Haemophilus influenzae and its pathogenicity

By

K. ZINNEMANN [1]

With 13 Figures

Table of contents

Introduction

It has been stated with some measure of justification that "the majority of bacteriologists at one time or another have come under the spell of the *Haemophilus* group" (ROSHER 1947). To a considerable extent this long continued interest is due to the puzzle set by PFEIFFER'S claim (1892) that the haemophilic Gram negative cocco-bacillus found by him in great numbers in the sputum of patients suffering from epidemic influenza and its after effects is the causative agent of that infection. His contention has been upheld by some (JOCHMANN 1903, HOLT 1910) and more or less violently contradicted by others (SACQUÉPÉE 1901, KLIENEBERGER 1905), and has continued as the subject of discussion for the better part of half a century.

Attempts to isolate *Haemophilus influenzae* during the period immediately after its discovery had been carried out on nutrient media containing fresh animal or human blood either smeared on top of the agar or incorporated in it. Growth on such media is notoriously poor and rather unreliable unless other organisms

[1] M. D. (Frankfurt), M. Sc. (Leeds); Senior Lecturer and Honorary Consultant Bacteriologist; School of Medicine, The University and The General Infirmary at Leeds, Yorkshire, England.

W. Kikuth et al. (eds.), *Ergebnisse der Mikrobiologie Immunitätsforschung und Experimentellen Therapie*

are also present, in which case the satellite phenomenon, first described by GRASSBERGER (1897), considerably facilitates recognition of the influenza bacillus. It must also be remembered that the claims and counterclaims regarding the role of *H. influenzae* in epidemic influenza, subsequent to PFEIFFER's original paper, were made during periods between pandemics and were probably related to local outbreaks of influenza and other respiratory infections.

The problem was not solved during the 1918 pandemic, probably in part because of inadequately developed techniques of isolation. The findings of LEVINTHAL (1918) and FILDES (1920) that blood treated carefully by heating or peptic digestion greatly improved growth of *H. influenzae* were published too late to be used universally by investigators during that pandemic. Those with experience, some of whom had used these new media, usually found *H. influenzae* in the majority of cases (FRÄNKEL 1918; LISTER 1918; TYTLER, JANES and DOBBIN 1919; DUVAL and HARRIS 1919; PFEIFFER 1919; LISTER and TAYLOR 1919; OLSON 1919, 1920; MCLEOD, RITCHIE and DOTTRIDGE 1921; OPIE, BLAKE, SMALL and RIVERS 1921; MCINTOSH 1922). However, PFEIFFER himself admitted (1893) that a disease closely resembling influenza could not be produced in animals infected with the bacillus he had discovered, and others after PFEIFFER failed similarly. On the other hand, during the 1918/19 pandemic successful inoculations in man were reported with filtrates of nasal and mouth washings from clinical cases and of lung juice from post mortem material (BRADFORD, BASHFORD and WILSON 1918/19; GIBSON, BOWMAN and CONNOR 1919). Yet, when similar inoculations were carried out with volunteers strictly isolated on an island untouched by the pandemic no clinical influenza resulted (LISTER and TAYLOR 1919). Nevertheless, CUMMINS (1919), though accepting the virus nature of the 1918/19 pandemic wrote when referring to the findings of GIBSON, BOWMAN and CONNOR: "Had it not been for these recent observations which have gone so far towards demonstrating the aetiological role of a filter passing organism, the evidence in favour of B. influenzae would probably have been regarded as conclusive. As it is, this bacillus must still be admitted to be the earliest and most constant associate, appearing on the scene within the first hours of attack."

The controversy persisted in a subdued fashion until 1933, when SMITH, ANDREWES and LAIDLAW isolated the influenza virus, with which they could regularly produce a febrile illness in ferrets. Since then PFEIFFER's claim that *H. influenzae* is the cause of epidemic influenza has been quietly abandoned by many workers in the field.

From 1943 onwards, when penicillin became available in the U.S.A. and Great Britain it was generally found that on media containing about 0.3 unit per ml. penicillin it is possible to isolate *H. influenzae* in small numbers from the respiratory tract of most healthy individuals (FLEMING and MACLEAN 1930; FLEMING 1937). For some years after that discovery it was a not uncommon experience to be asked, when reporting the presence of *H. influenzae* in a specimen, whether this organism was not now regarded as a saprophyte.

Since then the pendulum has swung slowly back with the recognition that, after all, *H. influenzae* is responsible for a number of infective conditions other than epidemic influenza. To-day few bacteriologists would deny the pathogenic

capacity of *H. influenzae* although they may hold divergent views on its relationship to epidemic influenza. BLAŠKOWIČ has given more historical details (1942) and those interested are referred to this part of his paper.

