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Influenza Immunization

Clinical Studies with Ether-split Subunit Vaccines *

F. B. BRANDON,[1] F. COX, E. QUINN, E. A. TIMM & I. W. McLEAN, JR

Clinical studies of ether-split influenza antigen vaccines have been in progress for almost a decade. One series of such studies, completed before the Hong Kong virus appeared, compared identically constituted conventional and antigen vaccines for serological effectiveness in 1700 vaccinees from the staff of a metropolitan hospital. A series of 6 annual trials included both "old" subjects (vaccinated the previous year) and "new" subjects (no vaccination the previous year). The serological response to the type A2 component of the antigen vaccines was 3–4 times better than that to intact virus in both the old and new populations. The response to either vaccine by new subjects significantly exceeded the response by the old subjects. The type B component of both vaccines induced an equivalent response in both populations. Monovalent Hong Kong vaccines, both conventional and antigen, given just prior to the Hong Kong epidemic induced an anamnestic response in a geriatric group. No influenza-like disease was seen in this high-risk group during the epidemic.

Although influenza virus vaccines have been in general use for almost a quarter of a century, only the last two years have seen significant improvements in the manufacturing methods of two decades ago. Recent innovations in continuous-flow centrifugal techniques applied on a production scale have provided influenza vaccines with lower levels of nonviral impurities than were previously attainable. However, the nature of the virus itself, where the immunizing surface proteins make up only a relatively small part of the total virus particle, indicates that intact virus vaccines, even those of extreme purity, are not without their drawbacks. Thus, while vaccine reactivity due to host cell and other nonviral impurities can be reduced or eliminated by physical purification, chemical refinement, which will allow the utilization of only those components of the virus particle essential for immunization, is needed to remove inherent viral toxicity.

The first steps pointing in the direction of such a vaccine were taken by Hoyle (1952), who showed that ether, while dissolving influenza virus lipids, destroyed the physical integrity of the virus particle along with its infective and pyrogenic properties and at the same time released certain biologically active antigens including the immunizing surface proteins.

Early clinical studies with the ether-treated vaccines confirmed the immunological effectiveness of the isolated antigens in children as reported by Davenport et al. (1964) and Brandon et al. (1967b), while demonstrating the virtual elimination of vaccine pyrogenicity in this young group.

Shortly after the appearance of the first type A2 virus strains, and in parallel with the early paediatric work, a series of adult studies with bivalent and polyvalent antigen vaccines was started among the staff of a Detroit metropolitan hospital. Emphasis in these studies, which compared the antigen vaccines with conventional vaccines of identical composition during non-epidemic years, was on the evaluation of the relative serological effectiveness of the two vaccines. In all, 6 annual studies with a total of 1700 vaccinees were completed before the 1968 respiratory disease season. In 1968 the appearance of the Hong Kong virus gave us an oppor-

* From the Medical and Scientific Affairs Division, Parke, Davis & Company, Detroit, Michigan; and the Department of Internal Medicine, Henry Ford Hospital, Detroit, Michigan, USA.

[1] Associate Laboratory Director, Department of Microbiology, Medical and Scientific Affairs Division, Parke, Davis & Company, P.O. 118 G.P.O., Detroit, Michigan 48232, USA.

tunity to compare, in an East Coast geriatric group,[1] the efficacy of conventional and antigen vaccines in the face of an epidemic. The serological results from these studies form the body of the following report.

METHODS AND MATERIALS

Vaccines

The pre-1968 studies utilized both bivalent and polyvalent vaccines, each of which contained the then current strains of types A2 and B influenza virus. Since these, and only these, strains were common to all vaccines in the series, the discussion will be limited to the type A2 and B results.

The 1968–69 Hong Kong influenza study differed from the earlier series in that only monovalent vaccines were utilized.

Throughout these studies, each antigen vaccine, whatever its composition,[2] was tested in parallel with a conventional vaccine of identical composition. Also, certain of the early, laboratory-scale antigen vaccines were prepared by processes which differed in detail from the process used today. However, the preparation of all vaccines included the basic steps of ether extraction and Sephadex gel filtration as reported by Brandon et al. (1967a).

Conventional vaccine. The conventional vaccine was prepared by formol inactivation following preliminary concentration and partial purification by differential centrifugation in the Sharples and International centrifuges. Preservative was added and the chicken-cell agglutinating (CCA) activity determined. An appropriate volume of the monovalent preparation was then diluted with 0.01 M phosphate-buffered isotonic saline at pH 7.2 to give the final desired concentration for use.

