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THE CONTROL OF INFLUENZA

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Summary

Influenza virus differs from the usual agents of epidemic disease by its extreme variability. Since consecutive outbreaks are caused by antigenically different viruses, both herd immunity and vaccination are largely ineffective and the epidemiology is characterized by pandemics, sensu stricto.

Severe pandemics occur every 10-12 years, followed by a period (subtype era) over which the evolution of the virus follows a predictably regular course. This process can be imitated in the laboratory, yielding mutant viruses which may serve as prospective vaccines.

The transition between subtypes is abrupt and hitherto unpredictable. There are indications, however, that the number of subtypes is limited and that they are linked in a secular cycle of about 70 years. If this proves to be correct, it should be possible to anticipate even the major antigenic shifts and thus eventually fully control the disease.

Zusammenfassung

Influenzaviren unterscheiden sich von den üblichen Erregern epidemischer Erkrankungen durch ihre aussergewöhnliche Variabilität. Da aufeinanderfolgende Epidemien durch Viren unterschiedlicher Antigenstruktur verursacht werden, sind sowohl Herdenimmunität als auch Schutzimpfungen kaum wirkungsvoll; die Epidemiologie ist somit eine Reihe von echten Pandemien.

Den schweren Pandemien, die alle 10 bis 12 Jahre auftreten, folgt eine Zeitperiode ("subtype era"), während der die Entwicklung des Virus einen voraussagbar regelmässigen Verlauf nimmt. Dieser Teilvorgang kann experimentell nachgeahmt werden und führt zur Gewinnung von Virusmutanten, die als Schutzimpfstoff eingesetzt werden können.

Der Uebergang zwischen den Subtypen erfolgt plötzlich, und die neu auftauchende Antigenstruktur ist bislang nicht voraussagbar. Es gibt jedoch Hinweise, dass die Anzahl der Subtypen beschränkt ist und dass sie in einem Zyklus von etwa 70 Jahren wieder auftreten. Wenn sich dies bewahrheitet, sollte es möglich sein, auch grundsätzliche Veränderungen der Antigenstruktur vorauszusagen und damit die Grippe schliesslich durch wirksame Prophylaxe unter Kontrolle zu bringen.

A memorial lecture is expected to start with homage to the man we are gathered to remember. Since my subject is influenza, I am in a position of paying the supreme compliment to the astuteness of the great epidemiologist Karl Meyer was: he never touched the stuff. In fact, if he were still with us, he would be the first to suggest a more appropriate title for my talk, to wit, "What hope do we have of ever controlling influenza?" Let us see then why we need this question mark after the title or, more specifically, what makes influenza so different from all the other viruses we have learnt to control.

As we all know, influenza comes around every year or two and, on top of that, we have memorable pandemics about every ten years. We also know that after each outbreak a good third to half of the population has mounted a potent immune response against the current strain, but neither that nor vaccination will stop them catching 'flu next year. This is a humiliating situation and we shall start therefore, humbly, by going back to square one.

We take a set of viruses from consecutive outbreaks, beginning with the HongKong virus which caused the last pandemic, and the set of corresponding antisera. With these reagents we perform all possible cross reactions and end up with a matrix of neutralizing titres. Since we are not interested in absolute values, the titres have been normalized to the homologous reaction; hence the values of 100 % in the main diagonal of Table 1.

What is remarkable about this Table is the asymmetry of cross reactions: all the high values are lying above the main diagonal and all the low ones below it. These antisera seem to be retrospectively active, but not prospectively. So we have a plausible explanation why we keep catching 'flu year after year and also an excuse for our vaccines which, made from last year's virus, are of little use against next year's epidemic strain.

At this stage we can either throw up our hands in despair or take up the challenge to beat Nature at her own game, by anticipating antigenic changes. This can be done in two ways. The practical man knows that the world is large and he has also worked out that epidemics must start somewhere. So he sets up an organization - the larger, the better - and starts isolating viruses from all local outbreaks, all over the world. If a new antigen turns up somewhere, it is taken as the next epidemic strain and goes straight into the vaccine.

Table 1. Cross reactions of influenza a viruses.
1. Hierarchic phase of subtype A3.

