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New Criteria for the Selection of Influenza Vaccine Strains

S. FAZEKAS DE ST. GROTH [1]

Antigenic relationships are defined by 2 criteria: distance and symmetry. Viruses within a subtype of influenza A are closely related, with the more junior antigens being superseded in time by senior ones. This phenomenon can be reproduced experimentally. Subtypes are distantly related to one another.

Immunity to a junior virus is not readily transformed into immunity to a senior antigen given later (original antigenic sin), whereas senior viruses do not jeopardize the efficiency of junior vaccines given subsequently.

It is proposed that future vaccines should contain senior mutants of a strain from the most recent epidemic (prospective vaccines), and no junior antigen.

With antigenically stable pathogens—the viruses of poliomyelitis, for example—prophylaxis reduces to finding a safe way of administering the required dose of viral antigen. Mutable pathogens—and there is no better example of these than the viruses of influenza—pose additional problems by the very fact of their mutability. It is my aim here to look into the mechanism of this variation and see what means we have of anticipating and countering antigenic shifts. Put crudely, I am calling the odds of beating the virus at its own game.

CRITERIA OF ANTIGENIC RELATIONSHIP

As a preliminary, we take a look at the operations by which antigenic relationships are defined. The elementary assay here is a checkerboard test; in its simplest form two viruses and their corresponding antisera are titrated against each other by haemagglutination-inhibition testing. If we choose, say, the A2/Kong Kong/1/68 (HK) strain and a strain—A2/NT/60/68 (NT)—isolated in the Northern Territory of Australia in the same year, we get results shown in Table 1.

For convenience, such a matrix can be made independent of the actual titres if each entry within a column is divided by the homologous titre. Such a derived *normal matrix* is also shown in Table 1, and it needs no elaborate mathematics to see that

TABLE 1
COMPARISON OF TWO HONG KONG-TYPE VIRUSES [a]

OBSERVED TITRES	anti-HK	anti-NT
HK	3 25	3 38
NT	3 20	3 38

NORMALIZED TITRES	anti-HK	anti-NT	EVALUATION
HK	0	0 00	SUM = 0 05
NT	0 05	0	DIFF = -0 05

CONCLUSION: HK and NT identical

[a] All entries are in $\log_{10}$ units.

HK and NT are *identical*: both the sum of normal titres and their difference (i.e., the log ratio of their average cross-reactions) fall within the error of the test (in this case ± 0.11 $\log_{10}$ unit).

We then take another pair, two earlier A2 strains, A2/Singapore/57 (Sing.) and A2/PR/1/64 (PR1). By looking down the columns of the normal matrix (Table 2) it is obvious that these two are pretty *closely related*, but their crossing is *asymmetric*. The anti-PR1 serum inhibits Sing. virus more effectively than does anti-Sing. serum the PR1 virus. I shall describe this relationship by calling PR1 *senior* to Sing.

Comparing the prototype strains of A1 and A2 (Table 3), we find that Cam (A1/Cam./46) and Sing. are *distantly related* and about *equivalent*: the homologous reactions are about 50-fold higher than the

[1] Chief Research Scientist, Division of Animal Genetics, Commonwealth Scientific and Industrial Research Organization, Epping, New South Wales, Australia.

heterologous, but their ratio falls within the error of these tests, indicating symmetric crossing.

A similar test performed on Sing. and, say, PR8 (Table 4) shows that these two are not only *distantly related*, but give also *asymmetric* crossing: Sing. is senior to PR8.

TABLE 2
COMPARISON OF TWO A2 VIRUSES [a]

OBSERVED TITRES

	anti-SING	anti-PR1
SING	4 20	2 83
PR1	3 02	3 33

NORMALIZED TITRES

	anti-SING	anti-PR1
SING	0	0 50
PR1	1 18	0

EVALUATION: SUM = 1 68; DIFF = -0 68 ***

CONCLUSION: SING and PR1 closely related
SING junior to PR1

[a] All entries are in $\log_{10}$ units. *** = P>0.001.