Antigen vaccine.[3] The concentrated, partially purified, active influenza virus preparation was treated with ether at 4°C for a period of about 5 hours. Monovalent preparations were further purified by Millipore and gel filtration and a preservative was added. In general, during developmental vaccine studies, CCA titrations of the ether-extracted preparations exhibited considerable variability. Hence, antigen vaccines were usually prepared by equivalent dilution to contain, assuming no loss, the same antigen mass as that contained in the conventional product, thus ensuring that an essentially equal quantity of immunizing antigen would be present in the two materials without regard to the CCA activity of the extracted preparations.

Subjects

All 1700 vaccinees in the pre-1968 studies were hospital staff volunteers, selected as being normal healthy adults between 18 and 65 years of age with no history of allergy or sensitivity to egg protein. The 1968–69 study was carried out in 437 residents in a retirement community with an average age of 78.3 years and of whom only 7 (1.6%) were less than 65 years old at the time of the study.

Treatment schedule

Early in the course of the hospital staff studies it became evident that an individual's serological response to vaccination was strongly influenced by whether or not he had received influenza immunization during the 12 months or so preceding the current programme. Accordingly, all subjects in each annual group were classified as being either "old" or "new", depending on whether or not they had been immunized with influenza vaccine during the preceding year. Thus, in effect, 4 treatment groups were created: (*a*) old subjects given conventional vaccine, (*b*) old subjects given antigen vaccine, (*c*) new subjects given conventional vaccine, and (*d*) new subjects given antigen vaccine.

Within each population (old *versus* new), subjects were assigned at random to the 2 vaccines (conventional *versus* antigen) and subsequent χ^2 testing showed that the randomization procedure had been successful in that the age and sex distribution between the paired vaccine groups (within each population) was not significantly different at the 5% level. A tabulation of assignments to treatment groups within each of the 6 hospital staff studies is shown in Table 1. In general, each subject received a 1-ml dose of the appropriate vaccine at day 0 and bloods for serological evaluation were drawn at day 0 and 2–4 weeks following immunization.

Similar procedures were followed with the geriatric group given the 1968 Hong Kong vaccines.

Serological testing

All sera were assayed for specific antibody against each of the vaccine viruses by the haemagglutina-

[1] The authors gratefully acknowledge the special contribution of Henry A. Cromwell, M.D., Director, Meadow Lakes Retirement Community, Hightstown, N. J., in directing this study.

[2] The composition varied from year to year according to the current recommendations of the Division of Biologics Standards of the US National Institutes of Health.

[3] Fluogen, Parke, Davis & Company.

tion-inhibition (HI) test, with pre- and post-vaccination sera being tested simultaneously to eliminate day-to-day variation in the test procedure as a factor in evaluating post-vaccination antibody changes. Hirst et al. (1942) have shown that the spread of human HI antibody titres approaches a normal frequency distribution when expressed as logarithms. Accordingly, geometric mean titres were derived from the data expressed in this form.

Subjective reactions

During the 1968 Hong Kong study only, each subject was given a subjective-reaction questionnaire on which he was asked to indicate by checks in the appropriate spaces which, if any, of several local or systemic reactions had occurred as a result of inoculation. This work is described in detail by Cromwell et al. (1969).

RESULTS

Pre-1968 hospital staff studies

Table 1 shows the assignment to treatment groups of subjects in each of the first 6 studies and the increase in geometric mean HI antibody titre against the type A2 virus by treatment group. Group response varied considerably from year to year; however, the over-all response pattern is quite clear. Within both the old and new populations, serological response to the antigen vaccine exceeded response induced by its conventional counterpart to a significant degree. Also, as expected because of their lower initial titres, titre increases of new subjects were higher than those of old subjects.

Table 2 shows the response by treatment group to the type B component of the 2 vaccines. In this case, the antigen and conventional vaccines produced almost identical responses within each population, although, again, a population response differential in favour of new subjects is evident.

To provide an over-all view, the response data within each treatment group were pooled across all 6 studies. Table 3 shows the frequency of pre- and post-vaccination HI antibody titres against the type A2 vaccine component within each of the 4 cumulative treatment groups.

It is again evident that within each population the antigen vaccine elicited a distinctly superior serological response when compared with the conventional product, and that new subjects, regardless of the vaccine given, responded better than old.

Table 4 gives analogous information for the type B strain.

The data in Tables 3 and 4 can be used to estimate not only the statistical significance of titre increases following vaccination but also the significance of differences between final titres attained by different treatment groups.