Viruses	Antisera against					
	NT 60	ENG 845	LAS	ENG 42	PRI	30c
NT 60/68	100	93	104	111	115	97
ENG 845/69	50	100	93	97	127	93
LAS/71	41	37	100	90	107	90
ENG 42/72	31	47	38	100	93	93
PRI/73	26	28	35	39	100	81
30c*	15	16	23	28	38	100

The titres are normalized to the homologous reaction (= 100 %), and represent means of 8–12 antisera per antigen.
* 30c, the senior laboratory mutant is included for purposes of comparison.

When I say "straight", I am not implying any ungentlemanly hurry. New isolates, as a rule, do not grow well. So they have to be adapted, and that takes time. Some of the more recalcitrant strains are loath to yield to simple serial passaging, so they have to be recombined with something more docile, and that takes some more time. Eventually the vaccine strain is distributed and the manufacturers start growing it. This is the risky part of the business. Not medically - an egg-adapted virus is quite harmless - but financially. It is understood that any excess vaccine will be unsaleable next year, so it makes no sense oversupplying the market. It is equally well understood that influenza vaccines are made over a 3-4 months' period, with costly equipment and staff standing idle over the rest of the year; so it makes no sense investing too heavily in this area. As a result too little vaccine is made, too late. What is made is, of course, as yet only a candidate for a vaccine: it still has to pass through the National Standards Laboratories before it is released, and that takes its time, too. Thus, to give a cautionary instance, the HongKong virus was clearly recognized four months before it reached the Western hemisphere, vaccine production was put into top gear, and American firms managed to turn out enough doses to vaccinate about one-fiftieth of the population. By the time the product reached the market, the epidemic was over.

It should be added that the manufacturers put in a remarkably fine effort and the Standards Authority cut all corners to release the vaccine promptly. The trouble was that the original HongKong virus happened to be a particularly poor grower and the actual vaccine strain, AICHI, became available only six weeks before the American epidemic started. The irony of the situation is that a high-yielding strain, NT60, has been available well before AICHI,

but this fact was hidden by the official statistics which list only antigenic comparisons and ignore growth rates and yields.

Clearly, catching the new virus in time was not a practical proposition, so the practice of using last year's virus as vaccine was given a new lease of life. As field strains hardly changed by '69, the old '68-vaccine was quite effective next year, performed poorly in '71 and failed altogether in '72. The formula was then changed, but the '72-antigen did not protect against '73-strains, the '73-antigen did not protect against '74-strains, and so on to the present day, with a new vaccine each year and no prophylaxis to speak of.

What happened to the virus in the field since 1973 is remarkable in itself. For the first four years after the HongKong pandemic we had widespread epidemics with essentially the same virus isolated in all parts of the world. The successive field strains formed a hierarchic order, as you have seen in Table 1. After '73 the outbreaks were less severe, were localized and were caused by readily distinguishable viruses in different parts of the world. Indeed, when we tested the strains isolated in France over the winter of 1975, there were six different antigenic groups some of them isolated from successive waves within a small community. If we compare these strains, we get a matrix like this:

Table 2. Cross reactions of influenza a viruses.
2. Bridging phase of subtype A3.

Viruses	Antisera against										
	NT 60	30c	PCH	SCOT	HAN	FIN	PR 2	ART	VIC 3	NG	VIC 112
NT 60/68*	100	97	123	76	62	68	46	57	55	62	41
30c*	15	100	19	12	15	13	24	19	24	16	18
PCH/73	12	23	100	23	22	19	26	52	18	14	22
SCOT/74	23	47	93	100	54	55	47	78	52	57	16
HAN/74	7	19	23	15	100	97	38	54	12	9	31
FIN/74	8	20	22	18	100	100	41	55	13	11	27
PR 2/74	13	25	27	24	35	30	100	35	20	18	28
ART/74	6	13	23	13	5	6	28	100	11	9	13
VIC 3/75	7	12	19	9	9	10	20	12	100	13	30
NG/75	9	15	26	13	13	12	23	19	14	100	21
VIC 112/76	7	18	24	25	10	9	19	38	35	19	100

Titres are normalized to the homologous reaction (= 100 %), and represent means of 4-6 sera per antigen.
*The junior and senior member of the hierarchic phase, included for comparison.

