DEGRADATION OF POLIO VIRIONS BY MERCURIALS. [1]

Lennart Philipson
Institute of Virology, University of Uppsala,
Uppsala, Sweden.

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In previous communications (Philipson and Choppin, 1960, Choppin and Philipson, 1961, Philipson and Choppin, 1962) it has been reported that a number of sulfhydryl reagents are capable of inactivating the biological activities of picornaviruses. The thermal stability of poliovirus is also influenced by di- or tetrasulfides (Pohjanpelto, 1958, Pons, 1964) suggesting that the tertiary structure of the virus capsid is strengthened by disulfide bridges between adjacent sulfhydryl residues. The disintegration of poliovirus by guanidine, urea or sodium dodecyl sulfate at an acid pH gives an insoluble protein, which after oxidation yields protein subunits of uniform size with a sedimentation coefficient of 2S with a strong tendency to aggregation if detergents are omitted from the buffers (Maizel, 1963, Scharff et al., 1964). Since sulfhydryl compounds have no effect on the dissociation of poliovirus capsides even at high concentrations (Scharff et al., 1964) it appears unlikely that disulfide linkages are involved in the assembly of structural protein into capsomers, but with regard to the effect of sulfhydryl reagents on virus activity and thermal stability, thiolester

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bonds may be of importance. Evidence is here presented that mercurials at alkaline pH will split the poliovirus capsid with a concomitant release of high molecular weight RNA. A partial dissociation of poliovirus capsid in smaller protein fragments can also be observed.

Virus and virus assays. The E206 strain of poliovirus type 1 was grown in KB cells in Eagle's medium (Eagle, 1959) as previously described (Philipson and Lind, 1964). The virus was purified according to Levintow and Darnell (1960) and the final product contained less than 5 per cent of the total amount of radioactivity in RNase susceptible and acid soluble products. Plaque assays of intact virus and viral RNA were performed in HeLa cells as described elsewhere (Philipson and Lind, 1964).

Procedure for virus degradation. Purified and labeled poliovirus in 0.05 M NaCl in 0.005 M phosphate-buffer pH 7.2 was incubated with equal amounts of different 0.2 M buffers containing varying concentrations of $HgCl_2$ or parachloromercuri benzoate (PCMB). After 2 minutes of incubation at 37°C the mixture was diluted tenfold in 0.1 M TRIS pH 7.2 with or without 1 μg of RNase/ml and incubated for an additional 30 minutes at 37°C unless otherwise stated. After cooling to 0°C, trichloroacetic acid (TCA) (final concentration of 10 % w/v) and bovine serum albumin (100 μg/ml) were added. After centrifugation at 2000 g for 10 minutes supernate and sediment were assayed for radioactivity. The difference in radioactivity between RNase treated and control samples were used as an expression for the liberation of high molecular weight RNA.

Results and Discussion. Purified and ^{32}P-labeled poliovirus was incubated in borate, glycine and TRIS buffers at different pH in the presence of 10^{-4}M PCMB or $HgCl_2$. The amount of RNase sensitive radioactivity was subsequently determined. Fig. 1 shows that a spontaneous release of poliovirus RNA occurs only above pH 10. In the presence of 10^{-4}M $HgCl_2$

complete release of RNA was observed in borate buffers already at pH 8.5. In glycine buffers $HgCl_2$ had a less pronounced effect. With PCMB a moderate release of RNA was observed with TRIS buffer at pH 8.0, which gradually increased at higher pH.

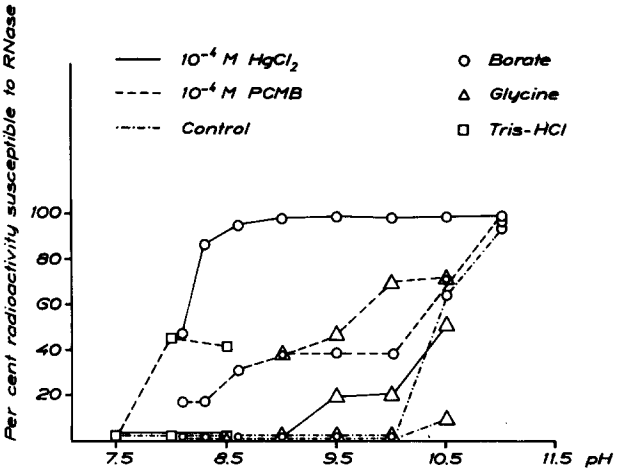

Fig. 1. RNase susceptible ^{32}P-activity of purified and labeled poliovirus type 1 after incubation at 37°C for 2 minutes in different buffers of alkaline pH.