Geometric mean titre increases against the A2 component following vaccination were, as expected, highly significant, with the smallest increase (in the old group given conventional vaccine) exceeding

TABLE 1

SUMMARY OF INCREASES IN GEOMETRIC MEAN HI ANTIBODY TITRE TO TYPE A2 VIRUS IN EACH OF 6 HOSPITAL STAFF STUDIES

Study in chronological order	"Old" subjects				"New" subjects			
	Conventional vaccine		Antigen vaccine		Conventional vaccine		Antigen vaccine	
	No. of subjects	Increase (x-fold)	No. of subjects	Increase (x-fold)	No. of subjects	Increase (x-fold)	No. of subjects	Increase (x-fold)
1	70	2.2	69	2.3	9	43.1	9	69.2
2	29	1.8	34	2.8	49	5.5	48	15.6
3	94	2.8	90	5.5	99	3.2	103	11.9
4	54	1.5	60	2.3	101	4.5	97	16.1
5	105	4.5	81	12.0	92	18.0	80	49.8
6	135	1.2	90	2.1	52	3.1	50	23.3
Total subjects	487	911	424		402	789	387	

TABLE 2

SUMMARY OF INCREASE IN GEOMETRIC MEAN HI ANTIBODY TITRE TO TYPE B VIRUS IN EACH OF 6 HOSPITAL STAFF STUDIES

Study in chronological order	"Old" subjects				"New" subjects			
	Conventional vaccine		Antigen vaccine		Conventional vaccine		Antigen vaccine	
	No. of subjects	Increase (*x*-fold)	No. of subjects	Increase (*x*-fold)	No. of subjects	Increase (*x*-fold)	No. of subjects	Increase (*x*-fold)
1	70	1.5	69	2.1	9	4.7	9	16.1
2	29	1.1	34	1.6	49	2.2	48	2.3
3	94	2.7	90	2.5	99	3.0	103	3.0
4	54	1.2	60	1.2	101	3.5	97	2.6
5	105	1.5	81	2.0	92	4.9	80	6.4
6	135	0.9	90	1.1	52	2.2	50	5.1
Total subjects	487	911	424		402	789	387	

its own standard error by a factor of about 8, and the largest (in new subjects given the extracted vaccine) being more than 20 times greater than its own standard error.

Of particular interest is the fact that within each population (old and new) the final titres attained by the groups given the antigen vaccine exceeded the corresponding conventional vaccine group titres to a highly significant degree ($P>0.01$), and that when the same vaccine (conventional or antigen) is compared in the 2 population groups (old *versus* new) it is found that new subjects have a significantly

TABLE 3

FREQUENCY DISTRIBUTION OF TYPE A2 PREVACCINATION HI ANTIBODY TITRES IN THE "OLD" AND "NEW" POPULATION GROUPS AND OF POST-VACCINATION TITRES IN EACH TREATMENT GROUP

	HI antibody titre [a]										Total subjects	Geometric mean titre (and *x*-fold increase)
	<5	5	10	20	40	80	160	320	640	≥1 280		
"Old" subjects												
Prevaccination	134	32	112	133	175	153	94	42	16	9	900	29.3
Post-vaccination with: Conventional vaccine	17	9	33	78	84	101	72	52	20	15	481	64.6 (2.20)
Antigen vaccine	13	7	23	44	49	76	75	59	37	36	419	108.7 (3.71)
"New" subjects												
Prevaccination	184	61	162	146	104	66	31	12	5	0	771	13.5
Post-vaccination with: Conventional vaccine	12	9	24	55	76	68	50	39	24	36	393	83.8 (6.19)
Antigen vaccine	14	4	8	18	45	44	53	31	46	115	378	265.5 (19.6)

[a] Reciprocal of dilution.

TABLE 4

FREQUENCY DISTRIBUTION OF TYPE B PREVACCINATION HI ANTIBODY TITRES IN THE "OLD" AND "NEW" POPULATION GROUPS AND OF POST-VACCINATION TITRES IN EACH TREATMENT GROUP

	HI antibody titre [a]										Total subjects	Geometric mean titre (and x-fold increase)
	<5	5	10	20	40	80	160	320	640	⩾1 280		
"Old" subjects												
Prevaccination	97	104		190	141	99	58	22	6	2	905	19.4
Post-vaccination with: Conventional vaccine	14	25	95	121	109	69	34	12	3	2	484	28.2 (1.45)
Antigen vaccine	14	21	66	98	89	77	33	15	8	0	421	32.4 (1.67)
"New" subjects												
Prevaccination	256	1	170	97	67	21	6	9	0	0	786	7.7
Post-vaccination with: Conventional vaccine	15	34	75	110	76	48	23	12	3	5	401	26.0 (3.38)
Antigen vaccine	16	31	77	100	66	43	26	19	6	1	385	26.5 (3.44)

[a] Reciprocal of dilution.