The pattern of neutralizing titres in Table 2 is irregular in two respects. First, we have viruses (PCH for instance) whose antibodies neutralize heterologous strains better than they neutralize themselves. This may seem paradoxical but is not a new phenomenon – it was well recognized in the early 'fifties, in the second half of the A1 era, and also occurred within subtypes A0 and A2. The second irregularity is that the main diagonal does not separate neatly the high and low values. Antisera against these late members of the subtype do not show the contrast of retrospective and prospective efficiency – they are mutually ineffective against each other. We have then a state of affairs here which is bound to further frustrate the practical man.

Let us see then what a theoretician would do in a situation like this. Being a theoretician, he wouldn't do anything, to begin with. He would just sit and muse about the fact that our virus has to survive in a hostile environment. Every infection, whether fatal or leaving behind an immune host, reduces the susceptible population. If the virus is to survive, it must either restrict itself to nonimmune subjects (as do most viruses which cause single-incidence, usually childhood disease), or it must find some way of escaping neutralization by existing antibodies. In these terms the epidemiology of influenza becomes an evolutionary problem, with the selective pressure represented by human herd immunity.

The working hypothesis is simple and can be readily tested in the laboratory: instead of growing the virus in a host system incapable of immune responses, we shall grow it in the presence of potent antibodies. What we find is that a small minority, of the order of 10^{-9}, thrives in the presence of antibodies which completely neutralize the parent population. These survivors can be isolated, shown to breed true – they are viable mutants.

On analysing our mutants we find – and this was a surprise – that most of them are not antigenic mutants at all. What they have done was to place an extra positive charge in the area which makes contact with the negatively charged cell surface. In an assay where antibodies and infectible cells are competing for the virus, this little trick tips the scales in favour of infection. In simple binary tests the difference disappears. Such adsorptive mutants give the paradoxical neutralization tests we have seen in Table 2: their antibodies are more than 100 % efficient against some heterologous strains, including their parent. The remaining isolates score as true antigenic mutants, each standing in asymmetric relation to its parent, just like early field strains of a subtype did.

By preparing antibodies against our mutants, we can carry them through several rounds of selection. We have done this, starting with the initial members of two subtypes. The results allow some general conclusions. First, the series is hierarchic, i.e., consecutive mutants give the asymmetric pattern of cross neutralization we saw among the early members of a

subtype. Second, the series is bounded, i.e., after three or four rounds of selection the same technique yields no further mutants. Third, the series is degenerate, i.e., the same mutants can be selected in one, two or three steps, or through different intermediates. Fourth, the series is convergent, i.e., while in the first and second generation there are a number of different mutants, they tend to give rise to the same terminal forms. Fifth, adsorptive mutants can arise at any point in the series, including the terminal stage.

We have also an exception to the general behaviour: during the earlier generations mutants arise which behave like terminal forms. These are readily distinguished from the standard hierarchic mutants: their frequency is about two orders of magnitude lower and they tend to give symmetric cross reactions with the senior members of the series, suggesting differences at two mutational loci. Occasionally they also cross-react with other subtypes and hence we call them bridging mutants.

The hour of truth for the theoretician comes when his findings are matched against the hard empirical facts.

When we do this with our series of hierarchic mutants (Figure 1), we find that all field isolated of the early, hierarchic phase of the present subtype have an equivalent among our laboratory strains. The Far Eastern sequence of HK1/68→AICHI/68→HK107/71 turns out to be a set of adsorptive mutants, while the Western strains are placed on two branches of the same family tree. There are also several laboratory mutants which either have not arisen in Nature or, just as likely, were missed by the conventional crude techniques of classification.

In practice, this means that we are in a position of anticipating antigenic changes over the first half of a subtype era. Indeed, a vaccine made from the senior laboratory mutant proved highly successful in the field.

The situation over the second, the bridging phase of a subtype is less satisfactory. Among our, admittedly, small number of bridging mutants there was no equivalent to the epidemic strains of 1973 and 1975, but we could match the strains of 1974 and 1976. Again, there are several laboratory mutants which did not turn up in Nature, so that the correspondence is imperfect in both directions. It is of practical importance, though, that the field strains of this phase cross-react only distantly (cf. Table 2) and the senior hierarchic mutant is still at least as useful as any non-homologous vaccine made up of an earlier epidemic strain.