Some of the contradictions of the early investigators must have been due to the lack of information on the existence of other species of the genus *Haemophilus* and the criteria used for their differentiation. Thus, for instance, KRISTENSEN (1922) in his extensive monograph on the haemophilic bacteria tabulates "typical" and "atypical" growth and colony characteristics of PFEIFFER'S bacillus (p. 145). Some of the characteristics of his "atypical" strains agree well with the species described in the same year by RIVERS as *H. parainfluenzae* and at the time of writing apparently unknown to KRISTENSEN. Even at the present time differentiation of the members of the genus *Haemophilus* presents certain difficulties and it is appropriate, therefore, to deal with their identification in some detail.

I. The genus *Haemophilus*

Definition: differentiation, isolation and typing of the species found in man. The most recent descriptions of the genus current in the U.S.A. and Great Britain differ slightly, but significantly. Therefore both are quoted here in full.

a) Minute, rod-shaped cells which are sometimes thread-forming and pleomorphic. Non-motile. Gram-negative. Strict parasites, growing only in the presence of certain growth accessory substances. May or may not be pathogenic. Found in various lesions and secretions, as well as in normal respiratory tracts, of vertebrates (PITTMAN in Bergey's Manual of Determinative Bacteriology, 1957).

b) Minute rods, sometimes almost coccal, sometimes thread-like; may be highly pleomorphic. Usually non-motile. Non-sporing. Gram-negative, non-acid-fast. On first isolation most species are dependent for growth on some factor, or factors, contained in blood or plant tissues. Some species retain this dependence after prolonged cultivation on laboratory media. Some species are obligatory aerobes, or grow very poorly under anaerobic conditions. All known species appear to be obligatory parasites, inhabiting particularly the upper respiratory tract; and most of the described species or types are pathogenic (WILSON and MILES in "Topley and Wilson's Principles of Bacteriology and Immunity" 1955).

X and V requirements. The growth factors or growth accessory substances, which provide the main means of differentiation of the *Haemophilus* species, are generally known as the X and V factors.

For some time after PFEIFFER'S original discovery that blood or mucous secretion is necessary for the successful culturing of the influenza bacillus, it was generally assumed that haemoglobin itself was essential (KRISTENSEN 1922). However, in 1921 FILDES found that haematin can be substituted for haemoglobin. This heat-stable factor is now generally labelled the "X" factor in accordance with the current usage relating to growth stimulating derivatives of blood pigments.

The fundamental observation for the recognition of the second essential growth factor was made by GRASSBERGER (1897) when describing the satellite

Table 1. *Characteristics of Haemophilus species occurring in man or to be distinguished from the species isolated from human sources*

Species	X	V	Haemolysis	Indole	Morphology	Serology	Other characteristics and habitat
H. influenzae (PFEIFFER 1892)	+	+	—	±	coccobacillary; filaments	non-capsulated: heterologous; capsulated: type-specific	strong odour of indole on solid blood-containing media (Levinthal and chocolate agar). In respiratory tract of man
H. aegyptius (KOCH 1883, WEEKS 1886, 1887)	+	+	—	—	coccobacillary; filaments	? homologous	comet-like colonies in 0.15% agar medium; haemagglutination of human red blood corpuscles; growth more feeble than H. I.; in human conjunctiva in warm climates
H. suis (LEWIS and SHOPE 1931)	?+	+	—	—	coccobacillary; filaments	non-capsulated: heterologous; capsulated: type-specific	growth more feeble than that of H. I.; in swine, causing swine influenza when together with swine influenza virus; ? occurrence in man
H. haemolyticus (PRITCHETT and STILLMAN 1919)	+	+	+	±	more bacillary than H. I.; contorted filaments	?	in upper respiratory tract of man; rarely in subacute endocarditis
H. parainfluenzae [RIVERS 1922 (2)]	—	+	—	±	usually bacillary, longer than H. I., frequently with tapered ends	non-capsulated: heterologous; capsulated: type-specific	slightly yellowish tinge of older cultures on chocolate agar, colonies more friable than H. I., characteristic odour
H. parahaemolyticus (PITTMAN 1953)	—	+	+	—	large, stout, bacillary with tangled filaments	?	large, friable colonies, strains die out easily; in upper respiratory tract in acute pharyngitis, occasionally in subacute endocarditis
H. haemoglobinophilus (canis) (FRIEDBERGER 1903)	+	—	—	+	coccobacillary, short filaments	?	feeble growth, catalase positive (ZINNEMANN, unpublished); in preputial secretions of dogs, occasionally from upper respiratory tract of man
H. influenzae-murium (MACKIE, VAN ROOYEN and GILROY 1933; KAIRIES and SCHWARTZER 1936; IVANOVICS and IVANOVICS 1937)	+	—	—	—	stoutish bacilli, may have tapered ends; filaments	?	copious growth, greenish pigment in old growth on chocolate agar, catalase negative (ZINNEMANN, unpublished); in upper respiratory tract of laboratory mice
H. ducreyi (DUCREY 1890)	+	—	(+)	?	long, slender rods, often in short chain formation	?	in lesions of soft chancre
H. aphrophilus (KHAIRAT 1940)	+	—	—	—	coccobacillary to filamentous	?	microaerophilic, grows best in 10% CO_2; in subacute endocarditis
H. vaginalis (GARDNER and DUKES 1955)	+	?	±	?	coccobacillary, filaments	?	feeble growth in 10% CO_2 and air only, oxidase negative; in human vagina

