

Purification of Influenza Viruses using Disulphide-Linked Immunosorbents Derived from Rabbit Antibody

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Abstract

Influenza viruses (A/Moscow/1019/65, A/England/344/68, PR/8-A/Hong Kong/68) were specifically adsorbed from crude egg grown suspensions by their homologous disulphide-linked immunosorbents prepared from the γ -globulin of rabbits immunized with influenza virus from calf-kidney tissue cultures. In batch experiments 20 mg of the appropriate 'non-avid' immunosorbents (from 4–7 ml of serum taken shortly after one dose of antigen) adsorbed approximately 6×10^9 , 3×10^9 and 3×10^{10} egg-bit infective dose₅₀ (approximately 1×10^{12} , 1.3×10^{12} and 1.9×10^{12} total particles as indicated by haemagglutination assays) of A/Moscow/1019/65, A/England/344/68 and PR/8-A/Hong Kong/68 respectively; and about 50 per cent of infective virus was eluted with alkaline buffers of pH 11.3, 12.0 and 12.5 respectively. After filtration to remove a trace of immunosorbent the eluted virus suspensions contained 40–91,000 HA/mg protein. 'Avid' immunosorbents (prepared from serum taken later after booster injections) had greater capacities (approximately 2–4 times) for infective virus than 'non-avid' immunosorbents but only 3–18 per cent of virus could be eluted from them. Both 'non-avid' and 'avid' immunosorbents adsorbed heterologous viruses of the same or different serotypes to those used to prepare the immunosorbents provided there was some similarity in either the haemagglutinin or neuraminidase antigens. However, their capacities for heterologous viruses were reduced in comparison to those for homologous viruses. Using a column containing a mixture of immunosorbent (100 mg) and Sephadex G-15 approximately 3.5×10^{12} total particles were adsorbed and 50 per cent eluted at each cycle at 5°C. Prior diafiltration of the crude virus suspensions was necessary to prevent column blockage but then the column could be recycled 10 times. The eluted virus suspension had 100,000 HA/mg protein and contained little or no egg or rabbit protein as judged by hypersensitivity reactions in suitably sensitised guinea-pigs.