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INFLUENZA: FOUR YEARS' PROGRESS*

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Influenza is a disease with which we are all familiar, either as doctors or as patients. Yet it is a disease concerning which exact knowledge has been lacking, whether from the clinical, the epidemiological, or the pathological point of view. The reason is this: the term "influenza" has been used to designate a scrap-heap of upper respiratory tract infections, and one can never be sure in reading any two descriptions in the literature that the writers are referring to the same disease.

In the absence of any exact method of clinical or pathological diagnosis the only attempts to define the disease have been on an epidemiological basis; and this is not very satisfactory for ordinary mortals, whatever it may be to epidemiologists. I propose to-day to try to convince you that it has recently become possible to separate off from the influenza scrap-heap a definite disease, one, moreover, which is probably responsible for the more important outbreaks labelled "influenza." The basis of separation is in the first place aetiological: one can recover from garglings in the early stages a virus which will infect ferrets and mice. My colleague Dr. Stuart-Harris will describe later in the discussion attempts made to recognize the disease from a clinical standpoint. The results I shall now describe are based chiefly on experimental work, some of it unpublished, carried out jointly by my colleagues Dr. Wilson Smith, Sir Patrick Laidlaw, and Dr. Stuart-Harris, and myself.

The Disease in Ferrets

To recover virus from a patient unfiltered garglings are dropped from a pipette on to a ferret's nose. When virus is present in the inoculated material the ferret shows fever and nasal symptoms after about forty-eight hours. It goes off its food, lies about apathetically, and develops nasal discharge, sneezing, and, later, pronounced nasal obstruction. The temperature chart often shows two spikes forty-eight hours apart. Influenza in the ferret is infectious by contact, and to guard against cross-infections we isolate each animal in a separate cubicle.

When we appear to have infected a ferret with material from a patient we usually kill the animal at the height of its disease on the third or fourth day. We then find that the mucous membrane over its turbinate bones is engorged and that the nasal cavities are full of muco-pus. We remove the turbinates and grind them up with sand; an emulsion is obtained which can be filtered and studied in other ways to prove that it really is influenza virus which has produced the symptoms.

As a rule influenza virus will only infect ferrets when introduced directly into the respiratory tract. If passages are made without the use of an anaesthetic no pneumonia will be produced, but by serial passages in anaesthetized ferrets one can obtain a modified virus which will produce extensive, often fatal, lung lesions (Shope, 1934). These lung lesions are commonly bacteriologically sterile. The affected lungs are firm and purplish red, and much moisture can be squeezed from their cut surfaces. Histologically bronchitis and bronchiolitis are prominent features. There is patchy atelectasis and exudation of fluid in the alveoli with peribronchial infiltration of cells, chiefly mononuclears. But polymorphonuclear infiltration is not a feature, nor are the alveoli packed with cells. It is of much interest to know that a biologically modified strain of virus can be obtained having such a predilection for the lungs; also that lung lesions of this type can be produced by the virus *alone*, without, so far as we can see, any assistance from secondarily invading bacteria.

Adaptation of the Virus to Mice

Virus adapted to ferret lungs will infect mice without any difficulty, but we have had varying success in our attempts to adapt recently isolated human influenza strains to mice. All but one of a number of strains isolated in 1935 infected mice readily after only two or three passages in ferrets (Andrewes, Laidlaw, and Smith, 1935). This year we have found such adaptation more difficult: several strains of virus have refused to infect mice even after a number of ferret passages. Hoyle and Fairbrother (1937) appear to have had the same experience. Lately we have discovered that the failure of some strains to infect mice is only apparent; there is actually merely a failure to produce macroscopic lesions. If inoculated mice are killed on the third day after infection, and more mice are infected with an emulsion of their lungs, and so on, one will ultimately succeed in obtaining visible lesions in the lungs of the mice perhaps after three, perhaps only after six, passages. Quite recently Francis and Magill (1937a), using such a passage-by-faith technique, have reported success in obtaining direct man-to-mouse isolation of influenza virus without the use of ferrets at all. The importance of this to workers in laboratories where ferrets are unobtainable is obvious.

The Disease in Mice

In mice the virus produces lesions of the lungs only; the nasal passages are not affected. Perhaps in relation to this fact influenza in mice is entirely non-contagious; normal mice kept in the same cage as infected ones neither contract the disease nor become immune. This makes work with mice much easier, but, on the other hand, we lose the opportunity of what would be a really exciting study in experimental epidemiology. When first adapted to mice influenza is a relatively non-fatal disease; but after

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many passages it becomes more lethal, and infections can be produced with one ten-millionth of a cubic centimetre of filtrate. Filtrates are readily neutralized by immune sera, and a neutralization test that has yielded much interesting knowledge is thus made available.

Evidence that the Virus is the Cause of Influenza in Man

I will now summarize the evidence which convinces us that the virus we are studying is the primary cause of epidemics of influenza in man.

