As could be expected, the low titres of the allantoic fluids are reflected in the concentrates; the mean titre of these is $10^{4.9}$ which is less than half of what is usually obtained with other influenza strains.

The low virus content in the concentrate is mainly due to the low titre in the allantoic fluids, but low recovery of virus in the final concentrate is a contributing factor. Only about 50% of the original HA activity was recovered in the concentrate. This is in contrast to results obtained with previous influenza strains, where the recovery rate has consistently been at least 70%. An analysis of the losses during centrifugation is shown in Table 2, which records the haemagglutinating activity in the allantoic fluids and in the Sharples centrifugal fractions for various vaccine preparations. Two columns show representative results as obtained with Asian 1957 virus and the B/Johannesburg strain. To the right are shown results obtained with 3 different batches of Hong Kong virus. The recovery rates of haemagglutinins in the final concentrate are listed in the bottom row. The recovery rates with the 3 Hong Kong preparations were only 54%, 48% and 38%, respectively, as compared with a recovery rate of more than 70% with both the Asian and the B strains.

Virus antigen is lost during centrifugation with the effluent liquids, but the loss is insignificant with all strains, and, if anything, it is lower with the Hong Kong virus than with the other strains. Some antigen is also lost in the pellet after the final low-speed centrifugation. This loss is very small with the Asian and B strains, but surprisingly high with all 3 preparations of Hong Kong virus. It appears likely that the comparatively poor recovery of Hong Kong virus in the final concentrate may be due to an aggregation of viral antigen which is sedimented and removed at low gravity.

In summary, the production of Hong Kong influenza vaccines was carried out at the Statens Seruminstitut by conventional methods without any particular difficulties, but with a relatively poor yield. This was due mainly to low titres of the allantoic fluids and partly to a lower than usual recovery of virus by differential concentration.

Most likely the adaptation to eggs of the Hong Kong virus strains we used was insufficient. I know of other smaller vaccine production units which have had similar experiences. For such smaller laboratories it would undoubtedly be a great advantage if the adaptation of new strains could somehow be centralized (for instance by the World Health Organization) so that virus could be made available which had been sufficiently adapted to eggs to be suitable for the preparation of seed virus for vaccine production.

Immunogenicity of Purified and Conventional Inactivated Influenza Virus Vaccines

by WILLIAM J. MOGABGAB [a]

Observations were made in the fall of 1968 on 2 purified influenza virus vaccines, one derived from calf kidney cell cultures and ether-treated after density-gradient centrifugation, and the second prepared with chicken-embryo-propagated viruses and purified by zonal centrifugation. Both contained 600 CCA units per 0.5-ml dose, of which 300 CCA units were of 1962 and 1964 influenza A2 strains and 300 CCA units were of 1966 influenza B. When these preparations were administered to adults subcutaneously, systemic manifestations were very uncommon and local reactions to both were quite mild. As shown in Table 1, haemagglutination-inhibition (HI) antibody responses to the viruses contained in the vaccine as well as those to the 1968 influenza A2 strain were similar after either vaccine. Larger incremental changes occurred in those individuals with lower prevaccination antibody titres. An unexpected finding was the lower titres, and of more significance in evaluating antigenicity of influenza vaccines were the smaller increments, obtained with the micro technique.

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TABLE 1

HI ANTIBODY RESPONSES TO PURIFIED BOVINE AND CHICKEN-EMBRYO INFLUENZA VACCINES [a] AS DETERMINED BY MACRO AND MICRO TECHNIQUES

HI technique	Viruses	No. of sera	Prevaccination titre range (reciprocal)	Geometric mean titres (reciprocal)		
				Pre	Post	Mean increase (x-fold)
			Bovine cell culture vaccine [b]			
Macro	A2/Japan/170/62	93	128–1 024	611	2 300	3.7
		31	2 048–>8 192	3 265	5 470	1.6
	A2/Taiwan/1/64	86	64–512	265	1 516	5.7
		38	1 024–>8 192	1 700	4 525	2.6
	A2/Aichi/2/68 [c]	46	<4–16	11	34	3.1
		77	32–1 024	63	102	1.6
	B/Mass./3/66	94	16–256	96	646	6.7
		30	512–>8 192	857	2 250	2.6
			Chicken-embryo vaccine [d]			
Macro	A2/Japan/170/62	55	128–512	338	1 205	3.5
		24	1 024–>8 192	1 624	3 555	2.2
	A2/Taiwan/1/64	36	128–512	355	1 218	3.7
		41	1 024–>8 192	2 800	8 470	3.0
	A2/Aichi/2/68 [c]	48	<4–32	24	68	2.8
		31	64–2 048	87	152	1.7
	B/Mass./3/66	37	<4–64	37	240	6.4
		42	128–>8 192	277	1 188	4.2
Micro	A2/Japan/170/62	54	4–16	14	34	2.3
		25	32–128	37	54	1.4
	A2/Taiwan/1/64	55	8–16	14	29	2.0
		24	32–64	33	47	1.4
	A2/Aichi/2/68 [c]	51	4–8	8	15	1.9
		28	16–64	17	19	1.1
	B/Mass./3/66	16	4–8	8	24	3.1
		63	16–128	19	42	2.1

[a] Containing 150 CCA units of A2/Japan/170/62, 150 CCA units of A2/Taiwan/1/64 and 300 CCA units of B/Massachusetts/3/66.