($P > 0.05$) higher final titre than old subjects. Thus, new subjects not only respond to vaccination with a greater increase in titre than old subjects (owing in part to the lower initial titre in the new group), but also attain a significantly higher final titre, indicating that response in the old group is not limited by a hypothetical response ceiling in the general population.

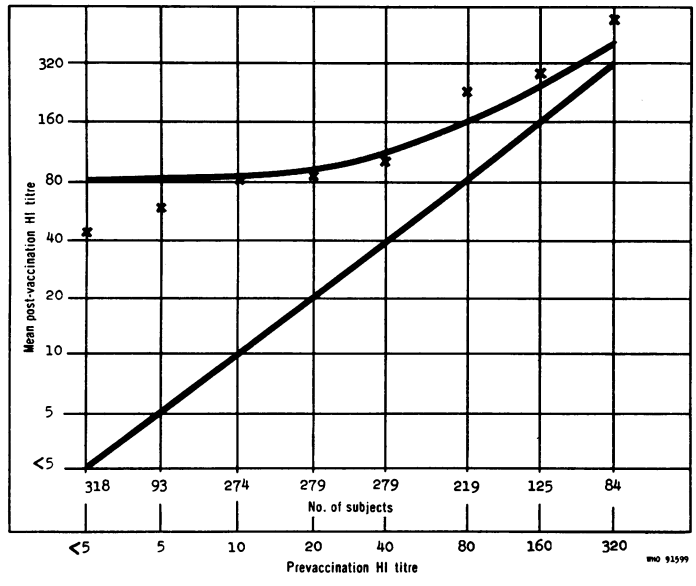
As with the type A2 vaccines, significant increases in geometric mean titre were seen in all type B treatment groups following vaccination. However, final titres in all groups were essentially identical regardless of the stimulatory vaccine.

Vaccines can be ranked with respect to their antigenicity in terms of titre increases and average final titres attained in a given population. Such values, however, do not tell us how much antibody is actually being elicited by vaccination. Since it was of interest to estimate this parameter, we turned to a procedure used by Hirst et al. (1942) over 25 years ago which showed that the average actual antibody production following vaccination (as opposed to increase in titre) was about the same for all subjects regardless of their prevaccination antibody level. The process is illustrated in the accompanying figure, where the data from all 1671 subjects providing pre- and post-vaccination A2 HI antibody titres have first been grouped by prevaccination antibody level and the post-vaccination geometric mean titre of each such group is shown as points in the figure. The straight line indicates the prevaccination titre level and the curved line shows the geometric mean antibody level that would have been achieved if 80 units of antibody had been added to each prevaccination specimen.

Table 5 shows the cumulated data plotted in the figure as well as the corresponding data from each of the 4 type A2 treatment groups. By plotting the data from each of the groups as in the figure and fitting (by inspection) the corresponding curved lines, it is estimated that 20, 60, 60 and 240 units of type A2 antibody were induced in the following treatment groups respectively: (*a*) old subjects given conventional vaccine, (*b*) old subjects given antigen vaccine, (*c*) new subjects given conventional vaccine, and (*d*) new subjects given antigen vaccine.

Similar procedures with the type B data (not shown) yielded estimates of type B antibody increases of about 10 and 20 units in the old and new populations respectively, regardless of the kind of vaccine used.

GEOMETRIC MEAN POST-VACCINATION HI ANTIBODY TITRES TO A2 VIRUS OF ALL VACCINEES AS A FUNCTION OF PREVACCINATION TITRE LEVEL [a]



[a] The straight line represents the prevaccination titre level and the curved line represents the post-vaccination titre level that would have been achieved if 80 units of antibody had been added to each specimen.

