Faculty of Pharmaceutical Sciences,
Nagasaki University,
Nagasaki-shi (Japan)

H. TANIYAMA
Y. SAWADA
T. KITAGAWA

1 T. SUGAI, *J. Antibiot., Ser. B*, 9 (1956) 170.
2 H. TANIYAMA AND S. TAKEMURA, *J. Pharm. Soc. Japan*, 77 (1957) 1210.
3 H. TANIYAMA, F. MIYOSHI AND K. KAGEYAMA, *J. Pharm. Soc. Japan*, 82 (1962) 87.
4 P. D. RESHETOV AND A. S. KHOKHLOV, *Antibiotiki*, 9 (3) (1964) 197.
5 A. S. KHOKHLOV AND P. D. RESHETOV, *J. Chromatogr.*, 14 (1964) 495.
6 O. I. VORONINA, K. I. SHUTOVA, I. I. TOVAROVA AND A. S. KHOKHLOV, *Antibiotiki*, 14 (1969) 1063.
7 P. D. RESHETOV, Z. A. JEGOROV AND A. S. KHOKHLOV, *Khim. Prir. Soedin. Akad. Nauk. Uz. SSR*, 2 (1965) 117; *C.A.*, 63 (1965) 2049g, 7258b.

Received December 21st, 1970

J. Chromatogr., 56 (1971) 360–362

CHROM. 5250

Treatment of controlled pore glass with poly(ethylene oxide) to prevent adsorption of rabies virus

When HALLER[1] in 1965 described a method for manufacturing porous glass of closely controlled pore size, it seemed apparent that this material would be valuable as a matrix for exclusion chromatography of biological substances. Since the pore sizes available (150–1500 Å) corresponded closely to the range of diameters of viruses and other subcellular particles, porous glass, with its dimensional stability, relative chemical inertness, and ease of sterilization, promised to be especially useful in the separation and purification of these substances.

Successful applications were in fact reported for several plant viruses, MS2 coliphage, and Kilham rat virus[2]. Purified preparations of ΦX-174, T4, and other bacteriophages were obtained in large quantities[3]. But an unknown number of investigators, including ourselves, found that other viruses adsorbed so avidly to the porous glass that exclusion chromatography was not operable. In our own prior experience, the list of viruses in this category included poliovirus, adenoviruses, vesicular exanthema virus, and the viruses of vaccinia, yellow fever, and rabies. Adsorption of poliovirus was reduced or eliminated by pretreatment of the glass with hemoglobin solutions followed by autoclaving to denature the hemoglobin *in situ*[4], but the same technique did not succeed in preventing adsorption of rabies virus. All of these experiments were conducted in buffered solutions at pH above the known or estimated isoelectric point of the virus, so the adsorption apparently entailed attachment of a negatively charged virion to a glass surface which itself is negative in net charge. Porous glass which had been surface-modified by covalent binding of propylamino groups*, conferring a net positive charge to the surface, did not adsorb

* Prepared by W. HALLER (1965 *et seq.*) by treatment of the dry glass powder with γ-amino propyltriethoxysilane.

poliovirus[4] in acetate buffer at pH 3 to 5, but rabies virus was either totally adsorbed or inactivated under these conditions.

We tested the effect of many different substances which might conceivably have altered the surface of the glass in such a way as to make it incapable of adsorbing rabies virus. Among these substances were cationic and non-ionic surfactants, quaternary ammonium compounds, peptides, alcohols, polyethylene glycols, polyvinylpyrrolidone, and choline, which were added in small amounts to the diluting fluid. Silanization of the dry glass powder by treatment with hexamethyldisilazane made the glass surface hydrophobic but failed to prevent virtually complete adsorption of virus.

We ultimately found, however, that low concentrations of poly(ethylene oxide), a polyether of molecular weight about 100,000** will prevent adsorption of rabies virus to porous glass of 1250 Å average pore diameter**. The polyether can either be

