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

BY

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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

in sera from patients with chronic bronchitis in both quiescent and active phases of the disease, from patients with non-infected asthma, and from controls with no respiratory disease.

Methods

Preparation of H. influenzae extract (modified from Tunevall, 1953).—A freshly isolated respiratory strain of *H. influenzae* grown on boiled blood-agar was harvested after eight hours into 1% sodium carbonate. After one hour at room temperature the residue was removed by centrifugation and the supernatant dialysed against running tap-water for 24 hours. The opalescent viscid solution was then centrifuged again and the supernatant, with merthiolate to a final concentration of 1/10,000, was stored at 4° C.

Extracts from three respiratory strains were used.

Nature of the Antigenic Extract.—The three extracts used contained 2, 1.9, and 1.2 mg. dry wt./ml., of which approximately 3% was carbohydrate (anthrone method) and 66% protein (Folin–Ciocalteau method). (I am indebted to Dr. C. Jenkin for the protein estimations.)

Preparation of Standard Antibody.—Three rabbits were each given two injections, four weeks apart, of 2 ml. (2 mg. dry wt./ml.) of an extract, prepared as described above, mixed with 2 ml. of incomplete Freund's adjuvant (Bayol F and arlacel A only). The rabbits were bled four weeks after the second injection and the sera pooled.

Tanning and Sensitization of Sheep Red Cells.—Two to 14-day-old sheep red cells in Alsevers solution (supplied by the Serum Research Institute, Carshalton) were treated as described by Boyden (1951) except that dilutions of *H. influenzae* extract were used as the antigen and that all procedures were carried out at room temperature instead of at 37° C.

Antigen Titration.—The dilution of antigen required was determined by a preliminary titration repeated weekly for each batch of sheep cells. The final dilution used was such as to give the standard rabbit antiserum a titre of 1/32,000, and was usually in the range 0.06–0.12 mg. dry wt./ml. This is somewhat lower than the optimal concentrations given by Stavitsky (1954) for diphtheria toxoid =0.125 mg. and egg albumen =0.25 mg. *protein*/ml.

Antibody Titration.—Dilutions of patients' sera with 1% normal rabbit serum were tested against sensitized and unsensitized tanned cells. The standard antiserum was titrated with each batch to check the sensitivity. The specificity of positive sera was checked by inhibition with the antigen extract. Controls of sensitized and unsensitized tanned cells in 1% normal rabbit serum were always put up.

Patients Tested

Sera were obtained from 90 patients with chronic bronchitis, from 33 with asthma, and from 50 controls.

All those with bronchitis had suffered from chronic, usually productive, cough and breathlessness for at least two years. In addition they had, with varying frequency, acute infective episodes, particularly in winter. Some of them also wheezed, and would no doubt be classified by some physicians as having "asthmatic bronchitis" or "infective asthma." The justification for including them all under the one heading, bronchitis, is that they all had predominant cough and sputum.

The asthmatic group consisted of patients with paroxysmal attacks of wheezing and dyspnoea, often with milder but more prolonged wheezing between attacks. They had no sputum, or had small amounts of mucus mainly during attacks. Infective episodes were absent or rare.

The control sera came from patients in two wards who had no bronchitis or asthma, together with a miscellaneous collection of sera, sent to the laboratory for routine investigations, from patients with non-respiratory diseases. In order to improve the age-matching of the control and bronchitis groups, laboratory sera were used only if from patients over 50 years old.

Results

The age distribution of the three groups is shown in Table I. Most of the patients with bronchitis were over 40, while those with asthma were on the whole younger. The controls covered the age range of both but were mostly over 40.

56% of those with bronchitis, 58% of the controls, but only 33% of those with asthma were men. The sex distribution was approximately the same at all ages.

