INOCULATION OF HUMAN VOLUNTEERS WITH PARAINFLUENZA VIRUSES TYPES 1 AND 3 (HA 2 AND HA 1)

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Chanock *et al.* (1958) have reported the isolation of two new myxoviruses which they named haemadsorption viruses type 1 and 2 (HA 1 and HA 2). They recovered the viruses from the respiratory secretions of small children with a variety of respiratory illnesses ranging from mild colds to pneumonia. They have confirmed these findings by further studies (Chanock *et al.*, 1959). Independently, one new virus (Cop 222) was isolated in Denmark, and several strains of another (Moss) were recovered in Sheffield, England. These were found to be serologically identical with HA 2 and HA 1 respectively (Birkum Petersen and von Magnus, 1958; Sutton *et al.*, 1959). HA 1 virus has recently been isolated in France also (Chany *et al.*, 1959).

The American workers found a significant association between virus recovery and respiratory disease. Because we had studied few cases and no control groups we had not been able to demonstrate such an association. We therefore wished to determine whether similar viruses which we had isolated in Europe were pathogenic for man by inoculating them to normal adult subjects living in strict isolation. In view of the recent statement of an international committee (Andrewes *et al.*, 1959) we shall refer to these viruses as parainfluenza viruses 1 (HA 2) and 3 (HA 1).

Methods

The volunteers were aged between 18 and 45 years. They were isolated in pairs at this unit, as described elsewhere (Andrewes, 1948). After a quarantine period of 2½ days the virus was administered as nasal drops in a volume of 1 ml. of Hanks's saline. Most volunteers also consented to have serum collected just before the inoculation was made and again about two weeks later, after they had left the unit. Throat swabs were collected two, four, and six days after inoculation. Until tested, each was stored at −60° C. in 3 ml. of 0.5% bovine plasma albumin in Hanks's saline.

Virus isolations and neutralization tests were performed in roller-tube cultures of human-embryo kidney or monkey kidney maintained in medium 199. The technique used was similar to that described by Chanock *et al.* (1958). Viruses from most volunteers were identified by neutralization or by haemagglutination inhibition tests with hyperimmune rabbit sera. For haemagglutination inhibition tests we used 4–8 units of virus and sera treated with cholera filtrate. Virus and serum were allowed to react for one hour before adding red cells, but otherwise the techniques were those employed with influenza viruses.

Complement-fixation tests were done in "perspex" haemagglutination trays, using 0.1-ml. volumes, 2 units of complement, and overnight fixation at 4° C. The antigens were fluids from monkey-kidney cultures infected with parainfluenza 1 and 3, standardized against human convalescent sera (kindly supplied by Dr. R. M. Chanock) and used in optimal dilutions. Neutralization tests were done by mixing a dilution containing about 100 infectious doses per 0.05 ml. of virus with equal volumes of serial dilutions of serum. About 20 minutes later 0.1 ml. of each dilution was inoculated to each of two monkey-kidney cultures. These were tested four or five days later by haemadsorption (Vogel and Shelokov, 1957), using human group O red cells. Virus infectivity titres and neutralization titres were expressed as interpolated 50% end-points. Recovery of virus or a fourfold or greater rise in antibody as measured by one or more techniques was taken to indicate infection of the subject.

Virus Strains.—Parainfluenza 1: We used Cop 222 isolated from a child in Copenhagen (Birkum Petersen and von Magnus, 1958) and serologically identical with HA 2. *Parainfluenza 3:* The Moss virus was isolated from a child in Sheffield, and is closely related to HA 1 (Sutton *et al.*, 1959). We also used a strain (K) of parainfluenza 3 virus isolated by one of us (M. S. P.) from a London child suffering from "febrile catarrh."

