

## The Immune Response of Hamsters to Purified Haemagglutinins and Whole Influenza Virus Vaccines Following Live Influenza Virus Infection

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**Abstract.** The ability of several, live type A influenza viruses to enhance the serum haemagglutination-inhibiting (HI) antibody response of hamsters to subsequent immunization with inactivated, heterotypic influenza virus vaccines was examined. Live influenza viruses were found to vary in their priming ability for a given vaccine, and a given virus was not able to prime for all inactivated vaccines to an equal extent. Common determinants in the haemagglutinin antigens of the priming virus and the vaccine virus were suggested as responsible for the enhancement of the antibody response to some of the vaccines, but for other pairs of viruses the haemagglutinin antigens were distinct. Thus, enhancement in these instances cannot be due to cross-reacting haemagglutinins. Pre-infection of hamsters by several influenza type A viruses was employed in an attempt to enhance the serum HI antibody response to purified, haemagglutinin antigens prepared from A/PR/8/34 and the MRC-2 recombinant strain of A/England/42/72 viruses. Although prior infection enhanced the antibody response to whole virus, this was not demonstrable for the purified haemagglutinin components of the virus. The possible reasons for this are discussed.

### Introduction

It was previously reported that an enhanced serum haemagglutination-inhibiting (HI) antibody response to inactivated influenza vaccines occurred in hamsters previously infected with a heterotypic influenza A virus (Potter *et al.*, 1973; Jennings and Potter, 1973). The priming infections could elicit an enhanced antibody response irrespective of which influenza A virus was used for the pre-infection, although it appeared that some viruses might prime better than others for a given influenza vaccine (Jennings and Potter, 1973). It was suggested that the priming effect was due to the production of an antibody to antigens common to all type A influenza viruses, such as the internal ribonucleoprotein or matrix antigen; subsequent immunization resulted in an exaggerated antibody response to the new haemagglutinin, (HA), be-

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cause this is introduced coupled with antigens experienced in the priming infection (Potter *et al.*, 1973). Other workers have reported similar findings in guinea pigs using antigens coupled to protein carriers (Katz *et al.*, 1970). The results obtained for influenza virus vaccines were not peculiar to hamsters; similar results were observed in mice and ferrets, and primed animals also showed an enhanced response for the neuraminidase antigen (Jennings *et al.*, 1974).

The present paper reports the variable ability of a number of type A influenza virus infections to prime for several inactivated influenza A vaccines. In addition, the antigenicity of purified haemagglutinin, produced from two viruses by treatment with the plant enzyme, bromelain (Brand and Skehel, 1972), was investigated in both normal and primed hamsters.

### Materials and Methods

**Viruses.** Influenza viruses A/Swine (HSw1N1), A/Equi-2/63 (Heq2Neq2), A/PR/8/34 (H0N1), A/FM/1/47 (H1N1), A/Singapore/1/57 (H2N2), and A/Hong Kong/1/68 (H3N2) were all strains of virus maintained in our laboratory. The MRC-2 recombinant (H3N2), prepared from influenza virus A/England/42/72 (H3N2) and A/PR/8/34 (H0N1) was kindly supplied by Dr. G. C. Schild, National Institute of Medical Research, Mill Hill, London.

Virus pools were prepared by the allantoic inoculation of 10-day embryonated hen's eggs. After incubation at 33°C for 48 h, the allantoic fluids were harvested, pooled and stored at -80°C. The identity of all viruses was confirmed by cross HI tests using monospecific ferret antisera.

**Vaccines.** Monovalent influenza virus A/Japan/305/57 vaccine containing 1,685 chick cell agglutinating (CCA)/ml, and A/Aichi/2/68 vaccine containing 1,714 CCA/ml were obtained from Professor W. M. Marine, Emory University, Atlanta, Georgia, U.S.A. These vaccines were prepared by Merrell-National Laboratories, Swiftwater, Pennsylvania, U.S.A. by formaldehyde treatment of virus purified by zonal centrifugation.