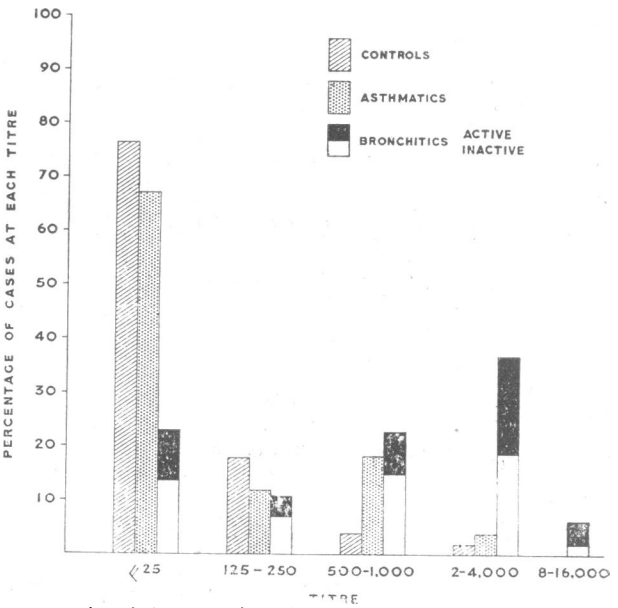
The distribution of antibody titres is given in Table II and the Chart. With a few exceptions the patients with

TABLE I.—*Age Distribution*

Age	Percentage at Each Age		
	Bronchitics	Asthmatics	Controls
0–19 years ..	0	12	2
20–39 ,, ..	11	33	6
40–59 ,, ..	51	52	54
60–79 ,, ..	38	3	38
No. in group ..	90	33	50

TABLE II.—*Anti-H. influenzae Titre in Three Groups of Patients*

Titre	No. of Patients		
	Bronchitics	Asthmatics	Controls
8,000–16,000 ..	5	0	0
2,000–4,000 ..	33	1	1
500–1,000 ..	21	6	2
125–250	10	4	9
<25	21	22	38
Total	90	33	50



Anti-H.-influenzae titres in three groups of patients.

asthma and the controls had very low titres, and the two groups were very similar. Most of the patients with bronchitis had high titres, 1/500 or more, though some were found over the whole scale. The difference between the patients with bronchitis and the controls is striking and significant.

The bronchitic group was then examined in more detail to see which factors, if any, were related to the antibody titre. No relation could be found between antibody titre and the age and sex of the patient or the duration of the disease.

Severity is difficult to measure objectively. A rough division was made into three grades—mild, moderate, and severe—based mainly on the amount of interference with work and normal living suffered by each patient. Patients in grade 1 were not off work from illness for more than three weeks a year and suffered little incapacity. Those in grade 3 were often in hospital or in bed at home and were unable to do their normal job. The rest were put in grade 2.

As always with such clinical classifications, grade 2 was rather large and covered too wide a range, but attempts at greater accuracy contained too great a subjective element to be of any value.

Using this crude measure of severity there was a slight but not statistically significant tendency for the more severe cases to have higher titres (Table III). When looked at individually conspicuous inconsistencies were found—both mild cases with high titres and, perhaps slightly more often, severe cases with low titres. It is not, of course, possible to say whether in fact they were severe because they had little antibody or mild because they had a lot.

Of the 90 patients with bronchitis 40 were tested two to three weeks after the onset of an acute infective attack in which *H. influenzae* was isolated from the sputum. The remaining 50 were tested during a quiescent phase. Comparison (Chart and Table IV) of antibody titres in the two groups shows no significant difference.

In 14 of the 40 patients with active infection paired sera were obtained, but in only one was there a significant rise over the period of two to three weeks (125–4,000). In two more there was a one-tube rise (1,000–2,000 and 2,000–4,000), while in one there was a one-tube fall (16,000–8,000). None of these changes can be regarded as significant.