TABLE 3
COMPARISON OF AN A1 AND AN A2 VIRUS [a]

OBSERVED TITRES

	anti-SING	anti-CAM
SING	4 20	1 81
CAM	2 42	3 55

NORMALIZED TITRES

	anti-SING	anti-CAM
SING	0	1 74
CAM	1 78	0

EVALUATION: SUM = 3 52 ***; DIFF = -0 04

CONCLUSION: SING and CAM distantly related
SING equivalent to CAM

[a] All entries are in $\log_{10}$ units. *** = P>0.001.

TABLE 4
COMPARISON OF AN A0 AND AN A2 VIRUS [a]

OBSERVED TITRES

	anti-SING	anti-PR8
SING	4 20	1 97
PR8	2 68	4 06

NORMALIZED TITRES

	anti-SING	anti-PR8
SING	0	2 09
PR8	1 52	0

EVALUATION: SUM = 3 61 ***; DIFF = +0 57 ***

CONCLUSION: SING and PR8 distantly related
SING senior to PR8

[a] All entries are in $\log_{10}$ units. *** = P>0.001.

A MODEL OF IMMUNOLOGICAL CROSSING

Within the framework of antigenic analysis defined above, the 4 examples exhaust the possibilities of antigenic similarity and symmetry. What we need now is some simple picture of antigen–antibody interaction which will account for the experimental facts. To this end we make use of a single premise only, and one that has been abundantly proven in practice, namely, that the response to any antigen is heterogeneous, and hence every antiserum contains several subpopulations of slightly different antibody molecules, each capable of binding, more or less firmly, its stimulating antigen.

An antigenic area on a viral coat protein may be thought of as a small number of amino-acid side-chains jutting out of the surface. Schematically, we represent these as a fork with prongs of variable length and width (Fig. 1), and the corresponding antibodies as cavities complementary to some or all of the prongs. On first principles, the firmness of combination (avidity) of a particular subclass within the antibody population should be proportional to its complementary area, and if we dissociate formed virus–antibody complexes the least avid antibodies should come off first. This experiment has been performed on several antigen–antibody systems—we have done it with influenza—and has given the expected results.

Now, what happens if we test such a serum against a related antigen? I have chosen one that differs by a single amino-acid only (Fig. 2), and it is plain that any antibody molecule that is complementary to spot x will be held at arm's length by side-chain x': part of antibody population A will be sterically hindered from reacting with antigen B, while the rest may make satisfactory contact. If we look at the reverse situation, all subclasses of antibody population B will also combine with antigen A but, on the whole, not as firmly as with their homologous antigen since the side-chain x will not fit snugly into the cavity corresponding to x'. Thus we shall have asymmetric crossing, with antigen B senior to antigen A.

Let us now take a third antigen which differs from the first two at locus y. Obviously, no member of antibody population B complementary to its antigen at y will be able to make full contact with C and, conversely, no antibody of population C complementary to x will combine with antigen B (Fig. 2). This excludes the bulk of the two populations from cross-reacting: the relationship will be distant. The

FIG. 1

SCHEMATIC REPRESENTATION OF AN ANTIGENIC DETERMINANT AND OF COMPLEMENTARY AREAS FROM THE POPULATION OF ANTIBODIES PRODUCED BY IMMUNIZATION WITH ANTIGEN A2