TABLE 5

GEOMETRIC MEAN POST-VACCINATION HI ANTIBODY TITRES TO A2 INFLUENZA VIRUS WITHIN EACH TREATMENT GROUP (AND OVER ALL TREATMENT GROUPS) AS A FUNCTION OF PREVACCINATION HI TITRE

Prevaccination HI antibody titre	" Old " subjects [a]		" New " subjects [a]		All subjects [a]
	Conventional vaccine	Antigen vaccine	Conventional vaccine	Antigen vaccine	
<5	15.5 (71)	26.6 (63)	44.1 (86)	138.4 (98)	44.8 (318)
5	14.8 (16)	41.7 (16)	41.7 (33)	190.3 (28)	55.1 (93)
10	27.5 (55)	45.7 (57)	102.0 (79)	242.5 (83)	85.7 (274)
20	37.3 (71)	72.6 (62)	69.7 (79)	380.6 (67)	90.2 (279)
40	79.5 (105)	115.5 (70)	96.5 (52)	280.5 (52)	115.0 (279)
80	125.6 (75)	249.4 (78)	231.1 (36)	584.9 (30)	218.9 (219)
160	251.1 (51)	320 (43)	231.1 (17)	580.8 (14)	296.1 (125)
≥320	425.2 (37)	844.5 (30)	362.5 (11)	1 018.3 (6)	565.3 (84)

[a] Numbers of subjects in parentheses.

One additional comment concerning the data in the figure is needed. It is apparent that the geometric mean post-vaccination titres of those subjects with relatively low prevaccination titres fall below the superimposed curved line, while the post-vaccination titres of those with high initial antibody fall above the line. At first glance this would seem to dilute the value of these observations. However, we interpret these data as showing that presorting has occurred in such a way that those subjects at the lower end of the pre-vaccination titre scale are there by virtue of a predisposition to respond relatively poorly to influenza vaccination or disease, while those on the upper part of the titre scale have a predisposition to respond relatively well to influenza antigen stimulation. Under these circumstances, it would be expected that those initially in the lower segment of the prevaccination titre scale would have a lower than average response to current vaccination while those in the upper part of the scale would have a better than average response.

1968–69 Hong Kong vaccine geriatric study

Serology. As reported by Cromwell et al. (1969), approximately 20% of the 437 geriatric subjects were bled, with a total of 76 individuals providing pre- and post-vaccination serum specimens. As expected, a relatively high proportion, almost 80%, of this older group had pre-existing HI antibody to the Hong Kong strain. However, prevaccination titres were generally low and the geometric mean prevaccination titre for the entire group was 17.8.

Unlike the hospital staff group, where each previously vaccinated or old subject was immunized with a strain or strains of virus closely related or identical to the strain(s) received the preceding year, the geriatric population had obviously never before been vaccinated with Hong Kong virus, although nearly half of them had been given influenza immunization the previous year. Recalling the effect that prior vaccination with homologous strains had on response in the hospital group, it was of considerable interest to determine what effect if any prior stimulation with a heterologous strain would have on response to the Hong Kong vaccines.

Table 6 shows the distribution of the 76 geriatric subjects with respect to vaccine assignment and immunization history. Clearly, the type of vaccine had little influence on response, with both the conventional and antigen vaccine groups having about a 20-fold increase in titre. However, vaccination history again seemed to play an important part in determining subject response, with subjects not immunized within the preceding year responding with more than twice the antibody titre of the previously vaccinated group.

Vaccine efficacy. The geriatric study was started during the week of 2 December 1968, just as excess pneumonia–influenza deaths (presumably associated with the Hong Kong influenza epidemic) began to appear in the Middle Atlantic geographical region as defined in the National Communicable Disease Center's morbidity and mortality weekly reports. Excess deaths continued through the ensuing 8–10-week period, reaching a peak rate about 3 times normal around the first of January. In spite of the fact that the retirement community remained open to the public and daily contacts occurred between all its members and the general public throughout the epidemic period, not a single case of influenza-like disease was seen in the resident group.

Subjective reactivity. Since the vaccinee himself is the best judge of vaccine reactivity, the post-immunization subjective-reaction questionnaire,

TABLE 6
DISTRIBUTION OF GERIATRIC SUBJECTS WITH RESPECT TO TYPE AND HISTORY OF VACCINATION, AND GEOMETRIC MEAN POST-VACCINATION TITRES

Vaccine type	Vaccination history			Geometric mean titre of group (and *x*-fold increase)
	Old	New	Total	
Conventional	18	22	40	320 (18.0)
Antigen	17	19	36	365 (20.5)
Total	35	41	76	
Geometric mean titre of group (and *x*-fold increase)	228 (12.8)	482 (27.1)		341 (19.2)