[b] Ether-treated after density-gradient centrifugation.

[c] Not included in vaccine.

[d] Purified by zonal centrifugation.

Also evaluated during 1968–69 were inactivated virus vaccines prepared by conventional procedures. The availability of unvaccinated military personnel and the impending epidemic of influenza A2 provided an opportunity to compare the efficacy of the 1967 military formula polyvalent vaccine with monovalent Hong Kong vaccine. In Table 2 are shown the pre- and post-vaccination levels of antibody to the major influenza A2 component and to the 1968 variant that was not included in the formula.[b] The

[b] Tables 2-5 are taken, by permission, from Mogabgab, W. J. & Leiderman, E., *Immunogenicity of polyvalent (1967) and monovalent (1968) influenza vaccines*, in press, *J. Amer. med. Ass.*

TABLE 2

DISTRIBUTION OF HI ANTIBODY TITRES BEFORE AND AFTER 1967 MILITARY FORMULA POLYVALENT INFLUENZA VACCINE,[a] KEESLER AIR FORCE BASE, 1968

Antigen	Airmen	Sera[b]	No. of sera with indicated titre (reciprocal)														Median titre	Mean titre	Increase (x-fold) in mean titre
			<8	8	16	32	64	128	256	512	1 024	2 048	4 096	8 192	16 384	Total			
A2/Taiwan/1/64	Trainees	Pre					2	5	18	15	7	0	2			49	256	379	1.4
		Post					1	1	15	16	11	1	4			49	512	548	
	Sergeants	Pre						10	10	15	6	1	1	1		44	512	399	1.2
		Post						6	15	11	6	2	2	1	1	44	512	495	
A2/Aichi/2/68	Trainees	Pre		2	12	20	7	1	2							44	32	31.4	1.4
		Post			4	23	13	1	1	2						44	32	45.2	
	Sergeants	Pre	2	23	15	9	3									52	16	12.9	1.9
		Post	1	9	15	14	10	2	1							52	32	24.2	

[a] Contains 100 CCA units of each of A/Swine/33, A/PR/8/34, A1/Ann Arbor/1/57 and B/Lee/40; 200 CCA units of B/Massachusetts/3/66; and 400 CCA units of A2/Taiwan/1/64.

[b] Before (Pre) and 3 weeks after (Post) vaccination.

TABLE 3

DISTRIBUTION OF HI ANTIBODY TITRES BEFORE AND AFTER MONOVALENT INFLUENZA VACCINE (HONG KONG) AND NATURAL ILLNESSES IN TRAINEES, KEESLER AIR FORCE BASE, 1968–69

Sera	No of sera with indicated titre (reciprocal)[a]														Median titre	Mean titre	Increase (x-fold) in mean titre
	<8	8	16	32	64	128	256	512	1 024	2 048	4 096	8 192	16 384	Total			
Vaccinated[b]																	
Pre	2	10	20	15	4	2								53	16	18.4	32
Post				3	6	5	8	8	9	0	9	2	3	53	512	590	
Natural illnesses																	
Acute	1	2	6	19	26	9	11	1			28[c]			75	64	58.8	17.4
Convalescent					2	5	14	9	13	4				75	1 024	1 024	

[a] With influenza A2/Aichi/2/68 antigen.

[b] Influenza A2/Aichi/2/68, 300 CCA units/ml, subcutaneously.

[c] These were >2048.

TABLE 4

COMPARISON OF EFFECTS OF INFLUENZA VACCINES ON OCCURRENCE OF ALL RESPIRATORY ILLNESSES DURING INFLUENZA OUTBREAK AMONG TRAINEES, KEESLER AIR FORCE BASE, WINTER 1968–69