TABLE III.—*Anti-H. influenzae Titre and Severity of Bronchitis*

Titre	Grade of Bronchitis		
	1	2	3
8,000–16,000 ..	0	3	2
2,000–4,000 ..	7	17	9
500–1,000 ..	5	10	6
125–250	2	8	0
<25	8	8	5
Total	22	46	22

TABLE IV.—*Anti-H. influenzae Titre in Active and Inactive Bronchitis*

Titre	Active	Inactive
8,000–16,000	3	2
2,000–4,000	16	17
500–1,000	8	13
125–250	4	6
<25	9	12
Total	40	50

In 10 patients further sera taken three to six months later all showed unchanged titres.

Discussion

The presence of antibody to *H. influenzae* to titres of 500 or more in 66% of patients with chronic bronchitis confirms the evidence already mentioned relating *H. influenzae* to this disease.

In the acute exacerbations of chronic bronchitis the position is less clear. If due to *H. influenzae* they might be followed by a rise in antibody titre. In the common more-prolonged states of active infection one might expect either a rise in antibody titre due to increased stimulation or possibly a fall if the antibody response were inadequate and circulating antibody were removed by increased antigen. The failure of the antibody titre to vary at all in acute attacks, while not supporting the haemophilus theory, does not disprove it. It is possible that absorption of antigen at the bronchial mucosa is poor, though Hers and Mulder (1953) have demonstrated *H. influenzae*, albeit in small numbers, between epithelial cells and occasionally deep to the basement membrane in cases of chronic bronchitis. The unchanged titre in some patients over periods of three to six months would fit with a steady slow absorption of antigen.

The work of Humphrey and Joules (1946) and of Wood *et al.* (1954) suggests that more rapid and more labile antibody responses can be detected with other, perhaps more superficial, antigens. In patients with chronic bronchitis Faunce (see May, 1958) found that an agglutinin titre of 1/20 or over against so-called rough or R strains of *H. influenzae* was more frequent among patients with purulent sputum than among those with mucoid sputum, but gave no figures of actual titres.

The relation between the antigens in sodium carbonate extracts as used in the present work and those responsible for the agglutination of Faunce's R organisms is unknown. Since the extracts were prepared from untypable respiratory strains they did not contain any specific polysaccharide. From Chen and Meyer's (1954) work using tanned red cells coated with protein and/or polysaccharide components of *Pasteurella pestis* it is probable that, under the conditions of sensitization used here (10 minutes at room temperature), protein would have been selectively adsorbed by the red cells and would have been the important antigen. However, some effect of the 3% carbohydrate present cannot entirely be excluded.

The present somewhat confused situation is unlikely to be clarified until much more is known about the markedly heterogeneous and poorly characterized antigens of respiratory strains of *H. influenzae*.

Summary

A method of detecting antibodies to *H. influenzae* by the agglutination of tanned red cells coated with a sodium carbonate extract of the organism is described.

With this method, antibodies in high titre (>500) have been found in 66% of patients with bronchitis compared with similar though never quite such high titres in 21% of asthmatic patients and 6% of controls.

Within the bronchitis group titres bore little or no relation to the duration or severity of the disease or to the presence of active infection.

The results confirm the close association of *H. influenzae* with chronic bronchitis but lend no support

to the theory that *H. influenzae* is responsible for the acute infective episodes which occur.

The need for further study of *H. influenzae* antigens is stressed.

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ACCIDENTAL INJECTION OF THIOPENTONE INTO ARTERIES

STUDIES OF PATHOLOGY AND TREATMENT

BY

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AND

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Thiopentone solution is sometimes injected accidentally into an artery in mistake for a vein during induction of anaesthesia. The clinical effects are well known. They range from gangrene and ischaemic contracture of muscles in the severe cases to minor degrees of anaesthesia of digits in the more fortunate patients. The clinical effects have been thoroughly described by Cohen in 1948, and by others whose publications are listed in the Bibliography. Uncertainty has existed about the nature of the vascular changes which follow the injection and which lead to the ischaemic end-results. This uncertainty has in turn led to some doubt on what treatment should be undertaken once the accident has happened.