Purified MRC-2 virus and a purified HA vaccine obtained from MRC-2 virus by bromelain treatment (Brand and Skehel, 1972) were prepared by one of us at Wellcome Laboratories, Beckenham, Kent. The purified virus preparation contained 10 mg/ml of whole virus protein, equivalent approximately to 80 000 CCA/ml of HA protein. The vaccine obtained using the enzyme bromelain, contained 0.675 mg/ml, (16 354 CCA/ml) of HA protein. The HA protein has been shown to represent approximately one third of the total protein content of the influenza virus particle (Skehel and Schild, 1971). Both materials were treated with a final dilution of 1 in 4000 formaldehyde and diluted in phosphate buffered saline (PBS), pH 7.2, to contain the equivalent of 1 600 CCA/ml. Purified A/PR/8/34 virus and a bromelain-split, purified HA vaccine obtained from it were also prepared at Wellcome Laboratories. The purified A/PR/8/34 virus contained 10 mg/ml of whole virus protein, equivalent to 80 000 CCA/ml of HA protein. The purified, enzyme-released HA vaccine contained 1 mg/ml (approximately 24 000 CCA/ml) of HA protein. Both materials were treated with formaldehyde as above and diluted in PBS to contain the equivalent of 1 600 CCA/ml.

The monovalent, formalin-inactivated A/FM/1/47 saline vaccine used in these experiments was prepared in our laboratory. Egg-grown virus was concentrated by

adsorption and elution with fowl erythrocytes followed by centrifugation at 35 000 g for 60 min; the pelleted virus was resuspended in PBS to give an approximate 100-fold concentration and inactivated with 1:4 000 formaldehyde. This vaccine contained 4 000 CCA units of HA per ml.

*Experimental Design.* All experiments were performed using 2–3 month-old Syrian hamsters. Blood was collected from the orbital sinus of each animal prior to its use, and groups of 3 or 4 hamsters inoculated intranasally with 0.2 ml of a live influenza virus preparation containing  $10^{5.5}$  to  $10^{6.5}$  egg infectious doses/ml (EID<sub>50</sub>/ml). Three weeks later a further blood sample was taken and the animals immunized intramuscularly with an 0.5 ml volume of killed virus vaccine appropriately diluted in PBS. A final blood specimen was collected from each animal three weeks after immunization. All sera were stored at  $-20^{\circ}\text{C}$ . In no case was serum HI antibody to any of the viruses used detected in sera obtained from the hamsters prior to these studies.

*Haemagglutination-Inhibition Tests.* These were performed by standard procedures and have been described in detail elsewhere (Jennings and Potter, 1973). The serum antibody response of hamsters to A/England/42/72 vaccination was kindly checked by Dr. J. S. Oxford, using the single radial diffusion method (Schild *et al.*, 1972).

## Results

### *Titration of A/PR/8/34 Vaccine in Normal and Infected Hamsters*

Groups of hamsters were bled, and infected intranasally with either A/Equi-2/63, A/FM/1/47, A/Hong Kong/1/68 or A/England/42/72 influenza viruses. Three weeks later, a further sample of blood was collected, and the hamsters immunized with varying doses of A/PR/8/34 vaccine. Three weeks later the animals were again bled and all sera tested for HI antibodies to both the infecting and the immunizing virus. The results are shown in Table 1.

It can be seen that normal hamsters produced detectable serum HI antibody in response to immunization with 200 CCA of A/PR/8/34 vaccine, but not in response to 80 CCA of the same vaccine. In contrast, two hamsters primed by pre-infection with heterotypic A/Equi-2/63 virus produced serum HI antibody in response to immunization with 20 CCA units of vaccine. Thus, the priming infection enhanced the response to this vaccine by 10-fold. There was also some evidence of priming for A/PR/8/34 vaccine by prior infection with A/England/42/72 virus; two of three primed hamsters produced serum HI antibody in response to 80 CCA units of vaccine. However, neither A/FM/1/47 nor A/Hong Kong/1/68 influenza viruses enhanced the response to A/PR/8/34 vaccination.

### *Titration of A/FM/1/47 Vaccine in Normal and Infected Hamsters*

Experiments similar to those described above for influenza A/PR/8/34 virus vaccine were performed using A/FM/1/47 vaccine. The results are presented in Table 2. Normal hamsters produced detectable serum HI

Table 1. Serum HI antibody response of normal and infected hamsters to immunization with varying doses of A/PR/8/34 vaccine

