BRITISH MEDICAL JOURNAL

LONDON SATURDAY OCTOBER 5 1957

THE PRESENT STATUS OF PROPHYLACTIC IMMUNIZATION AGAINST INFLUENZA

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It is now nearly a quarter of a century since the first recovery of an influenza virus was made in the laboratories of the Medical Research Council in London (Smith, Andrewes, and Laidlaw, 1933). Yet influenza vaccines remain essentially experimental in nature, and artificial immunization against influenza has not achieved the status of an accepted public-health measure as has, for instance, poliomyelitis vaccine.

The Justification of Present Attempts at Artificial Immunization Against Influenza

In the past twenty years epidemic influenza appears to have undergone an alteration in character, as shown by mortality statistics. In 1950 Martin pointed out that from 1920 onwards a downward trend had occurred both in the height of the peaks of influenza epidemics and in the intervening troughs of mortality, and drew an analogy with the experience of influenza from 1849 onwards, when for 40 years the disease carried negligible mortality. Is influenza really becoming less virulent at the present time, or are the changes in mortality due to some other reason, as for instance the use of chemotherapy? We cannot tell for certain, because we have almost no comparative information concerning morbidity. Such information as we have, however, lends little support to the view that morbidity is altering, for present outbreaks of influenza in the Far East have affected 20% or more of the population; yet mortality has been negligible in these outbreaks.

Meanwhile we still lack precise information concerning the causative virus of virulent pandemics of the 1890 or 1918 type, though there is evidence (Francis *et al.*, 1953) that the 1918 pandemic was due to the influenza virus A now found as a cause of epidemics of swine influenza (Shope, 1931). If this is true, then experiments on the immunization of man against the modern strains of influenza virus A may tell us something about how to protect against a future pandemic of virulent influenza, if indeed the latter should ever reappear. So one justification of present attempts to attain protection by immunization largely springs from the ever-present fear of a possible recurrence of virulent influenza. No one really believes that artificial protection against the mild influenza experienced in the last few years is worth while if it can only be attained by an annual injection of a vaccine as impure as are present-day egg vaccines.

Evolution of the Influenza Viruses and its Bearing on Immunization

It is common knowledge that the influenza virus A consists of many different strains bearing different antigenic groupings yet each possessing the same "soluble" antigen. Scientific opinion is still divided on the interpretation to be placed on these antigenic differences and on their classification. The American view as stated by Jensen (1957) emphasizes the sharing of antigens even between apparently different antigenic "families" so that an antigenic spectrum exists. Jensen considers that the findings indicate an almost random variation from year to year rather than a progressive chronological shift. The British view as restated recently by Andrewes (1956, 1957) is that the observed facts suggest that there is some form of progressive alteration which makes it unlikely that the antigens of old strains recovered twenty or so years ago, such as the W.S. and PR8 viruses, will ever reappear. Workers on both sides of the Atlantic agree that antigenic variation is probably due to propagation of the viruses in nature in hosts containing serum antibodies resulting from previous epidemics, and Andrewes (1956) calls this a form of "directed" mutation. This type of variation enables the virus to maintain itself by always being one jump ahead, evolutionarily speaking, compared with the human population's immunological reactions.

The importance of the antigenic differences among the influenza viruses in relation to immunization is due to the fact that vaccines made from killed or inactivated virus will not engender antibodies or give protection against antigenically remote strains. This fact became evident with influenza A in 1947, and more recently has also become true for influenza B (Davenport *et al.*, 1956). Thus we are seriously handicapped in the preparation of a vaccine by the lack of exact foreknowledge of what lies ahead. It is therefore essential to study the antigenic composition of viruses from current outbreaks in order to detect varieties which may be immunologically important. This has been done in the last nine years by the World Health Organization with its chain of co-operating laboratories. The emergence of new strains has thus been detected at a much earlier stage than it would have been without such an organization.

The Asian or Far East virus, which first appeared in outbreaks in Hong Kong and Singapore in April, 1957, is an excellent example of international collaboration and of the unpredictable behaviour of influenza A. This new virus has no apparent antigenic relationship to older strains of A though it possesses the Type A "soluble" complement-fixing antigen. It is a strain whose progress is therefore unhampered by human antibodies specifically directed against itself. Though not of a high order of virulence, it appears that this virus has some biological properties which differ from those of

older strains of influenza A. It seems most unlikely that vaccines prepared from such older strains will protect against the Far East virus.

Composition of Virus Vaccines

The human serological response to an influenza antigen is quite different from that obtained in animals. This was clearly shown by Davenport and co-authors (1953), whose doctrine of "original antigenic sin" arose out of the discovery that the antibodies found in persons of different ages is a spectrum made up of different components. Briefly, the older persons in the community possess sera richest in antibodies against the antigens of viruses which infected them in their youth. Children's sera are devoid of antibodies against antigens not experienced during their lifetime. This finding has been confirmed with sera from Great Britain (Davenport *et al.*, 1955), and so has the further discovery that a particular virus vaccine tends to enhance the antibodies found in the serum before immunization as well as to produce a response against its own antigenic component (Davenport and Hennessy, 1956; Jensen, 1957; Report of the M.R.C. Committee on Clinical Trials, 1957). The measurement of the serological effect of a particular vaccine is therefore a complicated affair and requires observations on persons of different ages. Meanwhile there is reasonable agreement that a vaccine must be prepared so as to produce antibodies against the virus likely to be encountered in the field. American opinion favours the use of a polyvalent vaccine containing old as well as recent virus strains. The British view is that a good monovalent vaccine will, because of the recall phenomenon, produce an enhancement of older antibodies and will also engender antibodies to the strain contained in the vaccine. These arguments concerning the merits of monovalent and polyvalent vaccine can be settled only by comparison of their relative protection in the field.