Results

Table I summarizes the outcome of the experiments in which volunteers received the Copenhagen 222 strain of parainfluenza 1 virus (Birkum Petersen and von Magnus, 1958). Eighteen subjects were given virus, and, in addition, three received inoculum in which virus had been neutralized by specific immune serum. Eight volunteers had detectable neutralizing antibody in the first serum specimen, but two of these became infected. Six subjects developed illnesses which, by the criteria used in this unit, were mild colds. All of these subjects were shown to be infected with the virus. Virus was isolated from the throats of nine volunteers in all. The positive specimens were those taken on the fourth or sixth day, about the time of onset of respiratory symptoms. All three volunteers who were infected by first-pass virus showed antibody rises. There was no antibody rise in the volunteers receiving fifth-passage virus, even in those from whom virus was isolated. No uninfected volunteers developed colds, but three who were infected had no symptoms, or those they had were not thought to amount to a significant cold. These

TABLE I.—*Effects of Inoculating Volunteers With a Danish Strain of Parainfluenza 1 Virus*

No. of Tissue-culture Passages of Inoculated Virus	Dose of Virus Given*	No. of Volunteers		No. of Infected Volunteers with Illness
		Infected†	Ill†	
1	15	4/5	3/5	3/4
2	1·5	2/2	1/2	1/2
5	15	0/6	0/6	0/0
5	150	3/5	2/5	2/3
5	0‡	0/3	0/3	0/0

* Expressed as tissue-culture infectious doses.
† Numerator = number of volunteers infected. Denominator = number inoculated.
‡ Same inoculum as in line above but mixed with rabbit immune serum against parainfluenza 1 virus.

results show that the virus infects man and apparently produces a mild illness of the upper respiratory tract or a subclinical infection. As little as 1.5 tissue-culture infectious doses initiated infection and caused symptoms.

Table II summarizes the results using the Moss and K strains of parainfluenza 3 virus. Sera collected before inoculation all neutralized Moss virus. Fifteen subjects were given the Moss virus and five developed illnesses. All those who became ill were infected with the virus, and one of those who remained well was infected. Virus was isolated from the throat of one volunteer from whom paired sera were not collected. There was a rise in antibody titre in the paired sera from five other volunteers when these were tested by one or more of the three methods used. Virus was recovered from three of these five volunteers. Virus was recovered from both volunteers receiving the K virus and both showed a rise in antibody titre. Fifteen TCD_{50} of these viruses failed to produce infection of the six volunteers used in the tests.

In the serological investigations of volunteers infected with types 1 and 3 virus, antibody rises were detected in nine cases by neutralization tests, in six cases by complement-fixation tests, and in three cases by haemagglutination inhibition tests. There were five cases in which an antibody rise was detected by only one method, but virus was recovered from the throat of each of these volunteers. Some of the combinations of laboratory results are shown in Table III, which also shows that some antibody responses would have been missed if we had not used a variety of tests.

TABLE II.—*Effects of Inoculating Volunteers With Two English Strains of Parainfluenza 3 Virus*

Strain and No. of Tissue-culture Passages of Virus Inoculated	Dose of Virus Given*	No. of Volunteers		No. of Infected Volunteers with Illness
		Infected†	Ill†	
Moss 5	150,000	3/4	3/4	3/3
,, 5	15,000	2/4	1/4	1/2
,, 5	1,500	1/4	1/4	1/1
,, 5	15	0/3	0/3	0/0
K 2	1,500	2/2	2/2	2/2
K 2	15	0/3	0/3	0/0

* Expressed as tissue-culture infectious doses.
† Numerator = number of volunteers infected. Denominator = number inoculated.

TABLE III.—*Representative Laboratory Results in Five Infected Volunteers*

Inoculum		Virus Isolations on Day			Homologous Antibody Titrated by		
Strain	Dose	2	4	6	Neutralization*	Haemagglutination Inhibition*	Complement Fixation*
Cop 222:							
1st pass.	15	0	+	+	<4/>16	64/64	5/160
2nd ,,	1·5	+	+	+	<4/<4	8/<8	<5/20
,, ,,	1·5	0	+	+	<4/<4	16/24	10/10
Moss ..	150,000	+	+	0	32/128	32/32	80/80
,, ..	15,000	0	0	0	16/64	<10/20	10/80

* The first figure represents the titre of serum collected before infection and the second of serum collected after infection.