Dose of vaccine (CCA units)	Hamster no.	Pre-infecting virus					
		None		A/Equi-2/63		A/HK 1/68	
		HI antibody response to A/PR8	HI antibody response to A/PR8	HI antibody response to A/PR8	HI antibody response to A/PR8	HI antibody response to A/Eng	HI antibody response to A/PR8
200	1	< 10-120 <sup>a</sup>	< 10-10	< 10-10	120-120	> 320- > 320	< 10- 15
	2	< 10- 15	15-40	< 10-30	120-120	> 320- > 320	< 10- 30
	3	< 10- 10	15-40	< 10-60	120- 30	> 320- > 320	< 10- 30
	4		60-40	< 10-60			
80	1	- <sup>b</sup>	15-60	< 10-30	240-160	> 320- > 320	< 10- 30
	2	-	20-30	< 10-15	120- 60	240- 240	-
	3	-	40-60	< 10-15	320- 80	> 320- 240	< 10-120
	4						
20	1	ND <sup>c</sup>	15-15	< 10-15	80- 60	> 320- > 320	-
	2		10-10	< 10-15	60- 40	< 320- > 320	-
	3				60- 60	> 320- > 320	-
	4						
8	1	-	ND	ND	60- 60		ND
	2	-			240- 80		ND
	3	-			240- 40		

<sup>a</sup> Serum HI antibody titres before and after immunization.<sup>b</sup> - = < 10- < 10.<sup>c</sup> ND = not done.

Table 2. Serum HI antibody response of normal and infected hamsters to immunization with varying doses of A/FM/1/47 vaccine

Dose of vaccine (CCA units)	Hamster no.	Pre-infecting virus					A/Hong Kong/1/68 HI antibody response to	
		None HI antibody response to A/FM1	A/Swine HI antibody response to		A/Equi-2/63 HI antibody response to			
			A/Swine	A/FM1		A/Equi		
5	1	< 10-20 <sup>a</sup>	40-30	< 10-15	15-15	< 10-15	15-15	< 10-15
	2	< 10-40	30-30	< 10-30	15-15	< 10-15	30-30	< 10-15
	3	< 10-80	30-30	< 10-30	30-15	< 10-15	30-30	< 10-15
	4	< 10-160	30-60	< 10-30	15-< 10	- <sup>b</sup>	60-30	< 10-15
0.5	1	< 10-80	30-60	< 10-15	15-15	< 10-15	30-20	< 10-15
	2	< 10-40	30-20	< 10-30	20-15	< 10-15	60-30	< 10-15
	3	< 10-80	30-20	< 10-15	15-15	< 10-15	30-30	< 10-15
	4	< 10-160	30-60	< 10-15	15-15	-	30-15	-
0.05	1	< 10-80	30-60	< 10-10	30-15	-	30-30	-
	2	-	30-60	< 10-40	15-15	< 10-15	60-30	-
	3	-	30-30	-	15-20	-	30-30	-
	4	-	30-60	< 10-15	15-30	-	-	-
0.005	1	-	80-80	-	15-15	-	20-15	-
	2	-	80-60	-	30-20	-	30-30	-
	3	-	60-40	-	15-15	-	80-30	-
	4	-	60-30	-	15-15	-	30-40	-

<sup>a</sup> Serum HI antibody titres before and after immunization.<sup>b</sup> - = < 10-10.

antibody in response to immunization with 0.5 CCA of A/FM/1/47 vaccine. Pre-infection of hamsters with influenza A/Equi-2/63 or A/Hong Kong/1/68 viruses failed to enhance the response of animals to this vaccine. On the other hand, there was some evidence that prior infection with influenza A/Swine virus did result in some enhancement, and three of four such hamsters responded to immunization with 0.05 CCA of A/FM/1/47 vaccine, whilst only one of four normal hamsters produced detectable serum HI antibody following this dose of vaccine.

*Titration of A/Japan/305/57 Vaccine in Normal and Infected Hamsters*

Table 3 shows that normal hamsters did not produce detectable levels of serum HI antibody following immunization with 1500 CCA of A/Jap/305/57 vaccine. However, hamsters pre-infected with either A/FM/1/47 or A/Hong Kong/1/68 virus responded to subsequent immunization with this vaccine. Thus, three of four hamsters primed with A/FM/1/47 influenza virus produced detectable levels of serum HI antibody in response to immunization with 1500 CCA units, but the response of primed hamsters to smaller doses of A/Jap/305/57 vaccine was irregular. Furthermore, hamsters primed by prior infection with A/Hong Kong/1/68 responded to 1000 CCA of A/Jap/305/57 vaccine, while some animals produced serum HI antibody in response to 500 and 250 CCA units (Table 3). However, infection of some hamsters with live influenza A/Hong Kong/1/68 virus induced serum HI antibodies to A/Singapore/1/57 virus; subsequent immunization with A/Japan/305/57 vaccine failed to boost the titre of this antibody. In other hamsters, HI antibody against A/Sing/1/57 virus did not develop following A/Hong Kong/1/68 infection, and for these animals subsequent immunization with A/Jap/305/57 did produce a serum HI antibody response.