What all this amounts to is that we have means of controlling influenza within a subtype era, but are apparently powerless against the major pandemics which mark the beginning of new subtypes.

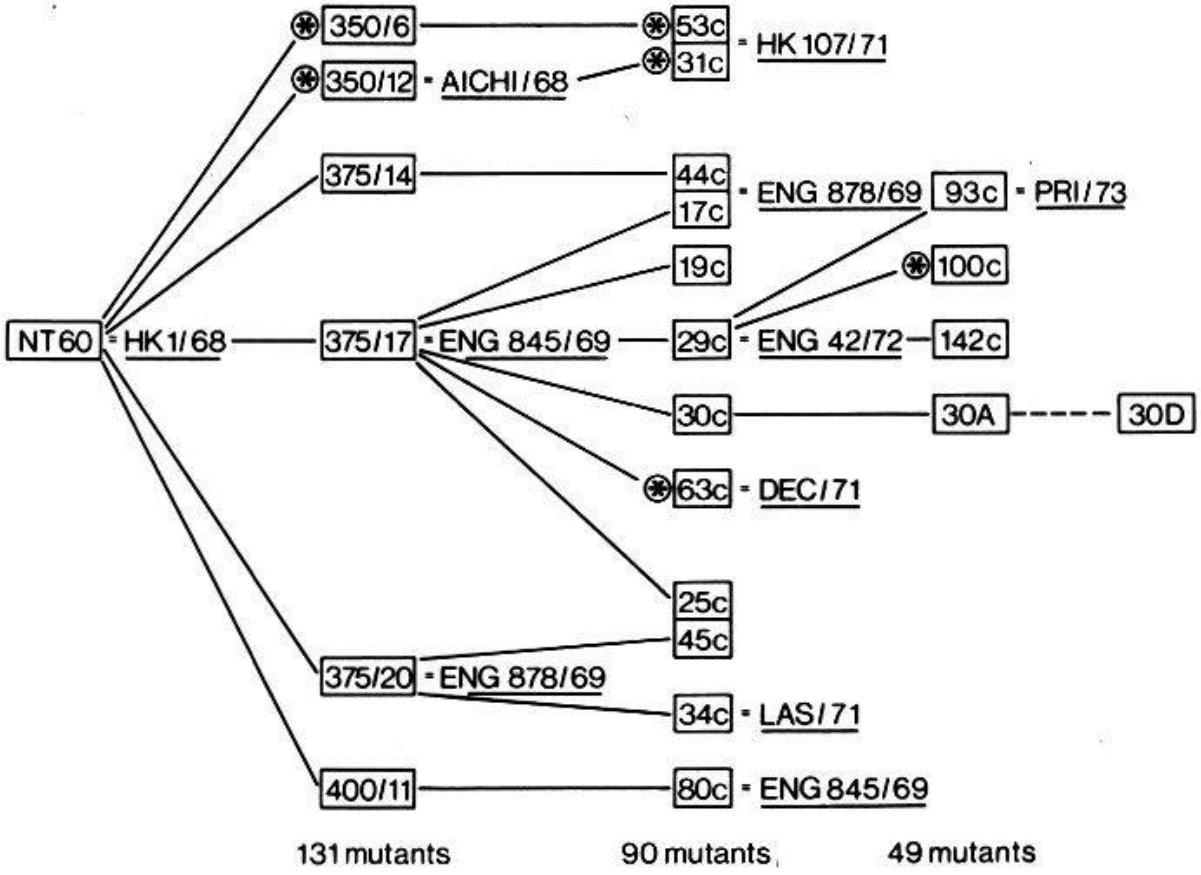

Figure 1. Family tree of laboratory mutants derived from A/NT 60/68 (H3N2) virus. Matching field strains (underlined) are equated with the corresponding mutant. * = adsorptive mutants