TABLE 7
PERCENTAGE OF RETIREMENT COMMUNITY RESIDENTS AND STAFF REPORTING SUBJECTIVE REACTIONS TO HONG KONG VACCINE

	Local reaction		Systemic reaction	
	Reported (%)	Not reported (%)	Reported (%)	Not reported (%)
Residents given:				
Conventional vaccine	24.5	75.5	6.7	93.3
Antigen vaccine	7.8	92.2	5.3	94.7
Employees given:				
Conventional vaccine	51.2	48.8	47.7	52.3
Antigen vaccine	26.9	73.1	18.1	81.9

which tabulates such judgements, provides a useful tool for determining the relative reactivity of different vaccines dispensed simultaneously through a homogeneous population. As demonstrated by Cromwell et al. (1969), this technique was successful in showing first that conventional vaccine reactivity has changed little over the last quarter of a century, and second that the antigen vaccines are from ½ to ¼ as reactive as their conventional counterparts both in working-age adults and in the geriatric cohort. The data are summarized in Table 7, which shows the percentage of retirement community residents (and community staff, a younger, working-age group not described elsewhere in this report) reporting local and systemic reactions to vaccination with either the conventional or antigen Hong Kong vaccines. The substantially reduced reactivity of the antigen vaccine is clearly evident in both the resident and staff groups.

DISCUSSION

Influenza virus and the problems of its control continue to engender widespread interest. Until quite recently the imperfections of influenza vaccines—i.e., their reactivity—have limited their use to the traditional high-risk groups, bypassing both the general public and the paediatric group where, if control is ever to be achieved, it must begin.

The antigen vaccines discussed here remedy many of the imperfections. Chemical treatment has virtually eliminated vaccine pyrogenicity, as demonstrated in children and in the geriatric group. Similarly, subjective reactions to vaccination induced by the antigen vaccines are much less frequent in adults and in the geriatric group than reactions following conventional immunization. Finally, the immunogenicity of the type B virus is fully retained during extraction while that of the type A2 virus is apparently enhanced by this process. Also, the A2 antigen vaccine appeared fully effective in the face of natural challenge as shown by the total absence of disease in the geriatric population during the Hong Kong influenza epidemic. However, the conditions of this study, where rapid and complete coverage of the high-risk population was the goal, precluded controlled epidemiological observations with an unimmunized group. Hence, the possibility that the high proportion of individuals with naturally occurring antibody also contributed to the lack of influenza illness in the total group cannot be excluded. Nevertheless, original titres to the Hong Kong variant were very low, and at the time of the epidemic peak had increased many-fold as a result of vaccination—an increase which almost certainly was accompanied by an increase in resistance to infection.

The reason for the enhanced immunogenicity of the A2 antigen preparations is not clear. It has been shown that the mean increase (x-fold) in neutralizing antibody titre following vaccination parallels the mean increase in HI titre. Thus, since the antigen vaccines were carefully prepared to contain the same mass of immunizing protein as their intact counterparts, there seems little question but that the extracted A2 antigens are more efficient stimulators

of both HI and neutralizing antibody than is intact virus.

The type B component of the antigen vaccines did not exhibit the enhanced immunogenicity associated with the type A2 antigens. Although a similar observation has been reported earlier by Hennessy & Davenport (1966), the reasons for the different response to the antigens derived from the 2 viruses are not known.

Old and new subjects, as defined in the context of this paper, are clearly different with respect to their responsiveness to influenza vaccination. At first glance we might expect that the apparent " refractoriness " of the old subjects is due simply to the more abundant preinjection antibody present in this group which might limit the effectiveness of injected antigens by combining with them, thus preventing stimulation of antibody-producing cells. However, when the prevaccination antibody titre is removed as a variable by comparing groups of new and old subjects of equal prevaccination titre (as in Table 5), it is apparent that, within each group, new subjects respond to vaccination with a greater increase and higher final titre than old subjects given identical stimulation.

It is of interest to note that the refractoriness of the old subjects is not strictly a homologous strain phenomenon since the geriatric group, who had never been given Hong Kong vaccine, displayed a similar response picture. On the other hand, it is likely that the lowered responsiveness of the old subjects applies only to subsequent influenza vaccination, since it has been determined (unpublished observations) that old and new subjects (with respect to influenza immunization) responded equally well to all components of a trivalent adenovirus vaccine.

On balance, the future of influenza immunization appears bright. Additional chemical refinement of virus preparations, already highly purified by physical means, introduces the concept of immunization with only the essential viral components, and seems the attainable goal of present technology—until genetic or other more fundamental controls of the virus become available. The antigen vaccine studied here approaches this goal.

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