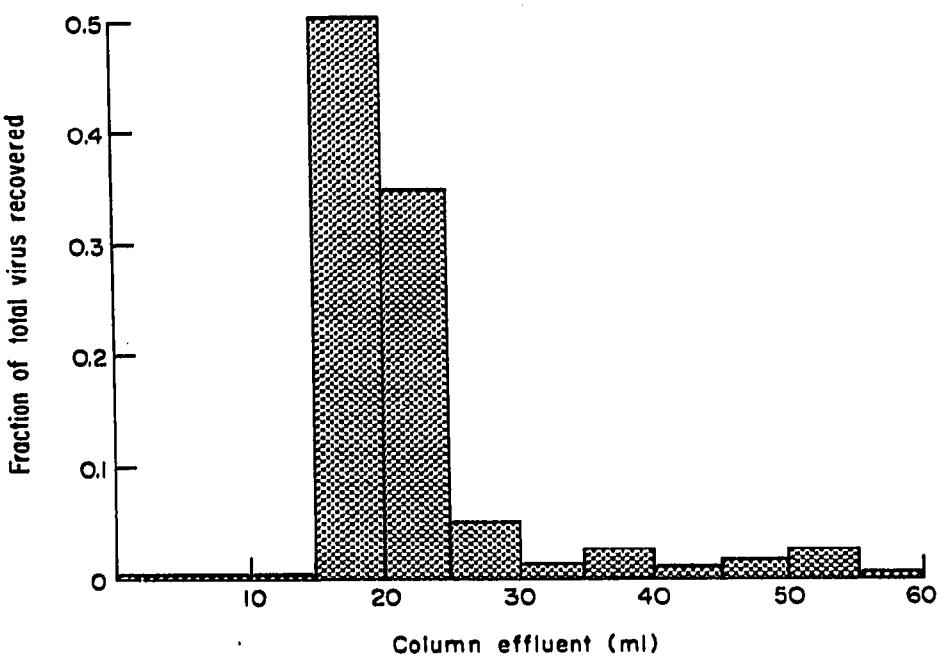

Fig. 1. Elution pattern of mouse-adapted rabies virus (fixed strain PV12) after passage over an 11 × 450 mm column of powdered glass of average pore diameter 1250 Å. The glass was conditioned by treatment with poly(ethylene oxide) (see text). Charge was 0.6 ml of 20% mouse brain suspension. Eluting fluid (isotonic phosphate buffered saline solution, pH 7.3) was pumped at a flow rate of 0.8 ml/min.

added to the eluting fluid at a concentration of 0.04%, or used to pre-condition the column by passage of one void volume of 0.4% solution followed by 5 or more void volumes of distilled water or buffered salt solution. As shown in Fig. 1, infectious rabies virus is excluded from the pores of 1250 Å porous glass and moves down the column as a single, relatively narrow zone. Substances of low molecular weight, presumably including undesirable constituents of brain tissue, are retarded in their passage through the porous glass and emerge as a discrete zone at eluant volumes of

* Polyox WSR N-10, Union Carbide Corporation, 270 Park Avenue, New York, N.Y. 10017, U.S.A.

** CPG 10-1250, Corning Glass Works, Corning, N.Y., U.S.A.

30 to 50 ml. Poly(ethylene oxide) of molecular weight 200,000 was also effective in preventing adsorption. Polyethers of lower molecular weight were not tested. Such compounds would be required to condition controlled pore glass of smaller pore size to ensure penetration.

A characteristic property of poly(ethylene oxide), in common with other polyethers, is a strong affinity for complex formation by hydrogen-bonding[5]. It seems probable that multiple hydrogen bonds between the polyether and the electronegative oxygen atoms in the –SiO– repeating structure of the glass account for the adsorption of poly(ethylene oxide). By competing efficiently for binding positions on the glass surface, the poly(ethylene oxide) thus effectively blocks adsorption of rabies virus.

This investigation was supported by NIH Contract 69-2049 sponsored by the U.S. Japan Program, Geographic Medicine Branch, National Institute of Allergy and Infectious Diseases. We thank Dr. W. HALLER, National Bureau of Standards for his helpful advice and Dr. EDWARD B. SELIGMANN, Jr., Division of Biologic Standards, National Institutes of Health, for providing the virus seed.

The University of Texas Medical School
at San Antonio, San Antonio, Texas 78229 (U.S.A.)

C. W. HIATT
ALEXIS SHELOKOV
EDWARD J. ROSENTHAL
JAMES M. GALIMORE

1 W. HALLER, *Nature*, 206 (1965) 693.
2 W. HALLER, *Virology*, 33 (1967) 740.
3 H. G. GSCHWENDER, W. HALLER AND P. H. HOFSCHNEIDER, *Biochim. Biophys. Acta*, 190 (1969) 460.
4 C. W. HIATT, N. NIELSEN AND W. HALLER, unpublished experiments (1965).
5 K. L. SMITH, A. E. WINSLOW AND D. E. PETERSON, *Ind. Eng. Chem.*, 51 (1959) 1361; M. RÖSCH, *Fette, Seifen, Anstrichm.*, 65 (1963) 223.

Received January 11th, 1971

J. Chromatogr., 56 (1971) 362–364