1. First, we have recovered the virus from garglings of fifty-three patients with the symptoms of influenza obtained at times of rather widespread prevalence of the disease. During the recent epidemic virus was isolated from thirty-one out of forty-one uncomplicated cases tested. Virus has only been obtained during the first few days of infection and not during convalescence. We have failed to recover the virus from normal people, and we have usually failed also with garglings from sporadic cases or patients in localized outbreaks diagnosed as influenza but occurring in the absence of a widespread epidemic.

2. The virus has been recovered as described above from influenza garglings taken during outbreaks not only in London (Smith, Andrewes, and Laidlaw, 1933), but from all over the world—in the West Indies, United States, and Alaska (Francis, 1934), in Australia (Burnet, 1935), in Russia (Smorodintseff, *et al.*, 1936a), in Holland, and, during the recent outbreak, in Manchester, France, Germany, Hungary, and again in the United States.

3. The serum of patients taken during the acute stages of infection is found to have little or no power to neutralize the influenza virus. But as early as the eighth day of the disease it will have acquired very definite neutralizing powers. No such neutralizing powers appear in the sera of patients suffering from respiratory diseases other than influenza. We now have some evidence that sera from the population at large, including people who have not recently suffered from influenza, contain better neutralizing antibodies just after an epidemic than just before one.

4. In 1933 we made two attempts to infect volunteers with virus which had been passed serially through ferrets; these were unsuccessful, but at the time we failed to obtain any volunteers who had not good antibodies in their sera, and such antibodies may have protected the two victims of this experiment. Last year an accident completed our chain of evidence for us. A ferret infected with a virus which had been passed in series through 196 ferrets sneezed upon Dr. Stuart-Harris and produced in him a typical attack of the disease. There was no influenza prevalent at the time, and the virus recovered was distinguishable by its biological effects from ordinary strains of human origin. There is therefore almost no room for doubt that Stuart-Harris's infection actually came from the ferret (Smith and Stuart-Harris, 1936). Smorodintseff and his co-workers (1936a) have recently recorded their success in infecting five human volunteers with the virus.

5. The irregularity with which Pfeiffer's bacillus has been recovered from influenza epidemics is notorious and cannot be explained away by blaming differences in technique. Smorodintseff *et al.* (1936) have lately carried out extensive attempts to infect volunteers with cultures of Pfeiffer's bacilli, but produced nothing comparable with influenza. Experimental "swine 'flu" has been demonstrated by Shope (1931) to be due to the combined action of a virus and a bacillus related to Pfeiffer's bacillus. On the other hand, experimental influenza in ferrets and mice is a pure virus disease in which bacteria ordinarily play no part. The evidence at present suggests that ordinary influenza in man resembles the disease in ferrets and mice in being purely a virus infection; further, it seems likely by analogy that the virus alone may cause pulmonary complications; but it is all too familiar to us that streptococci, pneumococci, staphylococci, and Pfeiffer's bacilli may on occasion play a very serious part as secondary invaders of the lung.

Immunological Aspects

Naturally we are all interested in possible practical applications of recent work on influenza, particularly in the possibility of prophylactic vaccination. In order to make the matter clearer I must first relate something about immunity in ferrets and mice.

IMMUNITY IN FERRETS

Ferrets which have recovered from an attack of influenza develop potent neutralizing antibodies in their sera and are completely resistant to reinfection for about three months. But after six months their immunity has definitely waned; antibodies, though still recognizable in their sera, are less than before, and the animals will develop some fever and nasal symptoms when a heavy dose of virus is given intranasally.

As already mentioned, influenza virus given subcutaneously or by routes other than into the respiratory tract does not infect—or only very exceptionally. It will, however, produce some immunity, especially when several doses are given. The immunity produced by vaccination is shown by: (i) the production of antibodies; (ii) the milder nature of the fever and symptoms; (iii) complete protection against the development of lung lesions; (iv) protection against the less severe test of exposure to contact with an infected ferret; (v) restoration of waned immunity to the complete state. By this is meant that ferrets whose immunity has fallen off six months after an infection are rendered once more completely immune by one dose of subcutaneous vaccine (Smith, Andrewes, and Laidlaw, 1935).

It would be more satisfactory if normal ferrets could be completely immunized by vaccination, but the results as they stand are not discouraging. It must be remembered: first, that human beings in an epidemic are exposed only to contact infection, against which vaccination does protect ferrets, not to massive intranasal instillations of virus; secondly, that almost all adult human beings have been exposed to influenza in the past and thus have some basic immunity which merely requires reinforcement. They are thus comparable rather to the ferrets with waned immunity than to fresh untreated ones.

IMMUNITY IN MICE

In mice influenza is a disease of the lungs; and just as vaccination is very effective in protecting ferrets against lung lesions, so it is in mice. Two subcutaneous or intraperitoneal injections give a substantial immunity; in many experiments all the mice of a vaccinated group have survived a dose of virus which has killed all, or almost all, controls. Further, when the vaccinated mice have been killed at the close of the experiment the lungs of most have appeared normal. After two doses of vaccine the immunity of mice appears to last for ten but not for sixteen weeks; this is as long as the immunity of mice which have actually recovered from an infection. Protection experiments in mice have lent themselves to the quantitative study of a number of problems, particularly those designed to find a suitable vaccine for use in man.