TABLE IV.—*Summary of Symptoms Produced by Parainfluenza Viruses and by a Typical Common Cold Virus*

	Frequency of Symptoms and Signs in Illnesses Produced by			
	Parainfluenza			Cold Washings H.G.P.
	Type 1 Cop 222	Type 3 Moss	Type 3 K	
>4 handkerchiefs/day* ..	5	5	2	12
Mucopurulent nasal discharge	1	3	0	5
Nasal stuffiness	6	4	2	11
Sore throat	5	4	1	10
Cough	0	1	1	9
Temperature >99·2° F. (>37·3° C.)	1	0	1	0
Incubation period (days) ..	1–7 (av. 4)	3–6 (av. 4)	3 and 4 (av. 3·5)	1–3 (av. 2·1)
Total no. of cases	6	5	2	12

* Indicates that volunteers used more than four paper handkerchiefs a day—an index of increased nasal secretion.

Symptoms and Signs in Infected Volunteers

Throughout these trials the clinical observer (M. L. B.) was unaware of the type of virus being used, and his impression was that, with one exception, the illnesses produced by these parainfluenza viruses fell within the range of illnesses produced by a "typical" cold virus—that is, nasal washings from an adult with afebrile coryza not transmissible to cultures (see Roden, 1958). This clinical impression was supported by an analysis summarized in Table IV. However, the incubation periods of colds produced by parainfluenza were longer than those in "typical" colds. In addition, one volunteer who received 1,500 TCD_{50} of the second tissue-culture passage of the K strain of parainfluenza type 3 virus developed a fever lasting four days (maximum temperature 101° F.; 38.3° C.), muscular aching, headache, coryza, cough, and a little sputum. She had, in short, clinical influenza, though her partner had a mild cold.

Discussion

We considered the possibility that the colds experienced by our volunteers were produced by a common-cold virus carried over from the washings from which the parainfluenza viruses were first isolated. It seemed unlikely that such a virus would survive five passages in monkey-kidney cultures and cause colds, since unpublished experiments designed to show this with a "pedigree" cold have been unsuccessful. The colds might have been produced by a non-cytopathic monkey-kidney virus which our tests might not have shown up. However, since the only volunteers to be ill had laboratory evidence of infection with parainfluenza viruses, and since no illness was found in volunteers who were not infected, we conclude that the parainfluenza viruses caused the illnesses.

The conclusion that our Danish parainfluenza 1 strain produced cold-like illnesses in adults is independent confirmation of the findings of Reichelderfer *et al.* (1958) in the U.S.A. We know of no previous published report that parainfluenza 3 strains cause illness in human volunteers, but our results with the Moss strain of parainfluenza 3 are very similar to those with parainfluenza 1. These experiments seem to fulfil the third clause of Koch's postulates for these viruses as causes of respiratory disease in man. It seems that parainfluenza 3 virus can cause colds and an influenza-like illness in adult volunteers, and Chanock *et al.* (1959) have impressive evidence that it may be concerned in mild and severe acute respiratory disease in children. Adults inoculated with parainfluenza 1 developed only colds, but this virus has recently been recovered in Ireland from an adult with an influenza-like illness (Meenan and Clark, personal communication). In contrast with these results, we have, in unpublished experiments, failed to produce illnesses like the typical common cold by inoculating Echo viruses (types 11 and 20) obtained from children with febrile respiratory infections.

We should also emphasize that we have failed to isolate parainfluenza, or any other known virus, from

washings containing typical pedigree cold viruses such as H.G.P. (Table III). We therefore feel that it has yet to be proved that parainfluenza viruses can under natural conditions produce a common-cold-like disease in adults.

Summary

Eighteen human volunteers were inoculated intranasally with various doses of a strain of parainfluenza 1 virus. Nine became infected, and six of these developed illnesses resembling the common cold.

Twenty volunteers received parainfluenza 3 virus. Eight became infected, of whom six developed cold-like illnesses, one had an illness like influenza, and one remained well. The colds produced were clinically indistinguishable from those occurring after instillation of washings containing a cold virus, but the incubation period was longer in the parainfluenza virus infections.