H. I. H. influenzae; + positive; — negative; ± may or may not be positive; ? not investigated, not known or unpublished.

phenomenon. He noticed that on fresh blood agar plates relatively large colonies of the influenza bacillus grow in the immediate vicinity of colonies of Gram positive cocci, while the rest of the culture distant from the contaminating coccus colonies consists of minute dew-drop colonies. This second factor is also present in the tissues of plants and animals and is synthesized by most bacterial species except some members of the *Haemophilus* group (DAVIS 1917). It was thought at first that this unidentified thermolabile factor was closely related to a vitamin and it was, therefore, labelled the "V" factor [THJØTTA 1921; THJØTTA and AVERY 1921 (1), (2); DAVIS 1921 (1), (2), (3); FILDES 1922, 1923, 1924; KRISTENSEN 1922]. Finally in 1937 the LWOFF (1), (2), (3) proved that the V factor is coenzyme I or phosphopyridine nucleotide after PITTMAN (1935) had shown that the V factor is involved in the respiratory processes of the bacterial cell.

Details of the X and V requirements of the *Haemophilus* species usually isolated from human sources, and of species to be distinguished from those occurring in man, are given in Table 1, together with other characteristics to which recourse may have to be taken for the correct identification of strains.

It will be seen that there are 4 species requiring X and V, 2 requiring V only and 4 requiring X only.

Sources of X and V factors. A watery solution of commercial haematin hydrochloride, in a final dilution of up to 1 in 100 million can be used as X factor. Most workers prefer either FILDES' peptic digest of blood (1920) or LEVINTHAL'S broth (1918), both autoclaved, to destroy their content of V factor, and refiltered.

As V factor a watery solution of pure coenzyme I, in a final dilution of up to 1 in 350 million can be used. For economy's sake a yeast extract is preferable and ALEXANDER'S (1948) modification of THJØTTA and AVERY'S method provides a relatively simple technique for its preparation: Emulsify 100 g. of baker's or brewer's yeast in 400 ml. of distilled water, adjust p_H to about 4.6 and boil for 10 min. Centrifuge or filter through paper, adjust p_H to 7.0, sterilize by filtration and keep in sterile glass container with glass stopper.

Media and methods for testing of X and V requirements. The growth factor preparations described are used for tests in fluid media, usually a 2% peptone water or a nutrient broth to which sufficient X or V is added to give the final concentrations of the pure preparations required. If FILDES' blood digest and yeast extract are used three to ten per cent of these are added. Turbidity after inoculation and incubation is taken as an indication of growth in the presence of one or both growth factors; it may be necessary to subculture the growth on to a suitable solid medium to check for purity of the culture. This tedious process may be avoided by testing on solid media directly. One of the difficulties in determining X and V requirements is the fact that yeast extract not infrequently contains small quantities of X factor sufficient to permit growth of some, presumably less fastidious, X or X and V dependent strains (GILDER and GRANICK 1947; NETER 1947). Also, most of the basic fluid and solid media may contain traces of X factor, including some brands and batches of peptone (TURNER and ZINNEMANN, unpublished).

A medium consistently free from X factor during several years of use has in our hands proved to be yeastrel agar as recommended for bacterial counts of water supplies (M. o. H. Report No. 71, 1956) as follows.

Yeastrel[1]	3g.
Peptone	5g.
Agar	15g.
Distilled water	1000ml.

[1] Manufactured by Brewers' Food Supply Co., Ltd, Edinburgh.

Dissolve the yeastrel and peptone in distilled water in the steamer, adjust reaction at room temperature to p_H 7.4, using phenol red as indicator. Wash agar in muslin bag under running water for 15 min. and add yeastrel-peptone mixture. Autoclave at 15 lb. pressure for 20 min., filter through paper pulp in

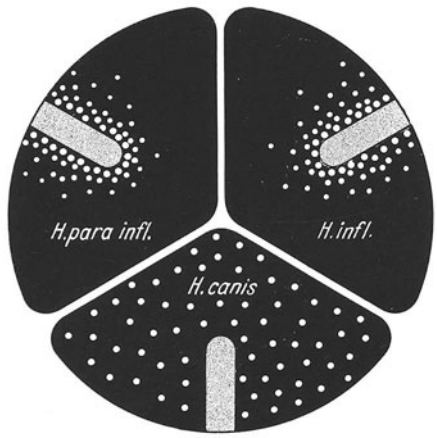

Fig. 1

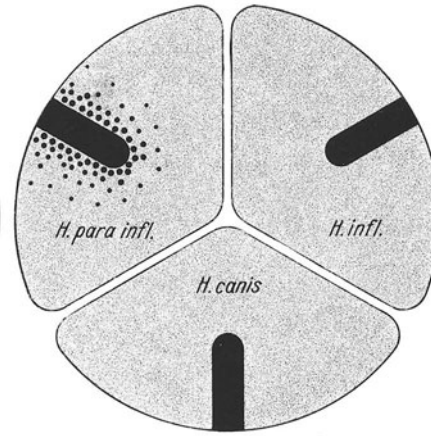

Fig. 2

Fig. 1. Growth of different Haemophilus species on autoclaved chocolate agar (*X* factor). Diagram. *V* factor supplied in restricted area by streak inoculation of Staphylococcus aureus