A most important result of such studies is the finding that virus inactivated by 1 in 5,000 formaldehyde is almost

if not quite as effective an antigen as is living virus. It is still the fashion, particularly in America, to make the statement that a "killed" virus cannot immunize. Influenza virus inactivated by formaldehyde appears by all the tests we can apply to be as dead as a doornail, and I feel that it is rather up to anyone who maintains that it is still alive to produce some supporting evidence.

It is satisfactory to be able to use a safely inactivated virus; it would be better still if we could use one freed from unwanted proteins. Virus filtrates have accordingly been washed on a collodion membrane with pores of such a size that the virus is all held back while the mouse proteins are washed through the membrane. Thus is obtained a water-clear fluid as effective in immunizing mice as the untreated filtrate. Most unfortunately it is found that while formolized virus is almost as effective an antigen as living virus, and purified virus is as effective as unpurified, yet virus which has been both formolized and purified fails to act as a good vaccine—we do not know why (Andrewes and Smith, 1937).

This is particularly unfortunate, since it appears that the presence of foreign protein interferes with the antigenic response to a virus. Thus we found that vaccine made from infected mouse tissues was very effective for immunizing mice, while vaccine made from infected ferret tissues was not. Shope (1936) has observed the same thing. Lately we have discovered that if mouse vaccine is mixed with an extract of normal ferret tissues the immunizing effect of the vaccine for mice is badly interfered with.

Vaccination of Man

Though we have not yet succeeded in eliminating the mouse protein from the vaccine available without spoiling it, we have made preliminary attempts at vaccinating human beings. Francis and Magill (1937) have made similar attempts, using living virus grown, as many viruses can be grown, on tissue cultures of chick embryo. They have succeeded with such a vaccine in inducing a rise in antibodies in the vaccinated subjects. Stokes *et al.* (1937) have also used a living vaccine made from mouse tissues, and claim that there was a lower incidence of influenza among the vaccinated members of the community they studied than in the control group; there is, however, no certainty that the respiratory disease which afflicted that community really was epidemic influenza. We have chosen to err on the side of caution by using only formalin-inactivated virus, and we too have followed the neutralizing antibodies in the people we have vaccinated. One dose of 2 c.cm. of vaccine subcutaneously has produced only a trifling local tenderness and has engendered a very satisfactory rise in antibodies. Among thirty volunteers, all soldiers at Woolwich, we obtained an average increase in antibodies of twenty-five-fold when we compared bleedings taken a fortnight after vaccination with those obtained beforehand. A few results of ours confirm those of Francis and Magill that not much rise occurs until after a week from the time of injection. A satisfactory feature was that one dose of vaccine produced as good an antibody rise as did two spaced a fortnight apart.

Encouraged by these results we began last December to make enough vaccine to inoculate larger numbers of people and to test whether an actual resistance to natural infection would be produced. It was arranged through the kind co-operation of the Army authorities to vaccinate five groups, each of about 100 men, in different units and to designate similar numbers as comparable controls. But scarcely had we begun when the epidemic burst upon us. We did not expect from the serological results that any active immunity would be produced until at least a week after injection, and it happened that in most of the vaccinated and control groups influenza appeared within a day or two of the vaccination. In two groups no influenza occurred amongst either the vaccinated or the unvaccinated controls. We thus have no certain evidence as to the efficacy of the vaccine. There is, however, suggestive evidence that the vaccine is not as good as we should like it to be. Four persons, vaccinated at least a fortnight beforehand, nevertheless developed clinical influenza, and from them we obtained the virus; so we at least know that the method is not 100 per cent. perfect.

And now there has arisen a new complicating factor. We no longer feel sure that all strains of human influenza virus are serologically identical. Magill and Francis (1936) reported lately that two strains of human influenza virus isolated by them in 1934 could be sharply distinguished from each other serologically by means of anti-sera made in rabbits by a particular method. We have been repeating this work, and have reason to suspect that the two strains which they have separated, both of them carried for many generations in tissue culture, have become antigenically changed in the course of this propagation. This might lead us to doubt the importance of the differences found. On the other hand, we have observed that some strains of virus isolated in England in 1937 are not identical with the W.S. strain with which we have worked since 1933 and which we used to prepare our vaccines. The difference is revealed by titrations of ferret sera as well as of rabbit sera, and also in cross-immunity experiments. There is a considerable antigenic overlap, and the strains are more closely related to each other than to the swine-influenza virus; but there are distinct differences. The importance of differences between strains to prophylaxis and to epidemiological studies one can at present only guess at. The tangle is not going to be an easy one to unravel.

Conclusion

I will conclude by re-emphasizing three main points:

First, epidemic influenza in man is caused by a virus which is transmissible to ferrets and mice.

Secondly, progress in our knowledge of influenza may largely depend on our recognition that this specific disease has hitherto been mixed up with other respiratory infections.

Thirdly, there are grounds for hope that an effective prophylactic against influenza may be found, though there are still many obstacles to overcome.

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