virus (No. of TCD_{50}/ml)	Survival rate in %
	of yolk	of saccharose		
1	0	0	$10^{3.5}$	0.07
	0	10	$10^{3.5}$	0.07
	5.9	1.2	$10^{4.5}$	0.7
	12.5	2.5	$10^{4.5}$	7.0
	25	5	$10^{5.6}$	10.0
2	25	5	$10^{5.33}$[1]	6.7
	33.2	6.6	$10^{6.33}$[2]	66.7
3	25	5	$10^{4.67}$[1]	1.5
	33.2	6.6	$10^{5.33}$[2]	6.7

Notes: 1. Original titer of material subjected to drying in 1st experiment — $10^{6.66}$, in 2nd and 3rd experiments — $10^{6.45}$.
2. The 1st, 2nd and 3rd experiments were performed on the 2nd, 7th and 28th days after drying respectively.

and with a condenser temperature of —76°. The material, in a volume of 0.5-1.0 ml per ampule, was frozen at a temperature of —48°. During a period of 5-6 hours (from the beginning of sublimation) the external temperature was gradually raised to room temperature. The whole process of drying lasted 10 hours. The ampules were sealed in vacuo.

The residual activity of the virus in the material was determined immediately after drying (and later, after 1-4 weeks and a few months) by titration of the contents of the ampules on a single-layer culture of renal epithelium of monkeys in test tubes. The dried material was diluted with ten times its volume of medium 199 in relation to the original volume of virus in the ampule. All the subsequent tenfold dilutions were prepared with the same medium. 1 ml of the material was added to the tubes containing the tissue (from 4-10 pieces to each diluted sample) after removal from its nutrient medium. The titration results were read on the 7th day.

EXPERIMENTAL RESULTS

The results obtained confirmed the observations of the workers cited above, that the titer of poliomyelitis virus in the dried preparations was, as a rule, considerably below the original. When the virus was dried without protective medium, for example in a solution of lactalbumin hydrolyzate, it retained less than 0.1% of its original activity. By the use of a protective medium, however, it was possible to ensure an appreciable increase in the survival of the poliomyelitis virus (to 10-66%; see table). A definite relationship was revealed between the survival rate and the concentration of the protective medium.

The stability of dry preparations is known to be higher than that of liquid preparations even when the latter are stored in the frozen state. Nevertheless the conditions and length of storage of dry poliomyelitis virus preparations must form the subject of quite independent study. In the table (see footnotes) are given the results of titration of the same material after storage at —20° for 1 and 4 weeks. Under these circumstances a fall in the virus titer was observed, in one case by one logarithmic point, and in the other by 0.66 $\log_{10}$. Further storage of this material under the same conditions for 4 months did not lead to any perceptible lowering of the activity of the material.

The methods of storage of virus material is of pressing importance to practical virology. The presence of a stable, easily transportable preparation of poliomyelitis vaccine would solve the problem of a standard antigen for diagnostic laboratories. The ability to dry the poliomyelitis virus is very important in the production of a stable, living vaccine against poliomyelitis from nonpathogenic strains, one of which was used in the present research.

The virus is preserved by drying a phosphatide-rich suspension of infected brain [3] and in a culture medium, artificially enriched with yolk, containing over 50% of lipids; this suggests the protective role of the phosphatides during the drying of the virus and gives grounds for further research along the lines of the choice of the most desirable medium for drying.

SUMMARY

The possibility of drying of the poliomyelitis virus is of great significance for obtainingnot only the standard antigen for the diagnostic investigations, but also the stable live vaccine against poliomyelitis from nonpathogenic strains, one of which (type 11 P – 712 [5]) was employed in the present work. The survival of the poliomyelitis virus during the process of drying of the culture fluid media was studied. Positive results were obtained. When drying the culture in the presence of egg yolk and saccharose the titer of the dry preparations was on the average lower than in the initial material by 1 point of ogarythim, viz., the viability reached 10%. A definite relationship between the survival of the virus and the concentration of the protective medium was revealed.

LITERATURE CITED

[1] S. Gard, Poliomyelitis, pp. 183-200, Moscow, 1957 [Russian translation].

[2] M. I. Sokolov, Zhur. Mikrobiol. Epidemiol. i Imminobiol., No. 10, 57-62 (1953).

[3] L. M. Kraft and E. Pollard, Proc. Soc. Exper. Biol. a Med., 1954, v. 86, p. 306-309.

[4] M. Pollard, Proc. Soc. Exper. Biol. and Med., 1951, v. 78, pp. 388-392.

[5] A. B. Sabin, J.A.M.A., 1956, v. 162, pp. 1589-1596.

[6] M. Theiler and S. Gard, J. Exper. Med., 1940, v. 72, pp. 49-67.