Fig. 2. Growth of different Haemophilus species on yeastrel agar (*X* and *V* deficient). Diagram. *V* factor supplied in restricted area by streak inoculation of Staphylococcus aureus

Büchner funnel, do not use egg white for clearing. Test reaction of filtrate at 50° C and, if necessary, adjust to p_H 7.0. Tube off in 10—15 ml. quantities and autoclave again at 15 lbs. for 20 min. The final reaction at room temperature should be p_H 7.2. As it has been autoclaved this medium is free from V factor as well. The medium is straw coloured and transparent.

For the determination of growth factor requirements it is used in conjunction with an autoclaved 10% fresh blood or chocolate, or an autoclaved LEVINTHAL agar plate all of which contain X factor only. The yeastrel agar and the opaque, dark brown coloured autoclaved blood agar plates are seeded uniformly with the *Haemophilus* strain under test. A staphylococcus is then streaked halfway across the inoculated area. Figs. 1 and 2 show diagrammatically the different readings that can be obtained with this method. Strains dependent on X factor alone grow all over the inoculated area of the autoclaved blood agar plate while on the same medium strains requiring either V alone or both X and V do not grow except as satellite growth in the vicinity of the staphylococcus streak.

On the yeastrel agar plate only V dependent strains show satellitism near the staphylococcus streak. Strains requiring either X only or both X and V show no growth at all on yeastrel agar. After more than 24 hours' incubation

V factor may diffuse from the staphylococcus streak up to the edge of the plate. To avoid erroneous readings the first should be taken on the day after setting up the test. A second reading two days after inoculation is only rarely required.

EVERALL (1953) soakes a sterile filter paper strip in an appr. 1 in 100 solution of commercial haematin hydrochloride (X) and one in undiluted yeast extract (V) and places the strips in a parallel fashion at a distance of 1 cm from each other on the surface of a nutrient agar plate containing a commercial meat extract (Lab-Lemco). Results are easy to read with this method, but the manipulation required for impregnation and placing of filter paper strips render the method prone to bacterial contamination.

PICKETT and STEWART (1953) rely for the identification of *H. influenzae* and *H. parainfluenzae* on their finding that catalase-positive bacteria induce satellite growth of both species whereas catalase-negative microorganisms induce satellite growth of *H. parainfluenzae* only. As basic medium they use either trypticase soy agar or heart infusion agar; in their hands the most satisfactory catalase producers are *Sarcina lutea, Micrococcus lysodeicticus, Neisseria catarrhalis* and *"Micrococcus pyogenes var. albus"* in descending order of effectiveness; the most satisfactory catalase-negative species is *S. faecalis*. The catalase- and non-catalase producers are streaked heavily in parallel lines across a plate previously inoculated uniformly with the *Haemophilus* strain under test. Using yeastrel agar these findings could not be confirmed by ZINNEMANN (unpublished).

Isolation of *H. influenzae*; media for isolation. Greater colony size facilitates recognition and isolation, and for this reason heated blood (chocolate) or LEVINTHAL agar plates are preferable to agar made with FILDES' peptic blood digest, on which *H. influenzae* colonies are rather smaller. The underlying principle, i.e. carefully controlled heating of a suspension of blood is the same in both chocolate and LEVINTHAL agar, the latter being merely a filtered version of the former. For the preparation of chocolate agar 10% of rabbit or horse blood is added to melted agar cooled down to 60° C, mixed well and the whole is then slowly brought to a temperature not exceeding 75—80° C in a water bath at which temperature it is kept until the colour of the mixture turns to that of milk chocolate. PITTMAN'S modification of LEVINTHAL agar (1931) is a simpler way of preparation than the original one by LEVINTHAL given in 1918 and 1922. It consists merely of making a 10% LEVINTHAL broth and mixing equal parts of that and of a 3—4% nutrient agar at 50—60° C before pouring plates. The broth is made by heating 10% fresh blood broth in the steamer for 5 min., filtering through paper and then through a Berkefeld candle. FILDES' agar (1920) contains 5% of his peptic blood digest, which is prepared in the following way. A mixture of 150 ml. saline solution, 6 ml. concentrated HCl, 50 ml. defibrinated rabbit, sheep or horse blood and 1 g of pepsin is heated at 55° C in a stoppered bottle for 2 to 24 hours. Then sufficient of a 20% sodium hydroxide solution (usually about 12 ml.) is added until a sample of the mixture diluted with water gives a permanganate-red colour with cresol-red indicator. Concentrated HCl is now added drop by drop until a sample of the mixture shows almost no change of colour with cresol-red, but a definite red tint with phenol red. It is important to avoid excess of acid. Chloroform is added to give a concentration of 0.25%

and the mixture is shaken vigorously. This peptic digest keeps for months. For use it is heated to 55° C for thirty minutes to remove the chloroform.