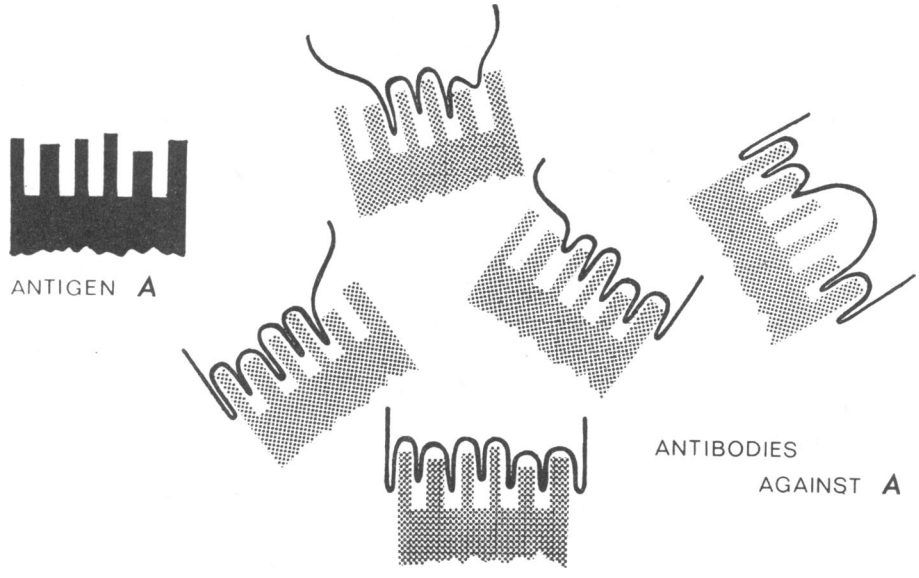
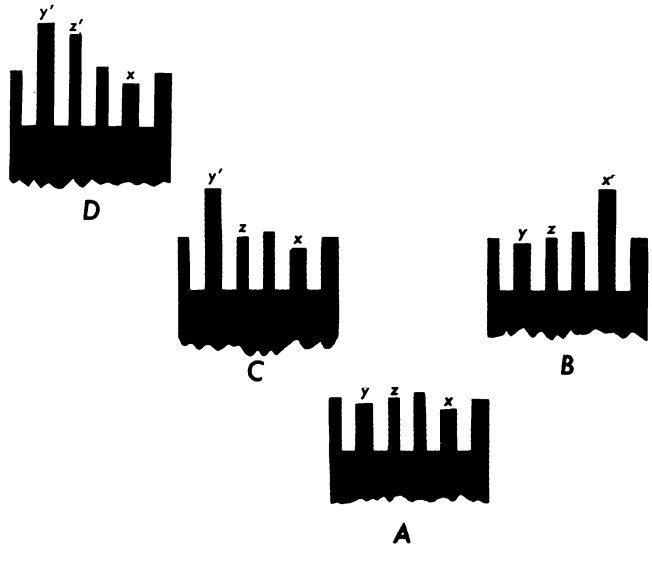


FIG. 2

SCHEMATIC REPRESENTATION OF THE EVOLUTION OF A FAMILY OF ANTIGENS[a]



[a] The side-chains of mutant amino-acids are indicated by the primed letters.

rest of the molecules may react symmetrically, making the two antigens equivalent in our terminology. And, by the same token, patterns *B* and *D* would lead to distant, asymmetric crossing.

EVIDENCE FOR THE MODEL

Cross-absorption of antisera

We take a closely related junior–senior pair of viruses, PR8 and Mel. for example, and exhaustively cross-absorb their antisera. We find that over 95% of the anti-Mel. population is bound to PR8, while the senior virus, Mel., binds less than 20% of the junior anti-PR8 antibody. This proves that asymmetry rests on the exclusion of certain classes of antibody from cross-reactions. Examples of this behaviour may be found throughout the literature, by extracting information from carefully conducted cross-absorption tests.

Mutants of junior viruses

A junior virus, PR8, is grown in the presence of homologous antiserum or, better, in the most avid fraction of such an antiserum, prepared by chromatography on the homologous antigen. The test system is so adjusted that about 5 out of 10^9 particles grow through. (In practice this amounts to inoculating 10^7 infective units per cup of tissue and adding to the medium 2.5–3.0 times the amount of antibody that would give 50% neutralization.) We find that all the isolated clones are antigenic mutants, and all are senior to the parental antigen. This proves that selection by antibody is for seniority, and gives us also a strong hint of what might be going on in the field where the virus has to survive in an environment increasingly saturated with antibody.