A second important matter connected with the antibody response to a vaccine has arisen from the development of vaccines containing adjuvant materials and existing as a water-in-oil emulsion. The simple virus vaccine suspended in formolized saline produces its maximum effect within two weeks, and the antibodies then slowly return to their previous level during the next year. Vaccines containing the virus adsorbed on to aluminium phosphate have been used in Great Britain chiefly because they do not cause such severe general or febrile reactions as do the ones with a similar virus content containing saline alone. Again the antibody response reaches a peak two to four weeks after inoculation and then slowly wanes during the next year. Vaccines emulsified in mineral oil by the aid of emulsifiers such as "arlacel" (Salk *et al.*, 1952) produce a slower rise in antibodies, but the peak, which is reached three months after inoculation, is much higher. A small amount of virus antigen amounting to one-tenth or even one-twentieth of that used in saline vaccine will, because of the adjuvant property of the emulsions, produce an excellent antibody response, which falls off more slowly than after saline vaccines. Unfortunately, although adjuvant vaccines appear to be ideal from the standpoint of economy of virus content and prolongation of the antibody response, they suffer from a serious disadvantage. Both in the U.S.A. and in this country occasional persons inoculated with emulsified vaccine have developed severe local reactions. The latter are really chemical abscesses, but they may require surgical intervention, and though relatively infrequent (0.1 to 1% of inoculated persons) they constitute a serious bar to the wider use of such vaccines. For the present it appears that we have to be content with the less potent but safer formolized saline vaccines.

Living virus vaccines can only be mentioned briefly. If it became possible to develop an attenuated virus strain with an inability to cause clinical reactions there would be much to be said for a trial of the degree of protection produced in the field. Russian workers are reported to have tested such vaccines and to have found that protection is induced with certain selected virus strains. We have no experience of the use of such a vaccine, but some basic preliminary work is being carried out (Isaacs and Roden, 1956), and we must await developments.

Evidence from Field Trials of Influenza Virus Vaccines

Field trials are the only way to test the relative protective effect of different vaccines and to establish a relationship between the potency of the vaccine in the laboratory and its clinical effect.

Field trials are governed by four important principles. Firstly, the selection of the population with a view to anticipating as high an attack rate as possible; secondly, control of the vaccine under test by a method free from bias; thirdly, avoidance of untoward reactions; and, fourthly, adequate ascertainment of clinical illnesses in both test and control populations subsequent to inoculation. In the U.S.A., Servicemen have been studied in this way by the Commission on Influenza for a number of years. But such groups are particularly liable to outbreaks of acute respiratory disease or febrile catarrh, which has its highest incidence in recruits and is due in part to infection by the adenoviruses. As clinical resemblances exist between influenza and febrile catarrh, it is necessary to rely upon laboratory evidence for proof that the illnesses at any one time are due to the influenza viruses. Such laboratory evidence confirmatory of influenza is harder to obtain in persons vaccinated against influenza than in inoculated persons (McDonald and Andrews, 1955), so that it is probable that a laboratory assessment will tend to bias the results in favour of the vaccine. Whether this is responsible in fact for the relatively good results reported by the U.S. Commission on Influenza cannot be stated for certain, but even these results have shown wide variation (Table I, after Francis, 1955).

TABLE I.—*U.S. Influenza Vaccine Trials (Commission on Influenza)*

Epidemic	Population	No.	% Attack Rate	Protection Ratio	Diagnosis
A (1943)	Controls	5,776	7·06	3·6	Clinical
	Vaccinated	5,806	1·96		
B (1945)	Controls	2,150	11·21	12·9	,,
	Vaccinated	1,150	0·87		
A (1947)	Controls	7,615	8·09	1·1	,,
	Vaccinated	10,328	7·19		
A (1950)	Controls	2,082	3·7	3·1	Serological
	Vaccinated	670	1·2		
A (1951)	Controls	5,228	2·01	4·0	,,
	Vaccinated	2,596	0·5		
B (1952)	Controls	430	19·32	2·7	,,
	Vaccinated	207	7·24		
A (1953)	Controls	5,527	5·7	6·0	,,
	Vaccinated	5,994	0·95		

Modified after Francis (1955).