These three media, if used in conjunction with a fresh blood agar plate, facilitate the recognition and isolation of *H. influenzae* and other members of the genus and in particular of the haemolytic species.

Non-capsulated and capsulated *H. influenzae* strains; surface antigenic structure. There had been a few observations in the literature to the effect that *H. influenzae* strains isolated from cases of meningitis differ morphologically and in some other respects from the usual type of strain found in the respiratory tract (COHEN 1909; HENRY 1912; WOLLSTEIN 1911, 1915; RIVERS and KOHN 1921; ENGERING 1923; GREKOWITZ 1929). Then, in 1931 PITTMAN discovered that as in the pneumococcus the surface of meningeal and certain other strains of *H. influenzae* is covered by a capsule; that such capsules act as specific antigens stimulating in immunized animals the production of precipito-agglutinins; that the concentration of these precipito-agglutinins in the serum can be determined by chemical methods (ALEXANDER and HEIDELBERGER 1940); that six different capsular antigens occur according to which six serotypes, subsequently labelled a to f, can be distinguished. These capsular antigens were reported to consist of six different polysaccharide combinations, five of which were analysed chemically (MACPHERSON, HEIDELBERGER, ALEXANDER and LEIDY 1946). Later, however, it was found that the specific substance of types a, b and c is a polyribophosphate (ZAMENHOF, LEIDY, FITZGERALD, ALEXANDER and CHARGAFF 1953; ZAMENHOF and LEIDY 1954). PITTMAN also established that capsulated strains are more pathogenic for white mice than non-capsulated strains (1931). Unless capsulated strains are kept in fresh rabbit blood at 4° C the capacity to form capsules may be lost, as such strains tend to dissociate into the commoner, non-capsulated ones. It is important, therefore, to distinguish between capsulated and non-capsulated strains.

Colonial characteristics of non-capsulated and capsulated *H. influenzae* strains. After 24 hours incubation on chocolate agar colonies of non-capsulated strains attain a diameter of 0.5—1.0 mm. with a pointed central portion, an intermediate flattened portion and a sharply bevelled periphery with a somewhat irregular edge, the whole surface showing very fine to medium granulation. In confluent growth lines of division between individual colonies can be seen frequently (Fig. 3).

Individual colonies of capsulated strains are usually larger and may attain a diameter of 1—3 mm. They are slightly whitish opaque, with a smooth, shiny surface, and appear mucoid. The edge is entire and after 24 hours its outline is a perfect circle. There is a great tendency for colonies to coalesce and no lines of division can be seen in confluent growth or in colonies growing close to each other (Fig. 3). The difference to touch with a wire loop between non-capsulated and capsulated strains can best be described as similar to that between margarine and butter on a warm day. ROSHER (1947) incubated single colonies of the two different growth forms for periods of up to 3 weeks in sealed FILDES' agar plates and noticed very marked differences between them.

On FILDES' or LEVINTHAL'S agar capsulated strains show iridescence in obliquely transmitted light; non-capsulated strains do not. The iridescence is due

to dispersion of light due to arrangement of the organisms in a sort of grid pattern like a diffraction grating [ENGBAEK 1950 (2)].

Serological identification. Non-capsulated strains are antigenically markedly heterogeneous as some workers noted with chagrin during the 1918 influenza pandemic (VALENTINE and COOPER 1919; PARK, WILLIAMS and COOPER 1918/19; KRISTENSEN 1922). The 6 capsulated *H. influenzae* types a to f, as already pointed out, are well defined serologically and high titre antisera giving sharp reactions in tests are obtained when using ALEXANDER, LEIDY and MACPHERSON'S (1946) or EATON, GERWE and LEONARD'S (1948) methods.

Slide agglutination test. Floccular precipito-agglutination due to the capsular antigen-antibody reaction occurs almost instantaneously in the slide agglutination

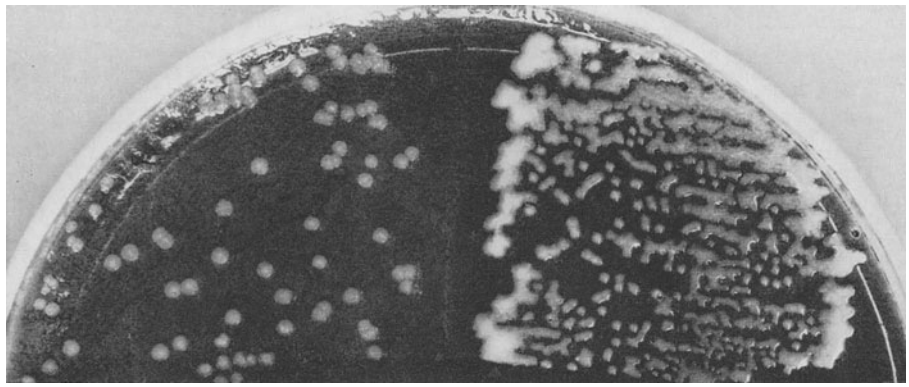

Fig. 3. Growth of non-capsulated (left) and capsulated (right) *H. influenzae* on chocolate agar