In contrast to the results obtained for A/FM/1/47 and A/Hong Kong/1/68 viruses, pre-infection of hamsters with either A/England/42/72 or A/Equi-2/63 failed to enhance the response of animals to subsequent A/Jap/305/57 vaccinations.

*Titration of A/England/42/72 (MRC-2) Vaccine in Normal and Infected Hamsters*

The antibody response of hamsters to immunization with influenza A/Eng/42/72 virus vaccine is shown in Table 4. Since this virus and influenza A/Hong Kong/1/68 possess similar HA antigens, the HI antibody response to both these viruses was determined. Serum HI antibody against A/Eng/42/72 virus was detected in sera from hamsters immunized with 800 CCA of this vaccine, but only one of four animals responded to

Table 3. Serum HI antibody response of normal and infected hamsters to immunization with varying doses of A/Japan/305/57 vaccine

Dose of vaccine (CCA units)	Hamster no.	Pre-infecting virus				A/Equi-2/63 HI antibody response to	A/Hong Kong/1/68 HI antibody response to	A/England/42/72 HI antibody response to		
		None	A/FM/1/47 HI antibody response to	A/Sing	A/FMI response A/Sing					
		HI anti-body response A/Sing			A/Equi	A/HK	A/Sing	A/Eng	A/Sing	
1500	1	- <sup>b</sup>	480-480 <sup>a</sup>	< 10-30	120-60	-	480-	< 10-120	> 320-320	< 10-15
	2	-	320-240	< 10-30	120-60	-	> 640-	30-60	> 320-320	-
	3	-	480-240	< 10-30			240-	40-< 10	> 320-320	-
	4	-	> 640-640	-						
1000	1	-	320-480	< 10-20	30-10	-	> 640->	< 10-60	60-60	< 10-15
	2	-	320-320	-	40-15	-	> 640-	120-80	60-60	-
	3	-	480-240	-	120-20	-	480-	< 10-30	120-240	-
	4	-					240-	< 10-60		
500	1	-	480-240	< 10-15	20-60	-	240-	120-60	> 320-120	-
	2	-	480-320	< 10-15	60-60	-	120-	120-	> 320-240	-
	3	-	480-320	-	80-60	-	240-	< 10-20	> 320-30	-
	4	-	> 640-480	-			240-			
250	1	-	480-480	< 10-30	120-120	-	640-	< 10-15	> 320-120	-
	2	-	> 640-320	-	60-60	-	120-	30-	> 320-320	-
	3	-	640-320	-	20-15	-	120-	< 10-60	> 320-320	-
	4	-	640-120	-						

<sup>a</sup> Serum HI antibody titres before and after immunization.<sup>b</sup> - = < 10 to < 10.

Table 4. Serum HI antibody response of normal and infected hamsters to

Vaccine dose (CCA units)	Hamster no.	Pre-infecting virus			
		None		A/Swine	
		HI antibody response to		HI antibody response to	
		A/Eng	A/HK	A/Swine	A/Eng
800	1	< 10-30 <sup>a</sup>	< 10-15	60- 30	< 10-30
	2	< 10-30	< 10-20	60- 80	< 10-20
	3	< 10-10	— <sup>b</sup>	60- 60	< 10-30
80	1	< 10-60	< 10-15	60- 80	< 10-15
	2	—	—	60- 60	< 10-30
	3	—	—	60- 60	—
	4	—	—	120- 80	—
8	1	—	—	60- 60	—
	2	—	—	60-120	< 10-15
	3	—	—	120- 80	—
	4	—	—	120-120	—
0.8	1	—	—	120- 60	—
	2	—	—	120- 60	< 10-10
	3	—	—	60- 60	—
	4	—	—	60- 30	—
0.08	1	—	—	60-120	—
	2	—	—	120- 60	—
	3	—	—	120- 30	—
	4	—	—	30- 20	—

<sup>a</sup> Serum HI antibody titre before and after immunization.

<sup>b</sup> — = <10- < 10.