When the effect of mercurials was tested on the infectivity of poliovirus type 1 it was found that 10^{-4}M $HgCl_2$ in borate buffers inactivated more than 4 log units of infectivity at pH 8.5. The effect of PCMB was the same as has been reported previously (Philipson and Choppin, 1960), 50 - 70 % of the virus infectivity being inactivated. In the absence of mercurials poliovirus infectivity was not significantly reduced at pH 10 for 2 minutes at 37°C.

To test if mercurials liberate infectious RNA from the virion, virus was mixed with equal amounts of 0.2 M borate buffer pH 8.0 with 10^{-4}M $HgCl_2$ and incubated for 5 minutes at 37°C, diluted in 2 M $MgSO_4$

and subsequently assayed for RNA activity with and without prior treatment with RNase (1 μg/ml) for 10 minutes at room temperature. As seen in Table 1 RNA infectivity is recovered with an efficiency of 0.05 % of the intact virus.

Table 1. Infectivity of RNA liberated from poliovirus type 1 degraded by mercury in borate buffer.

Virus infectivity of intact virus PFU/ml	Infectivity of degraded virus PFU/ml [x]		
	Plaque assay for intact virus	Assay for infectious RNA	
		Without RNase treatment	With RNase (1 μg /ml) treatment
1.1×10^7	1.2×10^1	3.8×10^4	1.0×10^1

[x] Virus was treated in 0.1 M borate buffer pH 8.0 with 10^{-4}M $HgCl_2$ for 5 minutes at 37°C.

Finally the effect of mercurials on the size of the capsid fragments was studied by gel chromatography of desintegrated ^{14}C-methionine labeled virus (0.1 M borate pH 8.5, 10^{-4}M $HgCl_2$, 15 minutes at 37°C) on Sephadex G-100 (AB Pharmacia, Uppsala, Sweden). ^{32}P-labeled virus was also investigated in the same manner. Eluting buffer was 0.1 M NaCl in 0.05 M phosphate buffer pH 7.2. Fig. 2 shows that the ^{32}P label of the desintegrated virus elutes with the void volume of the column as does intact virus. The ^{32}P-activity was, however, RNase susceptible in the virus preparation treated by $HgCl_2$ but resistant to this enzyme in the control.

The ^{14}C-methionine label elutes in the void volume together with the virus infectivity in the control but the major part of the ^{14}C-activity of the desintegrated material is retarded and elutes at a fraction of the volume of the gel (Kav) of 0.48, which corresponds to a molecular size similar to that of soybean trypsin inhibitor according

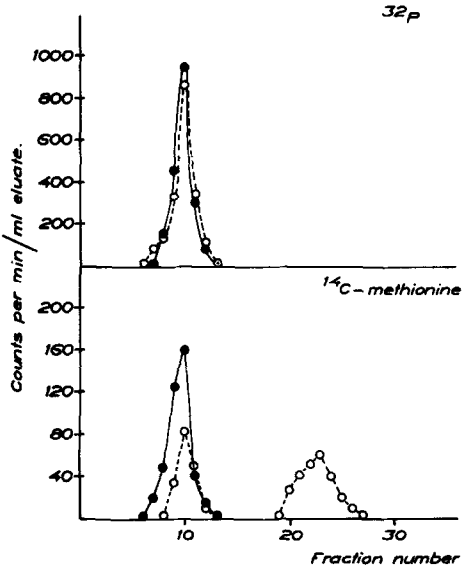

Fig. 2. Elution pattern of purified and labeled poliovirus type 1 treated for 15 minutes at 37°C at pH 8.5 in 0.1 M borate buffers with 10^{-4}M $HgCl_2$. o --- o and without $HgCl_2$ • - • from Sephadex G-100. Column size 1.8 x 29 cm and 2^2ml collected per fraction. Top row: ^{32}P-labeled virus. Bottom row: ^{14}C-methionine labeled virus.

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to Laurent and Killander (1964). Although the material is not homogeneous approximate molecular weight of 25,000 would be indicated by these results. The dissociation is, however, not complete since 35 per cent of the label elutes with the void volume.

The described method to degrade poliovirus might more specifically break the virus protein into subunits than high concentrations of urea or guanidine (Scharff et al., 1964). It remains to be seen if the polypeptide chains from virus degraded by mercurials are heterogeneous in electrophoresis as suggested for acid-degraded and oxidized poliovirus protein (Maizel, 1963). The results also indicate that thiolester bonds are involved in the assembly of protein subunits of the poliovirus capsid and this may help to elucidate the decoating mechanism of the minimal viruses.

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