Mutants of senior viruses

A senior virus, Bel. (A0/Bellamy/42), is grown in the presence of homologous antibody. If we use the most avid fraction of such an antiserum, the mutants that can be isolated differ from the parental strain not antigenically but by their affinity to the host cell: all of these mutants are significantly lower down in the receptor gradient (both for red cells and for the bronchial epithelium of mice) than is characteristic of the original Bel. strain. The practical consequence is that such mutants score as Q-phase variants in antihaemagglutinin tests: their antisera give higher titres against the parental Bel. virus than against the vaccinating Bel. mutant.

If we use a less avid fraction of the same antiserum, we do get antigenic mutants, and all of these score as distant-equivalent. Both classes of mutants are exactly as expected on the model.

Each of these tests is fairly critical, but even taken together they do not *prove* our hypothesis. For that we would need to know, in exact molecular terms, what an antigenic determinant is and what the combining areas of an antibody molecule are. In the meantime, since the tests are both critical and compatible with our model, we conclude that our hypothesis has at least a right to exist. And that is all the encouragement we need to look anew at the family of influenza viruses, this time not with an open mind but with a thorough bias: we set out to rank them in order of seniority.

HIERARCHY OF INFLUENZA A VIRUSES

The operations involved in hierarchic ordering are the same as the elementary 2×2 comparisons, but on an heroic scale: we take as many epidemic strains and their specific antisera as we can handle, and perform all possible cross-titrations. I have done this with 21 strains and about 10 times as many antisera, and the entries of Table 5 are the normalized titres of each group averaged over 4 to 8 bleeds of 2 rabbits each.

The hierarchic relations derived from Table 5 are shown in Fig. 3. The first thing we notice is that there are 4 groups which give distant cross-reactions with each other, but enclose viruses which are closely related. And this relationship is far from random. Within each subtype the strains climb the ladder of seniority, in much the same way as we can select senior mutants *in vitro* under the pressure of antibody. The initiator of a subtype is always the distant-equivalent of an historically earlier subtype. On this evidence I take the liberty of calling the Hong Kong-type strains subtype A3.

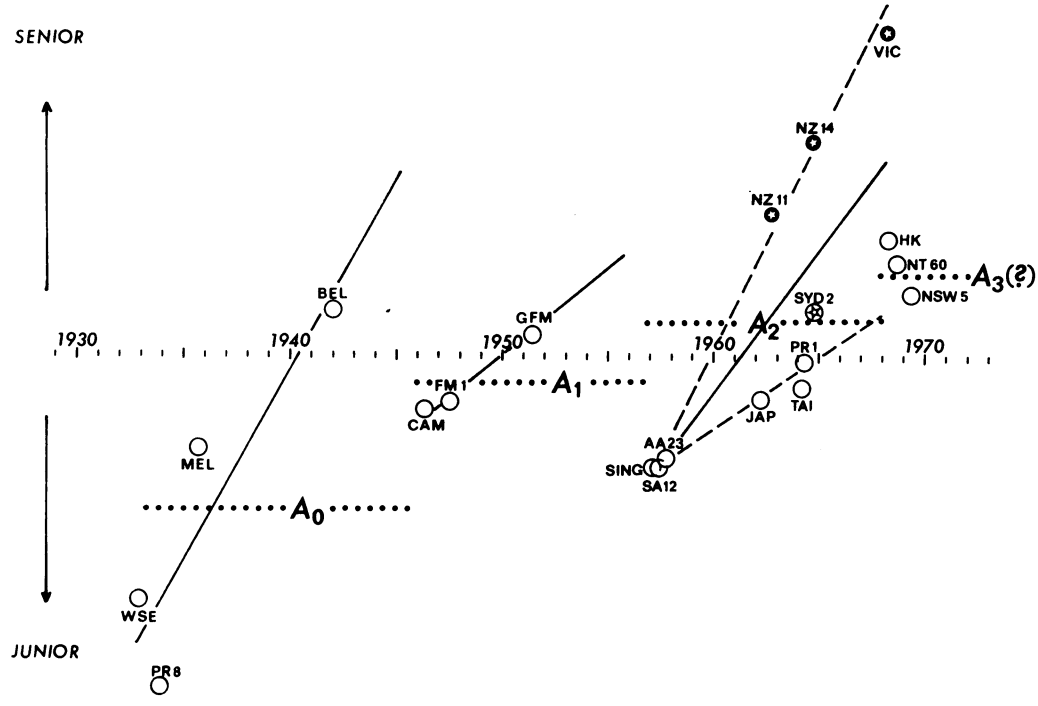
There is also another remarkable thing in Fig. 3. Towards the middle of the A2 era there is a striking separation between strains isolated in the South Pacific region and those coming from elsewhere. The former group (starting with A2/NZ/11/62 and A2/Sydney/2/64, and culminating in A2/Victoria/4/68) are the only strains which are senior to both the standard A2 viruses and the Hong Kong group. They do not fit comfortably into the A2 classification: their relationship to nearly all members is both highly asymmetric and more distant than one would expect within a subtype. I shall call them