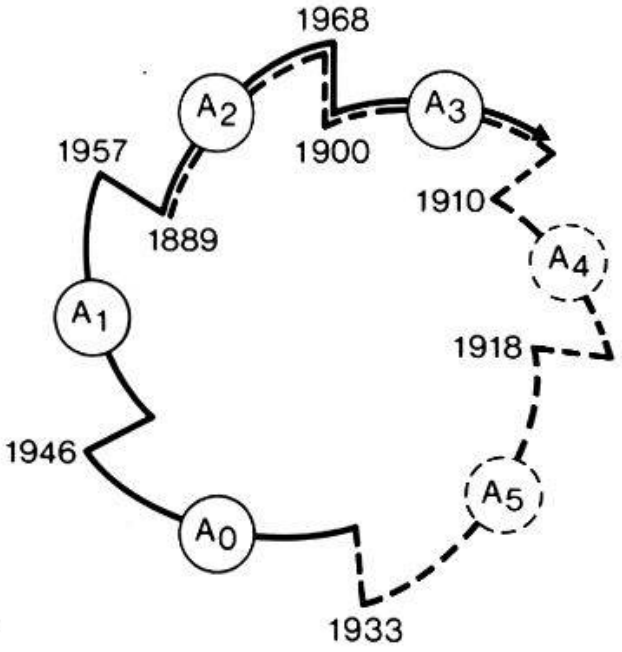

Figure 2. Schematic evolutionary cycle of influenza A viruses. The broken lines are based on serological evidence, the solid lines on virological studies. The dates mark major pandemics at the start of each subtype era (the symbols of subtypes are encircled).

Since 1933, when the first human influenza virus was isolated, we had four such pandemics (1933, 1946, 1957, 1968). By analogy, the great pandemics of 1889, 1900, 1910 and 1918 similarly define subtype periods. The number of subtypes, however, seems to be limited. There is solid serological evidence from four continents that people born in the decades immediately before and after the turn of the century had antibodies to the viruses which were to appear or, rather, reappear in 1957 and 1968, respectively. This suggests that there are only six subtypes and that these are linked in a secular cycle with a period of about seventy years (Figure 2).

Such circular evolution is utterly alien to Darwinian thought, yet it actually follows from the nature of the antigenic area of influenza A viruses. It has long been known from thermodynamic measurements that the influenza A virus-antibody union is entirely entropy-driven. This implies hydrophobic bonding, i.e., both combining regions must be made up largely, if not entirely, of hydrophobic amino acids. Such hydrophobic areas do not tolerate more than one large amino acid, since two long side chains could interact, eliminate the structured water ("ice caps") covering their tips and cause a hydrophobic flip, i.e., a fundamental conformational change. The permitted mutational substitutions automatically generate a hierarchic series under the pressure of antibody, while bridging mutants represent the maximal hydrophobic bulk compatible with the native conformation of the molecule. Bridging strains would be therefore terminal forms, by definition. Their back-mutants, however, should be fully viable since they amount to junior members of one or another subtype. Survival of such back-mutants depends, of course, on the state of the herd immunity which, in its turn, imposes the secular cycle corresponding to the lifespan of humans.

We have increasing evidence that something like this is in fact happening in Nature. The extensive survey sponsored by WHO last year has demonstrated that antibodies against SW virus (a strain believed to be closely related to the 1918-1919 pandemic agent) are present in cohorts born well after the termination of that subtype era. The incidence of such antibodies shows great regional variation, precisely as should be expected from the emergence of rare bridging mutants. We have conducted a similar survey on Australian blood donors and not only confirmed the WHO findings on anti-SW antibodies, but both extended it to the next two subtypes and showed that these illegitimate antibodies were actually directed against bridging strains rather than against the common hierarchic mutants.

The recognition of a self-renewing human reservoir of all possible subtypes immediately suggests an experiment. We have to find out whether some of our bridging strains or their back-mutants would react with the sera of people born before 1918. These age groups should

still have antibodies against the viruses current between 1910 and 1918 and, on the only rational hypothesis we have, that is the subtype likely to return and cause the next pandemic.

We have started this work and, indeed, have a family of laboratory mutants which gave the expected reactions. Granted, the sample we have tested to date is small and perhaps not representative, but it is a promising enough start to allow me ending this talk on a more optimistic note. We certainly do not have influenza under control yet, but armed with the theoretical and technical knowledge gained recently, perhaps the next battle will end the embarrassing series of defeats we have suffered in the past.

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