We wish to thank the volunteers whose willing and conscientious help made these experiments possible, Dr. C. H. Andrewes for his interest and help in the clinical assessment of the volunteers, and Miss J. B. Macdonald, S.R.N., and Miss S. Witt for other assistance. The monkey-kidney tissue and medium 199 used were kindly supplied by the Medical Research Council Laboratories at Holly Hill, Hampstead.

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The Planning Committee of the Royal Commonwealth Society for the Blind was appointed in the winter of 1956 to "review the principles underlying the Society's present activities and to advise on the principles which should govern the next phase of development," and they have now summarized their recommendations in a report entitled *The Next Five Years*. In drawing up the Society's medical programme, the Committee were advised by the Society's panel of medical consultants, under the chairmanship of Sir Stewart Duke-Elder, who recommended that the Society should offer to undertake field trials, probably in Africa, following on the isolation of the trachoma virus; that it should award medical scholarships, make grants to individual investigators, and finance a mobile eye institute staffed by a medical orderly to bring drugs against conjunctivitis to Kenya villages. The importance of medical work in the Society's programme, say the Committee, is underlined by the fact that at least two thirds of the blindness in the Commonwealth is preventable, and that present facilities for research, prevention, and treatment are wholly inadequate. The Society's funds should be channelled into a few well-defined schemes, such as those described, which can make a large impact on the problem, but it should not itself become involved in schemes which are so general that they require long-term action by Governments, or so specialized that they can be undertaken effectively only by an organization exclusively devoted to medical work.

ANTIBODIES TO HAEMOPHILUS INFLUENZAE IN CHRONIC BRONCHITIS

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The association of *Haemophilus influenzae* with respiratory disease other than epidemic influenza was recognized quite early (Kretz, 1897) but tended to be overlooked, particularly after the 1918 influenza pandemic. The shift of emphasis in the next few years to the influenza virus increased the neglect of the bacillus. Writing about chronic bronchitis, Marshall (1931) stressed the importance of exogenous infection by various organisms but made no mention whatever of *H. influenzae*. Mulder (1938) pointed out the frequency with which *H. influenzae* could be isolated from the sputum of patients with chronic bronchitis, and with the recent development of interest in this disease his results have been confirmed and extended (Mulder, 1956; May, 1958).

Several kinds of evidence demonstrate the closeness of the relation between chronic bronchitis and *H. influenzae*. It is the organism most commonly found in the sputum in this condition (Mulder, 1956), and is often present in pure or almost pure culture. If swabs are taken direct from the bronchi, so avoiding contamination with throat organisms, this predominance becomes even more marked (Brumfitt, Willoughby, and Bromley, 1957). Positive cultures are more common in patients with purulent than in those with mucoid sputum (May, 1958). Nevertheless the exact significance of *H. influenzae* in chronic bronchitis, and more particularly in the acute exacerbations of chronic bronchitis, remains uncertain. It was thought worth while examining the antibody response to *H. influenzae* in various stages of the disease to see whether further information on this point could be obtained.

Transient specific agglutinins to the autogenous strains of *H. influenzae* have been described in influenzal pneumonia (Wilson, Dunn, and Blair, 1924) and in bronchopneumonia (Humphrey and Joules, 1946). Wood, Buddingh, and Abberger (1954), using a slide-agglutination technique, showed a rise in titre lasting two to three weeks in 46 out of 51 infants with acute bronchiolitis. The titre was not related to the duration or severity of the disease. This problem differed from that of chronic bronchitis as all 32 strains of *H. influenzae* isolated belonged to one or other of Pittman's types. Similarly the extensive work on the immunology of *H. influenzae* meningitis is largely concerned with specific capsular polysaccharide antigens. The more relevant work of Faunce (see May, 1958) is discussed below.

Most respiratory strains of *H. influenzae* do not belong to specific Pittman types, but show marked antigenic heterogeneity (Wilson and Miles, 1955). Because of this, in all the agglutination reactions so far mentioned the patient's serum has been tested against his own strain of *H. influenzae*. In the present work all sera have been tested against extracts of *H. influenzae* prepared as described by Tunevall (1953) and shown by him to contain species-specific antigens common to all strains of *H. influenzae*.

By the use of tanned sheep red cells coated with such extracts, antibodies have been looked for and estimated