<sup>c</sup> ND = not done.

80 CCA units. Evidence of some priming for A/Eng/42/72 vaccine was obtained in hamsters pre-infected with A/FM/1/47, since these hamsters produced HI antibody in response to immunization with 80 CCA of vaccine. No priming effect for A/England/42/72 vaccine was observed in hamsters pre-infected with influenza A/Equi-2/63, or A/PR/8/34 viruses, and only slight evidence of priming in hamsters pre-infected with A/Swine.

When the sera were tested for HI antibody using A/Hong Kong/1/68 the results were distinct, despite the fact that the HA antigens of A/Hong Kong/1/68 and A/Eng/42/72 viruses cross-react. Thus, of the normal hamsters immunized with 800 CCA of A/England/42/72 vaccine, two of three responded, and only one of four animals inoculated with 80 CCA produced serum antibody (Table 4). Pre-infection of the ham-

immunization with varying doses of A/England/42/72 (MRC-2) vaccine

A/PR/8/34 HI antibody response to		A/FM/1/47 HI antibody response to		A/Equi-2/63 HI antibody response to		
A/PR8	A/Eng	A/FM1	A/Eng	A/Equi	A/Eng	A/HK
240—120	< 10—240	160—120	< 10—80	10—15	< 10—15	< 10—15
240—60	< 10—15	30—40	< 10—80	15—30	< 10—60	< 10—15
120—30	< 10—60	120—60	< 10—60	< 10—10	< 10—15	—
320—240	< 10—60	240—120	< 10—15	15—15	—	—
120—120	—	120—60	< 10—20	< 10—10	—	< 10—15
240—120	—	60—30	< 10—15			
120—60	—	60—15	< 10—15			
320—80	—	120—120	—	30—60	—	< 10—15
480—120	—	60—15	—	60—60	—	< 10—30
120—80	—	240—160	—	20—30	—	< 10—30
240—120	—	120—120	< 10—15	20—20	—	—
120—120	—	40—120	—	15—15	—	—
320—120	—	120—120	—	30—60	> 10—30	< 10—40
240—120	—	120—60	—	60—60	—	< 10—15
240—240	—	160—30	—	30—15	—	—
120—120	—			60—80	—	< 10—10
120—120	—	ND <sup>c</sup>	ND	60—60	—	< 10—40
480—240	—			15—10	—	—
60—60	—					

sters with A/FM/1/47 and A/Swine influenza viruses had no effect on the A/Hong Kong/1/68 antibody response. In contrast, pre-infection with A/Equi-2/63 considerably enhanced the HI antibody response to A/Hong Kong/1/68 and serum antibody to this virus was detected following immunization with both 0.8 and 0.08 CCA of A/England/42/72 vaccine.

*Antibody Response of Normal and Infected Hamsters  
to Immunization with A/England/42/72 (MRC-2)  
and A/PR/8/34 Haemagglutinin Vaccines*

Hamsters immunized with purified haemagglutinin from influenza virus A/Eng/42/72, obtained by bromelain treatment did not produce detectable HI antibody (Table 5). The equivalent amount of antigen did induce antibody when inoculated as whole virus (Table 4). In addition,

Table 5. Serum HI antibody response of normal and infected hamsters to immunization with A/Eng/(MRC-2) purified haemagglutinin vaccine

Dose of bromelain- split vaccine (CCA units)	Hamster no.	Pre-infecting virus				vaccine			
		None HI antibody response to	A/Swine HI antibody response to		A/Eng	A/Eng/(MRC-2) HI antibody response to		A/Sing HI antibody response to	A/Eng
			A/Swine	A/Eng		A/Eng	A/Eng		
800	1	—	120—240 <sup>a</sup>	—	—	60—60	—	ND <sup>c</sup>	ND
	2	— <sup>b</sup>	240—240	—	—	80—30	—	—	—
	3	—	120—60	—	—	160—80	—	—	—
	4	—	160—320	—	—	—	—	—	—
80	1	—	240—120	—	—	80—120	—	40—60 <sup>a</sup>	—
	2	—	120—120	—	—	120—80	—	80—60	—
	3	—	240—240	—	—	320—160	—	60—30	—
	4	—	240—240	—	—	120—120	—	120—60	—
8	1	—	80—120	—	—	120—120	—	120—30	—
	2	—	240—240	—	—	160—120	—	80—30	—
	3	—	240—30	—	—	160—240	—	160—30	—
	4	—	240—120	—	—	120—120	—	80—30	—
0.8	1	—	160—60	—	—	80—120	—	80—40	—
	2	—	40—120	—	—	60—80	—	120—80	—
	3	—	240—120	—	—	120—120	—	160—60	—
	4	—	120—60	—	—	120—60	—	120—120	—

<sup>a</sup> Serum HI antibody titres before and after immunization.<sup>b</sup> — = < 10—< 10.<sup>c</sup> ND = not done.