test. Slower, granular, non-type specific agglutinations occur with some strains and sera; they are presumably due to somatic antigens and their agglutinins, and make the slide agglutination test less than 100% reliable [ENGBAEK 1950 (1)]. It is important, therefore, to obtain confirmation by one of the other available methods.

Formal tube precipito-agglutination. Best results are obtained when an appropriate amount of an overnight LEVINTHAL broth culture is added to the serum dilutions, the tubes are incubated at 37° C for 4 hours and are then placed in a refrigerator overnight (ALLISON, GORDON and ZINNEMANN 1943). In serum dilutions up to 1 in 20 a firm disk of precipitate is often seen at the bottom of the tube and this disk can be broken up into coarse floccules by shaking. Higher dilutions show all degrees of floccular to finely granular agglutination. No non-specific or cross-reactions occur between the 6 capsular types with this method.

Precipitin reaction. The specific capsular substances go easily into solution in culture media and body fluids, and can be extracted easily by suspending cultures from solid media in physiological saline or distilled water. They can be identified in body fluids by the precipitation ring test which is best carried out in capillary tubes. This test may be useful when *H. influenzae*-like organisms can be seen in direct films, but can no longer be isolated owing to chemotherapy having been started. Both serum and body fluid must be clear for the test. In a Pasteur pipette one or two drops of the body fluid are slowly layered over a

small amount of antiserum; a white ring will form within a few minutes if antigen and antibody match. If ring formation is delayed it can be speeded up by short incubation at 37° C. On rare occasions interpretation of the results may be difficult because cross reactions occur between the specific capsular substances of *H. influenzae* type b and the pneumococcus subgroups 6, 6A, 6B, 15A, 29, 35B, *H. influenzae* type a and pneumococcus group 6, *H. influenzae* type c and pneumococcus groups 11, 11B, 19A, 21 and 33B [ALEXANDER 1948, TUNEVALL 1952 (3)] as shown in Table 2 adapted from TUNEVALL.

Table 2. *Cross-reactions between H. influenzae and pneumococcus type antigens and the corresponding type-specific antisera.* [After TUNEVALL 1952 (3)]

H. influenzae	Type of	Pneumococcus	Authors
a	⟷	6B	CHAPMAN and OSBORNE (1942), TUNEVALL [1952 (3)]
b	⟷	6	ZEPP and HODES (1943), NETER (1943)
	⟷	6A, 6B	TUNEVALL [1952 (3)]
	——→	15A	ALEXANDER, LEIDY and MACPHERSON (1946)
	⟷	29	ZEPP and HODES (1943)
	——→	35B	ALEXANDER et al. (1946), TUNEVALL [1952 (3)]
c	——→	11, 11B, 19A	ALEXANDER et al. (1946)
	←——	21, 33B	ENGBAEK (1949)

The ring precipitation test is usually carried out with cerebrospinal fluid of treated cases of meningitis, with serous exudates of the pleural or abdominal cavities and aspiration fluids from joints. Positive results are sometimes obtained with blood serum in severe infections of some duration. Specific polysaccharides are excreted in the urine where they can be detected with the test.

Neufeld reaction (Capsule swelling test). This technique was first applied to *H. influenzae* type strains by ALEXANDER (1939). A hanging drop preparation is made with a) one loopful of a young LEVINTHAL broth culture, b) one loopful of type-specific antiserum and c) one discharged loopful of LOEFFLER'S methylene blue. Capsules around the blue stained bacillary bodies are well defined when viewed through a microscope with well corrected tube length (Fig. 4).

Cross-reactions between *H. influenzae* type sera do not occur with this technique. LEVINTHAL broth cultures should not be older than five to six hours as most of the capsular substance goes into solution during longer incubation. The age of a LEVINTHAL broth culture can be reduced considerably if a small amount of medium (1—2 ml.) is pre-warmed to 37° C before inoculation with an overnight chocolate or LEVINTHAL agar culture immediately after the latter has been removed from the incubator. In this way the lag period can be circumvented (ROGERS and ZINNEMANN 1958). Capsules are fully developed when the LEVINTHAL broth culture shows the first signs of turbidity, which occurs after one to two hours under the conditions just described.

The patient's own serum can be used for typing the infecting *H. influenzae* strain if the infection has lasted for a week or more. Sufficient specific antibody is circulating in the blood by that time to make the serum suitable for the NEUFELD test with laboratory type strains a to f. Occasions for this technique are rare, but it can be useful when a *H. influenzae* strain has been isolated and has died

before it could be typed. Errors are possible with this technique owing to cross-reactions between *H. influenzae* and pneumococcus type strains.