The problem of ascertainment of illness amongst the ordinary population is not a simple one. All sorts of illnesses are labelled influenza, and there are always cases of acute respiratory disease occurring endemically in the population. Furthermore, direct laboratory study of particular persons in the general population is difficult to organize. In Britain a compromise arrangement has been to use a clinical record of illnesses subsequent to inoculation which is kept by the family doctor and by medical officers working in industry. A parallel spotting scheme for evidence of influenza, and organized by the Public Health Laboratory Service, has given the times when the viruses could be detected in the community. In practice this method has worked better than might be expected. There is no doubt that we have obtained precise information of the period of time when the respective influenza viruses have been active in the community. We also have a record in simple diagnostic terms of illnesses causing at least two days' absence from work and diagnosed as clinical influenza, other respiratory disease, and non-respiratory illness. Only the attacks labelled clinical influenza have shown any reduction in the vaccinated compared with the control groups. But the incidence of such influenza over a three-

months period of observation has never been higher in the control population than 5%. At times of such minor prevalence of influenza, a dilution of the latter by the inclusion of other diagnostically confusing illness seems inevitable. So that, on the whole, it has been surprising that a reduction of illness has been demonstrated at all (Table II). Yet

TABLE II.—*British Influenza Vaccine Trials*

Epidemic	Population	No.	Vaccine	% Attack Rate	Protection Ratio	Diagnosis
(1) A (1953)	Industry, Services nurses, students	6,370	B	4·9	1·63	Clinical
		6,340	A	3·0		
(2) A (1956)	Industry	2,499	B	5·0	1·4	,,
		2,487	A (monovalent)	3·5	1·5	
		2,509	A (polyvalent)	3·3		
(3) A (1956)	School	344	Unvaccinated	20·0	2·5	Clinical and laboratory
		100	A (1955)	8·0	10·0	
		100	A (1954+1955)	2·0		

(1) Report of M.R.C. Committee on Clinical Trials of Influenza Vaccine (1953).
(2) Report of M.R.C. Committee on Clinical Trials of Influenza Vaccine (1957).
(3) Hawkins, Hatch, and McDonald (1956).

though vaccination in 1953 and in 1956 lowered the attack rate only by 30 to 40%, the effect was statistically significant. In contrast, a single trial in 1956 at a boys' school, where the attack rate of influenza A in the uninoculated boys was 20%, showed a ratio of protection closely similar to that found by the U.S. Commission (Hawkins, Hatch, and McDonald, 1956). Perhaps part of the disappointment afforded by the M.R.C. field trials may thus be blamed on nature, but some at least is undoubtedly due to our poor methods of measurement of protection.

The Future Prospect

In view of what has thus far been accomplished, it is obviously unrealistic to visualize the control of influenza with immunizing agents such as those at present available. "Control" is a word which implies ability to curtail spread as well as to limit manifestations of the infectious process. The present era may indeed be the first phase in our gropings towards control of influenza, and so we must continue to experiment with different sorts of vaccines and also with field trials.

So far as the present threat of Asian influenza is concerned, it must be obvious that in the short time available we can do little but prepare for as good a field trial of protection by formolized egg vaccine as can be devised. Such a trial must be preceded by tests of the antibody-forming power of an inactivated vaccine made from the Asian virus. As the latter has certain unusual properties, it is clear that we cannot be sure that the facts gathered about vaccine made from older viruses will necessarily hold good for the future.

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THE NATURE OF CANCER ANAEMIA AND ITS BEARING ON THE IMMUNOLOGICAL THEORY OF CANCER

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A preliminary account of this work has already been reported (Green, 1957b) in a general survey of the immunological theory of cancer (Green, 1954). This ascribes the neoplastic change to loss of tissue-specific antigen(s). An important part of these investigations was to study the nature of tumour haemolysins and haemagglutinins and determine the response of many kinds of experimental tumours to the parenteral or intratumoral injection of foreign and autologous red cells. Some of these results have been reported (Green, 1957b), and a more detailed account will appear in a separate publication. Here we present the results of tests on the blood of cancer patients and discuss them in the light that these and other findings throw on the immunological theory in general.

This theory implied that an immune reaction of the tumour to certain cells of the host was a possibility. If so, the anaemia of cancer, often so prominent a feature at some stage of human and experimental cancer, might be due to an "auto-antibody" produced by the tumour mass. Such an antibody, if coating the red cell, might be detected by the use of specific antiglobulin sera. Accordingly direct Coombs tests were made on the blood of cancer patients, but only very occasionally was a positive result obtained at a titre positive with sensitized Rh+ cells. However, on using much lower dilutions of antisera it was found in many cases that the speed of agglutination was much greater than that of the red cells of normal subjects. While this work was in progress it was becoming evident (see Discussion) that the anaemia of human cancer was not primarily a by-product of haemorrhage, infection, malnutrition, etc., but a true haemolytic anaemia induced by an agent of unknown origin. In terms of the immunological theory this suggested that the tumour itself could on occasion react against cells of the host possessing an antigen which the tumour cells lacked. Such a possibility made the occurrence of what seemed a relatively feeble antibody coating the red cell a matter of great practical and theoretical interest.

Methods

Direct Coombs Test.—This is performed in the standard way (Dunsford and Bowley, 1955), mixing 1 drop of 50% packed cells and 1 drop of diluted antiglobulin serum on an