Table 6. Serum HI antibody response of normal and infected hamsters to immunization with A/PR/8/34 purified haemagglutinin vaccine

Dose of brome- lain split vaccine (CCA units)	Hamster no.	Pre-infecting virus					
		None		A/HK/1/68		A/Eng/42/72	
		HI antibody A/PR8	HI antibody A/EM1	HI antibody response to A/PR8	HI antibody response to A/HK	HI antibody response to A/Eng	HI antibody response to A/PR8
800 CCA	1	— <sup>b</sup>	120—60 <sup>a</sup>	—	160—120 320—120	40—40 120—120	20—30 30—60
	2	—	—	—	—	—	—
	3	—	—	—	—	—	—
	4	—	—	—	—	—	—
80 CCA	1	—	60—30	—	160—80	30—30	30—60
	2	—	60—80	—	240—240	60—60	15—30
	3	—	60—40	—	120—60	80—60	15—40
	4	—	—	—	120—120	120—30	60—40
8 CCA	1	—	240—480	—	160—120	120—80	40—60
	2	—	120—480	—	160—120	60—120	30—60
	3	—	240—480	—	160—120	60—40	40—60
	4	—	> 640—240	—	160—60	60—40	15—60
0.8 CCA	1	—	60—60	—	120—120	60—40	120—120
	2	—	60—30	—	60—120	120—30	30—30
	3	—	120—120	—	120—120	15—15	15—15
	4	—	120—30	—	120—120	60—15	15—15

<sup>a</sup> Serum HI antibody titres before and after immunization.<sup>b</sup> — = < 10 < σ0.

hamsters previously infected with influenza virus A/Sing/1/57, A/FM/1/47 or A/Swine influenza viruses did not produce detectable serum HI antibody in response to subsequent immunization with enzyme-released A/England/42/72 HA vaccine, although prior infection with A/FM/1/47 virus has been shown to enhance the response to A/Eng/42/72 whole virus vaccine.

Similar results were obtained for the purified HA from influenza virus A/PR/8/34 (Table 6). Thus, the equivalent of 800 CCA of purified HA from this virus failed to elicit a detectable serum HI antibody response, whereas the equivalent CCA of whole virus vaccine was able to do so (Table 1). Hamsters primed by infection with A/Eng/42/72, A/Hong Kong/1/68 or A/FM/1/47 influenza viruses also failed to respond to the equivalent of 800 CCA of purified, bromelain-released HA.

### Discussion

It was previously reported that the serum HI antibody response of hamsters to type A influenza vaccines was enhanced by pre-infection of these animals with live, heterotypic influenza A viruses (Potter *et al.*, 1973; Jennings and Potter, 1973). However, some viruses appeared less effective priming agents than others. The results presented here show that enhancement of the antibody response to influenza vaccines in hamsters primed with various heterotypic influenza A viruses depends on both vaccine virus and priming virus. The priming ability of live influenza A viruses varies considerably, although serum HI antibody to intranasal infection indicated that infection had taken place in every case. Earlier reports (Friedewald and Hook, 1948) and recent studies in this laboratory (Jennings, unpublished observations) show that influenza viruses do replicate in hamster lung, and high titres of virus can be recovered from this tissue 2–4 days after inoculation.

As reported earlier (Jennings and Potter, 1973), A/Hong Kong/1/68 virus did not prime for A/PR/8/34 vaccine and the same virus did not enhance the response to the A/FM/1/47 vaccine used in these studies. The infection induced in hamsters by A/Hong Kong/1/68 may be less severe than that induced by other influenza viruses and the stimulation of the immunological system of the animal insufficient for priming to occur. However, the same virus effectively primes hamsters to respond to A/Jap/305/57 vaccine, but there is evidence for cross-reactivity of HA antigenic determinants between these viruses. Thus, Dowdle *et al.* (1972), using HA-specific recombinants and anti-sera prepared against them in ferrets, showed cross-reactions between these two haemagglutinins and similar cross-reactions were found in cross-infection studies. Furthermore, Marine *et al.* (1969) reported that immunization of man with A/Hong Kong/1/68 vaccine induces an increase in the titre of A/Japan/305/57