Vaccine group (and no. of men)	Clinical type [a]	No. of illnesses in indicated week													Rate per 1 000	Reduction (%)	Proportion (and 95% confidence interval)
		Vaccination		2 Dec. 68	Epidemic period									Total			
		18 Nov. 68	25 Nov. 68		9 Dec. 68	16 Dec. 68	23 Dec. 68	30 Dec. 68	6 Jan. 69	13 Jan. 69	20 Jan. 69	27 Jan. 69	3 Feb. 69				
Out-patients																	
Placebo (1 042)	V																
	IV	1		1	2	1	1	3	4	8	6		2	27	25.9		
	III		2	1		1					1		1	3	2.9		
	II	3	3	3	4	7	4	4	8	10	5	5	1	48	46.0		
	I	7	12	14	15	18	13	11	14	18	10	7	9	115	110.3		
	Total	11	17	19	21	27	18	18	26	36	22	12	13	193	185.2		0.1852 (0.1614–0.2210)
Polyvalent [b] (1 030)	V		1								1			1	1.0		
	IV		2	1	1			3	4	7	3			18	17.4	32.8	
	III			1	1				2	1	1			5	4.9		
	II	4	2	5	6	3	3	5	7	12	2	5	2	45	43.7	5.0	
	I	7	7	4	19	16	4	4	16	18	12	6	7	103	100.0	9.3	
	Total	11	12	11	27	19	8	12	29	38	19	11	9	172	167.0	9.8	0.1670 (0.1440–0.1916)
Hong Kong [c] (881)	V		1														
	IV	2		3	6	1				3		1	1	12	13.6	47.5	
	III				1									1	1.1	62.0	
	II	4	3	5	4	4	1	1	3		1	5		19	21.6	53.0	
	I	6	8	9	9	15	4	2	8	12	2	5	3	60	68.1	38.3	
	Total	12	12	17	20	20	5	3	11	15	3	11	4	92	104.4	43.6	0.1044 (0.0857–0.1245)
Hospitalized patients																	
Placebo (1 042)	V	1	1			1		1	1	9	1		1	2	1.9		
	IV					1		1	1	9	1		1	14	13.4		
	III																
	II						1							1	0.96		
	Total	1	1			2	1	1	2	9	1		1	17	16.3		0.0163 (0.0095–0.0263)
Polyvalent [b] (1 030)	V							1						1	0.97		
	IV								6	7				13	12.6	5.9	
	III																
	II																
	I																
	Total							1	6	7				14	13.6	16.6	0.0136 (0.0074–0.0229)
Hong Kong [c] (881)	V		1	1						1				1	1.1		
	IV	1						1	1	2				4	4.5	66.4	
	III																
	II																
	I																
	Total	1	1	1				1	1	3				5	5.7	65.1	0.0057 (0.0020–0.0127)

[a] Clinical types: I = afebrile URI; II = pharyngitis; III = bronchitis; IV = febrile URI; V = pneumonia.

[b] 1967 military formula.

[c] 1968 monovalent influenza A2/Aichi/2/68, 300 CCA units/ml.

TABLE 5

SEROLOGICAL EVIDENCE OF INFECTION WITH HONG KONG INFLUENZA IN VACCINATED AIRMEN REPORTING WITH RESPIRATORY ILNESSES

Vaccine group (and no. of men)	Serological response (≥4-fold HI titre rise)	No. of illnesses studied in indicated week													Positive (%)	Proportion (and 95% confidence interval)
		Vaccination		2 Dec. 68	Epidemic period									Total		
		18 Nov. 68	25 Nov. 68		9 Dec. 68	16 Dec. 68	23 Dec. 68	30 Dec. 68	9 Jan. 69	13 Jan. 69	20 Jan. 69	27 Jan. 69	3 Feb. 69			
Placebo (1 042)	Rise		0	0	0	0	4	2	4	12	7	2	1	32	56.1	0.5614 (0.4236–0.6926)
	No rise		0	3	2	5	4	2	4	3	2	2	1	25		
Polyvalent [a] (not in study)	Rise		0	0	4	4	3	7	3	1	0	1	0	23	35.4	0.3538 (0.2392–0.4823)
	No rise		12	10	10	9	4	4	1	1	3	8	2	42		
Polyvalent [a] (1 030)	Rise		0	0	0	0	0	1	2	10	2	0	0	15	36.6	0.3659 (0.2212–0.5306)
	No rise		0	2	3	1	3	2	4	4	3	4	2	26		
Hong Kong [b] (881)	Rise		1	1	0	0	0	0	0	1	1	0	0	2	7.7	0.0769 (0.0095–0.2513)
	No rise		1	1	3	0	3	2	2	9	1	2	2	24		

[a] 1967 military formula.

[b] 1968 monovalent influenza A2/Aichi/2/68, 300 CCA units/ml.

post-vaccination titres of the latter were considerably smaller than those produced by a 300-CCA-unit Hong Kong vaccine, as shown in Table 3. These were similar to the antibody increments caused by natural infection.

The protective effect of this monovalent Hong Kong vaccine was measurable at a significant level on the basis of out-patient visits alone, as shown in the upper part of Table 4. There was a 43.6% reduction in rates of all types of respiratory illnesses in vaccinated personnel. An even greater reduction in rate, 65.1%, of those hospitalized was observed, as shown in the lower part of Table 4. That statistically significant results could be obtained by clinical and epidemiological observations alone was partly due to the fact that 56% of the respiratory illnesses were caused by influenza A2 virus during the outbreak.

The effectiveness of the monovalent vaccine was even more strikingly shown when the results were analysed on the basis of serologically confirmed influenza, as shown in Table 5. Only 2 Hong Kong vaccine failures were detected among the sample studied. There were also fewer serological responses in the group that received the 1967 polyvalent vaccine as compared with those receiving placebo, but the reduction was not large enough for statistical significance.