As Table 1 shows non-capsulated and capsulated strains occur also with the species *H. suis* and *H. parainfluenzae*. These species have not been studied as intensively as *H. influenzae* and, therefore, relatively little is known of the number of serological types within these species or of their frequency and significance.

Complexity of *H. influenzae* capsule. The cross reactions between certain *H. influenzae* and pneumococcus type antigens and their sera, as seen in Table 2,

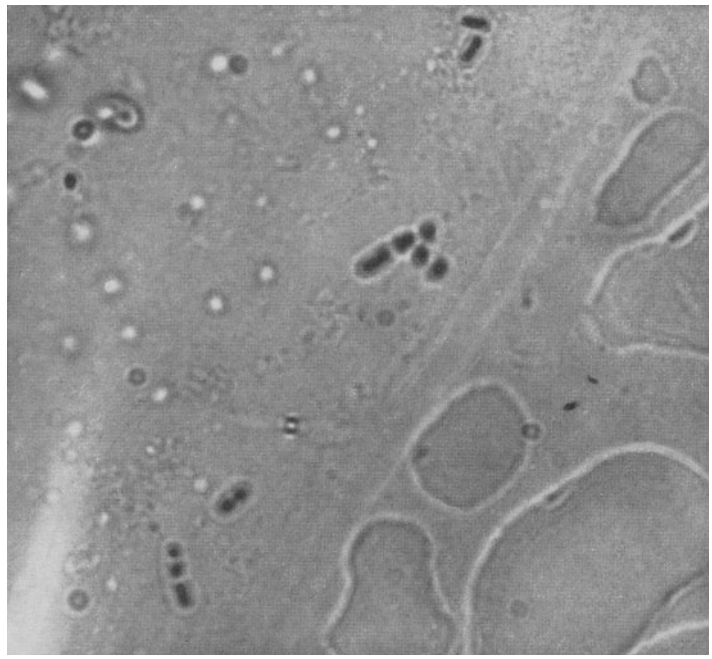

Fig. 4. Capsule visible in Neufeld test with type b *H. influenzae* strain. Hanging drop preparation of 2 hour LEVINTHAL broth culture, with 1 loopful of type specific antiserum and 1 loopful of methylene blue added and mixed; microscope focused on edge of drop, evaporation zone in lower right half of picture. Oil immersion

suggest that more than one polysaccharide complex may be present in the capsular material of a given *H. influenzae* type strain. By immunological means WILLIAMSON and ZINNEMANN (1951, 1954) have shown that the capsular antigens of most type e strains contain two components, whilst that of some strains is composed of only one of the two antigens. They labelled the two antigens e1 and e2 and regarded strains with e2 antigen only as degraded, although such strains may be isolated from patients. No strain with e1 antigen only has as yet been found. There is no reason why capsular antigens of other *H. influenzae* types should not be similarly complex.

When a non-capsulated strain of *H. influenzae* originally derived from a capsulated strain is exposed to desoxyribonucleic acid (DNA) of its original parent or of a different type, a small proportion of the cells is transformed into a capsulated strain of the type from which the DNA was prepared (ALEXANDER and LEIDY 1950). Probably such susceptible non-capsulated bacterial cells arise

as the result of mutation, the transformation of susceptible cells taking place almost immediately on exposure to DNA (ALEXANDER and LEIDY 1951). With this hitherto unknown technique new types of *H. influenzae* can be produced in vitro which combine the type specific substances of two types within the same cell (LEIDY, HAHN and ALEXANDER 1953). These findings raise the question whether the predominance of type b strains in *H. influenzae* meningitis of children is not due to the organisms acquiring the type b capsular substance through the substrate on which they are growing within the patients' body rather than to the greater virulence for man of type b strains as has been postulated by DAWSON and ZINNEMANN (1952).

Differentiation of H. influenzae from other X and V dependent species. This may present difficulties. In fact, until the careful work of PITTMAN and DAVIS (1950) and DAVIS, PITTMAN and GRIFFITS (1950) there were no means of distinguishing between *H. influenzae* and the KOCH-WEEKS bacillus, *H. aegyptius*, other than the knowledge of the site from which the strains had been isolated. They showed that colony appearances in a semifluid medium containing 0.15% agar are characteristically different. *H. aegyptius* colonies develop a comet-like, short, downward, fluffy projection after 24—36 hours' growth. Non-capsulated *H. influenzae* colonies are granular, without the fluffy elongation, and capsulated strains always produce a large, fluffy colony without any projection. Occasionally non-capsulated strains grow in small, fluffy colonies which makes this means of differentiation not entirely reliable. However, cultures of *H. aegyptius* in beef heart infusion broth containing FILDES' peptic digest of blood, or saline suspensions of cultures on beef heart infusion agar with FILDES peptic digest of blood give a marked agglutination of a 0.5% suspension of washed human red blood corpuscles of all blood groups if an equal volume of the suspension of red blood corpuscles is added and the mixture is left at room temperature. No haemagglutination occurs with any of the other *Haemophilus* species. Haemagglutination can be inhibited to varying titre by *H. aegyptius* rabbit antiserum; antisera against other haemophili have no such inhibiting effect. Less useful differences are that *H. aegyptius* is serologically more homogenous than non-capsulated *H. influenzae*, that growth is slower and consequently colonies are smaller, and that *H. aegyptius* never forms indole or ferments xylose, both of which biochemical activities may or may not be present in strains of *H. influenzae*. OLITZKI and SULITZEANU (1958) have recently shown that the different antigenic composition of *H. aegyptius* and *H. influenzae* can be demonstrated very well by the agar diffusion technique and this method may well provide one of the most convincing procedures for the differentiatial diagnosis of *H. influenzae* and *H. aegyptius*.