antibody. Webster and Laver (1972), however, were unable to detect any direct relationship between the purified HA antigens of Hong Kong and Asian influenza viruses. In the present study, infection of hamsters with A/Hong Kong/1/68 virus induced antibody in some hamsters to A/Sing/1/57 virus, suggesting some relationship between their HA antigens. Thus, the priming for A/Jap/305/57 vaccine by A/Hong Kong/1/68 virus may simply be the anamnestic effect produced by further contact of the animals with common HA determinants.

A similar relationship has been shown for the HA antigens of A/Hong Kong/1/68 and A/Equi-2/63 viruses (Coleman *et al.*, 1968; Marine *et al.*, 1969; Tumova and Easterday, 1969), and this may explain the increased A/Hong Kong/1/68 antibody response observed following A/England/42/72 vaccination of hamsters preinfected with A/Equi-2/63 virus. Pre-infection with this virus failed to enhance the response to A/Eng/42/72 vaccine, presumable reflecting a greater HA antigen disparity between A/Equi-2/63 and A/England/42/72 viruses compared to that between A/Equi-2/63 and A/Hong Kong/1/68 viruses.

However, enhancement of influenza vaccination by prior heterotypic infection of hamsters with live influenza viruses can occur even though no relationship between the HA antigens of the viruses concerned is detectable, and the priming ability of influenza viruses for several influenza vaccines is shown in Table 7. This table has been prepared using earlier results (Potter *et al.*, 1973; Jennings and Potter, 1973), and results

Table 7. Priming ability of influenza type A viruses for heterotypic influenza vaccines

Immunizing virus	Pre-infecting virus						
	A/Swine (H5W1)	A/Equi-2 (Heq2)	A/PR8 (H0N0)	A/FM1 (H1N1)	A/Sing (H2H2)	A/Hong Kong (H3N2)	A/Eng (H3N2)
A/PR8 (H0N0)	— <sup>a</sup>	++ <sup>c</sup>		+	+	—	+
A1/FM1 (H1N1)	+ <sup>b</sup>	—	+		++	—	
A/Japan (H2N2)	—	—	—	—		++	—
A/Hong Kong (H3N2)	+	+	+	++	+		
A/England (H3N2)	—	—	—	+			

<sup>a</sup> — = Pre-infecting virus fails to enhance serum HI antibody response to the vaccine shown.

<sup>b</sup> + = Pre-infecting virus enhances the serum HI antibody response to the vaccine shown by 5 to 10-fold.

<sup>c</sup> ++ = Pre-infecting virus enhances the serum HI antibody response to the vaccine shown by > 10-fold.

presented in this paper, and shows that both A/FM/1/47 and A/Sing/1/57 are relatively effective priming agents, in that A/FM/1/47 primed for three of four heterotypic vaccines, while A/Sing/1/57 primed for A/PR/8/34, A/FM/1/47 and A/Hong Kong/1/68 vaccines; this priming was in many cases for the haemagglutinin of a vaccine virus having no demonstrable cross-reaction with that of the infecting virus.

The ability to elicit a priming reaction may also depend on the immunogenicity of the vaccine. The response of hamsters to A/Hong Kong/1/68 vaccine (Potter *et al.*, 1973), and to A/Jap/305/57 and A/England/42/72 vaccines was poor. In the present study no animal produced serum HI antibody following 1500 CCA of A/Jap/305/57 vaccine and furthermore no virus infection could prime hamsters to induce a response to this vaccine with the exception of A/Hong Kong/1/68 and this can be explained by cross-reactivity between the two viruses. It was also difficult to prime for A/England/42/72 vaccine and Table 4 shows that levels of 80 CCA of this vaccine are required to induce serum HI antibody in normal hamsters. However, both A/PR/8/34 and A/FM/1/47 vaccines are more immunogenic in hamsters (Tables 1 and 2), and both these vaccines are enhanced by pre-infection with several heterotypic viruses. Thus, it appears that the greater the immunogenicity of a vaccine, the more readily it is enhanced by prior heterotypic infection. On the other hand, A/Hong Kong/1/68 vaccine could be enhanced by priming with each virus tested, but is a relatively poor vaccine in hamsters.