TABLE 5
NORMALIZED MATRIX OF INFLUENZA A VIRUSES[a]

Serum	Test antigens																				
	WSE	PR8	Mel.	Bel.	Cam.	FM1	GFM	Sing.	SA/12	AA	Jap.	NZ/11	Taiw.	PR/1	NZ/14	Syd.	Vic.	HK	NT	NSW	Swine
A0/WSE/33	0.00	0.62	0.75	2.14	0.88	0.79	1.46	2.40	2.08	1.26	1.04	2.59	1.59	0.73	2.38	1.07	2.46	2.41	2.40	2.45	1.39
A0/PR/8/34	1.18	0.00	1.26	1.71	1.41	1.48	1.69	2.09	1.59	1.34	1.52	2.31	1.79	1.30	1.93	1.35	2.39	2.29	2.29	2.18	1.42
A0/Mel./35	0.59	0.34	0.00	1.36	1.11	0.90	1.38	2.26	1.49	1.17	1.32	2.44	1.57	1.05	2.22	1.12	2.13	2.18	2.19	2.06	1.21
A0/Bellamy/42	1.13	0.05	1.05	0.00	1.34	1.24	1.73	2.02	1.53	1.21	1.41	2.22	1.61	1.31	1.97	1.20	2.28	2.07	2.16	1.87	1.34
A1/Cam./46	0.62	0.65	0.91	1.61	0.00	0.37	1.23	1.74	1.10	0.64	1.05	2.19	1.29	0.67	1.80	0.70	2.09	2.15	2.17	2.01	0.85
A1/FM/1/47	0.39	0.52	0.76	1.53	0.53	0.00	0.78	1.42	0.88	0.47	1.03	2.16	1.16	0.44	1.43	0.45	2.02	2.04	2.06	1.77	0.61
A1/GFM/51	0.24	0.24	0.54	1.60	0.47	0.36	0.00	1.88	0.87	0.43	0.70	1.97	0.95	0.36	1.79	0.56	1.90	1.63	1.66	1.52	0.06
A2/Singapore/57	1.49	1.52	1.72	2.71	1.78	1.72	1.92	0.00	0.30	0.28	0.57	1.43	1.21	1.19	1.63	0.61	2.13	2.14	2.14	2.08	1.75
A2/SA/12/57	1.51	1.19	2.17	2.11	1.91	1.63	1.41	0.36	0.00	0.55	0.58	1.13	1.11	1.26	1.47	0.45	1.23	1.55	1.56	1.42	1.65
A2/AA/23/57	0.92	1.06	1.23	2.49	1.30	1.10	1.36	0.11	−0.18	0.00	0.07	1.05	0.80	0.79	1.16	0.20	1.62	1.90	1.87	1.87	1.71
A2/Japan/170/62	1.01	0.77	1.39	1.51	1.16	1.02	0.98	0.40	0.49	0.50	0.00	0.50	0.71	0.62	0.90	0.43	1.06	0.93	0.96	0.70	1.16
A2/NZ/11/62	0.75	0.36	1.35	1.52	1.16	0.87	0.60	0.93	1.35	1.01	0.50	0.00	1.29	1.11	1.81	1.09	1.96	1.50	1.54	1.36	1.04
A2/Taiwan/64	1.38	1.00	1.61	1.58	1.41	1.22	1.19	0.84	0.98	1.01	0.66	1.42	0.00	1.02	1.38	0.71	1.57	1.22	1.22	0.98	1.36
A2/PR/1/64	0.83	0.74	1.28	1.67	0.95	0.90	1.01	0.50	0.56	0.47	0.06	0.97	0.50	0.00	0.65	0.28	1.25	1.39	1.39	0.92	1.08
A2/NZ/14/64	0.76	0.52	1.36	1.47	1.05	0.89	0.67	0.66	0.76	0.83	0.29	1.02	0.69	0.03	0.00	0.61	0.88	1.04	1.10	0.87	0.99
A2/Sydney/2/64	0.90	0.65	1.27	1.23	0.98	0.82	0.83	−0.09	−0.12	−0.03	−0.10	0.68	0.28	0.49	0.90	0.00	1.21	0.54	0.64	0.23	1.02