Various factors have been suggested to explain the pathology. Prolonged arterial spasm has been postulated. It has also been suggested that the intimal layer of the artery is damaged and that thrombosis follows. The extreme alkalinity of thiopentone solution (usually about *p*H 10.9) has been blamed for these things. Widely differing treatments based on the uncertain nature of the pathology have been advocated or attempted. They have included the following measures:

(1) Injection into the damaged artery of a vasodilator drug. It has been advised that this be done immediately the accident has been discovered and, if possible, through the same needle without removing it. (2) The use of sympathetic or brachial-plexus block with local analgesic solution such as procaine (these measures being intended to relieve a postulated spasm of the injured artery). (3) Administration of anticoagulant drugs to prevent arterial thrombosis. (4) Arteriotomy and removal of clot from the vessel. (5) Arteriectomy: excision of a portion of the damaged vessel to relieve a theoretical reflex spasm of collateral branches.

Variable degrees of success have been reported after these measures, and it has been difficult to judge their efficacy under clinical conditions.

Laboratory Experiments

Investigations of the circulatory changes after intra-arterial thiopentone injections were for obvious reasons impossible in human subjects, so studies were undertaken in animals. The question of spasm was first investigated, employing a method similar to that described and used by Kinmonth and others in 1949, 1952, and 1957 in the rabbit and other animals. The intra-arterial injections were made into the femoral artery through a fine needle inserted just below the inguinal ligament. The needle-point was directed in a proximal direction as it would be under the clinical conditions of an accidental intra-arterial injection, and the needle was connected by a length of fine polyethylene tubing to a syringe to eliminate artifacts due to movement of the needle during injection. The artery was observed through a dissecting microscope a short distance below the site of injection, and changes in its diameter were measured with a micrometer eyepiece. Typical findings after an injection of 5% thiopentone are shown in Fig. 1.

There is a contraction of the vessel lasting about 30 seconds immediately after the injection of thiopentone. It is followed by a rapid return to the original diameter, after which a slight dilatation occurs for approximately one minute. On no occasion was any prolonged contraction observed nor anything at all resembling the spasm which follows mechanical trauma to an artery. Injections of buffered solution of alkalinity equal to that of thiopentone (0.1 M sodium carbonate/0.1 N hydrochloric acid, giving *p*H 10.9) produced no changes in the arterial diameter. This experiment was repeated many times with consistent results. On no occasion was anything other than transient contraction produced by thiopentone solutions, and alkaline solutions of equal *p*H never produced any change in arterial diameter.

The nature of the short-lived constriction and the ensuing vasodilatation after the thiopentone injection are of interest. They were also observed in animals where the femoral nerve had been divided and the femoral artery dissected free for a distance with the

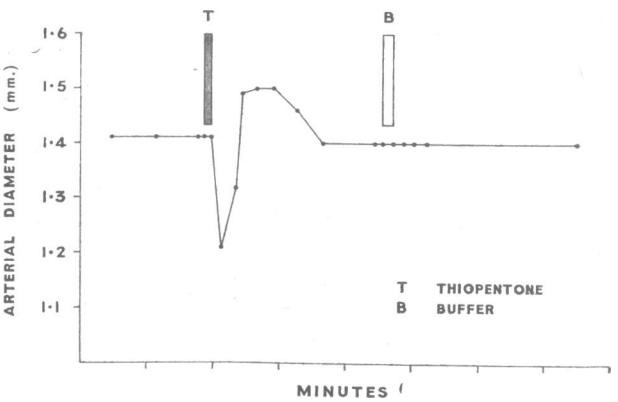

FIG. 1.—Injections into femoral artery of rabbits of (*a*) 0.5 ml. of 5% thiopentone at T, and (*b*) 0.3 ml. buffer solution of equal alkalinity (*p*H 10.9) at B.