The suggestion was put forward earlier (Potter *et al.*, 1973), that common internal antigens are responsible for the enhancement of the HI antibody response to vaccination in primed hamsters; this was examined using purified HA antigens prepared using the enzyme bromelain (Brand and Skehel, 1972). An earlier report from this laboratory (Potter *et al.*, 1973a), showed that hamsters pre-infected with A/FM/1/47 virus were primed for subsequent immunization with an ether-Tween split A/Hong Kong/1/68 vaccine, suggesting that the response of these hamsters was not dependent on the heterotypic HA being complexed with carrier antigens. However, the composition of the split vaccine was not known with certainty. The results presented here show that no antibody response to A/PR/8/34 or A/England/42/72 purified, enzyme-released HA vaccines occurred in either normal or pre-infected hamsters. Similar results have been obtained in ferrets (McLaren *et al.*, unpublished observations). However, purified, whole virus A/England/42/72 vaccine from which the HA vaccine was prepared, was enhanced by prior heterotypic virus infection with A/FM/1/47 virus. This result could be predicted from the above explanation of the priming reaction. Thus, purified HA antigen obtained using bromelain has been removed from carrier protein which stimulated the enhanced antibody response in primed animals.

## References

- Brand, C. M., Skehel, J. J.: Crystalline antigen from the influenza virus envelope. *Nature New Biol.* **238**, 145—147 (1972)
- Coleman, M., Dowdle, W. R., Pereira, H. G., Schild, G. C., Chang, W. K.: The Hong Kong/68 influenza A2 variant. *Lancet* **1968 II**, 1384—1386
- Dowdle, W. R., Marine, W. M., Coleman, M., Knez, V.: Haemagglutinin relationships of Hong Kong (H3) and Asian (H2) influenza strains delineated by antigen-specific recombinants. *J. Gen. Virol.* **16**, 127—134 (1972)
- Friedewald, W. F., Hook, E. W., Jr.: Influenza virus infections in the hamster. A study of inapparent virus infection and virus adaptation. *J. exp. Med.* **88**, 343—353 (1948)
- Jennings, R., Potter, C. W.: Enhanced response to influenza A vaccines in hamsters primed by prior heterotype influenza infection. *Arch. ges. Virusforsch.* **42**, 197—206 (1973)
- Jennings, R., Potter, C. W., McLaren, C.: Effect of pre-infection and pre-immunization on the serum antibody response to subsequent immunization with heterotypic influenza vaccines. *J. Immunol.* (in press, 1974)
- Katz, D. H., Paul, W. E., Goidl, E. A., Benacerraf, B.: Carrier function in anti-hapten immune responses. I. Enhancement of primary and secondary anti-hapten antibody responses by carrier preimmunization. *J. exp. Med.* **132**, 261—282 (1970)
- Marine, W. M., Workman, W. M., Webster, R. G.: Immunological interrelationship of Hong Kong, Asian and Equi-2 influenza viruses in man. *Bull. Wld Hlth Org.* **41**, 475—482 (1969)
- Potter, C. W., Jennings, R., McLaren, C., Marine, W. M.: The potentiation of the antibody response to inactivated A2/Hong Kong vaccines by previous heterotypic influenza virus infection. *Microbios* **8**, 101—110 (1973)
- Potter, C. W., Jennings, R., Rees, R. C., McLaren, C.: Antibody response of hamsters to A2/Hong Kong virus vaccine after priming by heterotypic virus infection. *Infect. Immun.* **8**, 137—144 (1973a)
- Schild, G. C., Henry-Aymard, M., Pereira, H. G.: A quantitative single-radial-diffusion test for immunological studies with influenza virus. *J. gen. Virol.* **16**, 231—236 (1972)
- Skehel, J. J., Schild, G. C.: The polypeptide composition of influenza A viruses. *Virology* **44**, 396—408 (1971)
- Tumova, B., Easterday, B. C.: Relationship of envelope antigens of animal influenza viruses to human A2 influenza strains isolated in the years 1957—68. *Bull. Wld Hlth Org.* **41**, 429—435 (1969)
- Webster, R. G., Laver, W. G.: Studies on the origin of pandemic influenza. 1. Antigenic analysis of A2 influenza viruses isolated before and after the appearance of Hong Kong influenza using antisera to the isolated haemagglutinin subunits. *Virology* **48**, 433—444 (1972)

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