A2/Victoria/4/68	0.49	0.18	1.30	1.17	0.98	0.71	0.41	0.62	0.79	0.93	0.33	1.08	0.67	0.56	0.85	0.82	0.00	0.25	0.26	0.54	0.73
A2/Hong Kong/1/68	0.78	0.49	1.05	1.44	1.08	0.75	0.68	1.35	1.31	1.11	0.88	1.73	1.12	1.27	1.44	1.00	1.43	0.00	0.04	0.23	0.84
A2/NT/60/68	1.08	1.03	1.52	1.76	1.30	1.19	1.04	1.19	1.25	1.29	1.07	1.73	1.26	1.31	1.62	1.22	1.44	−0.01	0.00	−0.18	1.28
A2/NSW/5/69	0.79	0.42	1.37	1.43	1.08	0.90	0.64	1.03	1.24	1.06	1.02	2.10	1.20	1.20	1.86	1.08	2.03	0.39	0.43	0.00	0.97
A/Swine/31	0.06	0.27	0.37	1.60	0.36	0.55	−0.06	1.45	0.85	0.36	0.47	1.81	0.89	0.23	1.43	0.40	1.76	1.56	1.58	1.47	0.00

[a] Each entry represents the mean HI titres of 4–8 bleeds taken from 2 rabbits each and is given in $\log_{10}$ units.

FIG. 3
HIERARCHIC PROGRESSION OF INFLUENZA STRAINS[a]



[a] Positions on the hierarchic scale (ordinate) are calculated by taking the difference of the element in row *i* and column *j* and the element in row *j* and column *i* of Table 5. Dotted lines mark the mean hierarchic position and span of a subtype, continuous lines the trend within a subtype. Starred symbols represent the " bridging strains " within subtype A2, while the divergent trend in this group is shown by broken lines.

bridging strains, as they can be shown to have anticipated the mutation which separates the A3 group, yet carry also the mutation characteristic of A2 viruses.

The existence of such bridging antigens would suggest a very attractive model for accounting for the molecular mechanisms at work in maintaining the constitution of a subtype over an extended period, as well as for allowing the next subtype to be viable, i.e., senior to its immediate predecessors. Whether bridging strains occur as a rule, or arose as a freak of the A2 subtype only, becomes thus one of the fundamental questions we have to answer before the evolution of influenza viruses can be understood, let alone anticipated. At any rate, such a bifurcation has occurred at least once, and was sufficient for predicting that Australia will not have a Hong Kong-type epidemic of the proportions experienced throughout the northern hemisphere.

VARIETIES OF ORIGINAL ANTIGENIC SIN

Finally, we shall consider one more piece of evidence. If an adult is given a shot of the present influenza A vaccine, he will produce antibodies, and those antibodies will usually react better with one of the older strains than with the actual vaccinating antigen. This is the original antigenic sin, and a major nuisance to all who like to keep their antibodies up to date. The situation is actually not as bad as it looked at first sight, and I can best demonstrate this by reference to an experiment we did recently. We immunized 30 rabbits each with the most junior virus (PR8), or with one of the most senior ones (Bel.). These animals were then boosted, in groups of 5, with one of 6 related antigens, spanning the whole width of the seniority scale, and eventually all sera were tested for cross-reactivity against the whole range of influenza antigens.

Results relevant to the present discussion are summarized in Table 6. It is evident that when primary immunity was established against the junior virus, i.e., by use of vaccine I, all responses were characteristic of original antigenic sin; when the primary antigen was senior to the booster, i.e., vaccine II, the response was at least as good against the second antigen as against the first. We need not go into the whys and wherefores of this phenomenon—a complete analysis will be given in a separate publication—but conclude that if you must sin, it is sounder to start with a senior antigen as partner.

TABLE 6

RESPONSE OF RABBITS TO CROSS-IMMUNIZATION WITH INFLUENZA VIRUSES [a]

VACCINE		RESPONSE					
		PRIMARY			SECONDARY		
I	II	vs.I	vs.II	diff.	vs.I	vs.II	diff.
PR8	PR8	3·15		0	3·75		0
	WSE	3·41	1·95	+1·46	3·77	3·36	+0·41
	CAM	3·44	1·36	+2·08	3·76	3·30	+0·46
	FM1	3·72	1·40	+2·32	4·03	2·95	+1·08
	MEL	3·33	1·69	+1·64	3·92	3·26	+0·66
	BEL	3·19	1·01	+2·18	3·99	2·81	+1·18
BEL	PR8	2·26	1·97	+0·29	3·32	3·57	−0·25
	WSE	2·65	1·87	+0·78	2·99	3·30	−0·31
	CAM	2·60	1·67	+0·93	3·24	3·25	−0·01
	FM1	2·40	1·46	+0·94	2·81	2·87	−0·06
	MEL	2·75	1·56	+1·19	3·43	3·50	−0·07
	BEL	2·70		0	3·46		0

[a] All entries are in $\log_{10}$ units.

CONCLUSIONS

What remains is to see what all this means to the prophylaxis of influenza. To begin with, we note that the evolution of this pathogen follows a regular course, and we further note, with dismay, that the new strains are always one step ahead of the producers of vaccines. For this reason, we should think twice before including junior antigens into a vaccine formula, both because they are not likely to do much good in themselves and because they jeopardize the efficiency of the more senior antigens. Instead, since the evolutionary progression can be imitated in the laboratory, we might once take an epidemic strain, select its senior mutants and use a mixture of these *prospective antigens* as vaccine. Technically there is no problem here, and it should also leave more time for the manufacture of a sufficient number of doses. A second point is that even the best formula will not perform uniformly well in the field, as the older cohorts will tend to produce old-fashioned antibodies. This can be overcome by a massive dose of non-toxic subunit vaccines. So my propositions come to this: let us outsmart the virus by anticipating its mutations, and let us give everybody a chance to respond once to the vaccine we are giving.

POSTSCRIPT

It is purely a matter of convenience that I have used my own observations to illustrate what I had to say. I could have demonstrated the phenomenon of selection for seniority by the data of Archetti and Horsfall, of Isaacs and Edney, of Hamre, Loosli and their collaborators, or of Quilligan; and the hierarchic arrangements within influenza could have been derived from the data of Dowdle, of Minuse, of Jensen and Francis, or of Hirst. I do not claim any originality in observation, only in interpretation.