The differentiation of *H. suis* from *H. influenzae* is less easy. According to LEWIS and SHOPE (1931) *H. suis* strains grow more feebly, are indole negative and do not ferment carbohydrates. Others record *H. suis* as fermenting maltose and saccharose and as unable to utilise galactose whilst *H. influenzae* ferments galactose but not maltose and saccharose (PITTMAN and DAVIS 1950). Thus it is doubtful whether the presence or absence of enzymic activities can be regarded as a good and reliable way of separating the two species. So far, apart from the more feeble growth of *H. suis* there does not appear to be any useful differentiating

characteristic, and as a result one cannot tell whether *H. suis* occurs in man, as was suggested by LEWIS and SHOPE.

Production of haemolysis and the somewhat large colonies of *H. haemolyticus* make it easy to separate this species from *H. influenzae*.

Differentiation between X or V dependent species. With most species no difficulties should arise if the tests for X and V requirements are supplemented by the indole and haemolysin tests.

It may, however, not be easy to distinguish between *H. haemoglobinophilus* (canis) and *H. influenzae-murium*, particularly as the former may occasionally be isolated from man (ZINNEMANN, unpublished). There is adequate information in the literature on *H. haemoglobinophilus*, but that on *H. influenzae-murium* requires extension, as only a small number of strains have been investigated and these not very thoroughly (MACKIE, VAN ROOYEN and GILROY 1933; KAIRIES and SCHWARTZER 1936; IVANOVICS and IVANOVICS 1937; LWOFF 1939). So far it appears that *H. haemoglobinophilus* (canis) grows more feebly than *H. influenzae-murium*, and produces indole and catalase; *H. influenzae-murium* does not possess these characteristics, and in addition old cultures of *H. influenzae-murium* on chocolate agar may show a small amount of a greenish-yellow pigment (ZINNEMANN, unpublished).

Somatic antigens and antibodies. Little is known of the somatic antigens. It is obvious from the heterogeneity of the non-capsulated strains that the antigenic composition of the surface of the non-capsulated bacillary body of *H. influenzae* must be very complex. On the other hand, TUNEVALL [1953 (2)] showed with the agar diffusion technique that growing cultures, and LEVINTHAL agar cultures dissolved in 1% sodium carbonate, contain one, and possibly more than one antigenic component common to capsulated and non-capsulated strains of the species, or at least widely distributed among all strains.

The multiple precipitation lines in some of this author's experiments may be due to the fact that during the first three to five days of incubation the basins in the agar gel were refilled daily with antigen and antiserum. This is likely to produce repetitions of the same line at various distances from the basins (WILLIAMSON and ZINNEMANN, unpublished).

TUNEVALL also demonstrated the presence of a complement fixing antibody in a considerable percentage of children with *H. influenzae* sinusitis or otitis, in *H. influenzae* carriers and in about 70% of healthy adults when a sodium carbonate extract of *H. influenzae* was used as antigen [1952 (1), (2), 1953 (1)]. Moreover, complement fixing antibody could often be demonstrated in children during the first three months of life whilst it was absent between the ages of three months to two years. In the 2—3 year age groups 60% were still without complement fixing antibody whilst between the ages of four to seven only 20% were negative. Titres in this last group, when present, were about twice as high as in adults. In this context it is of some interest to recall that blood of children 2 months to 4 years old has no bactericidal power against *H. influenzae* (FOTHERGILL and WRIGHT 1933), and this fact is usually quoted to explain the predominance of severe *H. influenzae* infections in infants and young children. However, on the basis of experiments with white mice REITLER (1954) suggested that immature cells are more vulnerable to the action of the endotoxin of *H. influenzae* and

that this may be an additional factor in the high susceptibility of infants. So far, little is known about toxins produced by *H. influenzae* although large doses of culture filtrates injected into laboratory animals produce toxic death. DUBOS (1941) obtained an endotoxin from a type b strain by means of alkaline extraction. This endotoxin caused generalized haemorrhagic lesions in rabbits in a dose of 0.1 mg. or less of the desiccated material. Rabbits immunized with it became completely resistant to it and to injections of large amounts of live broth cultures

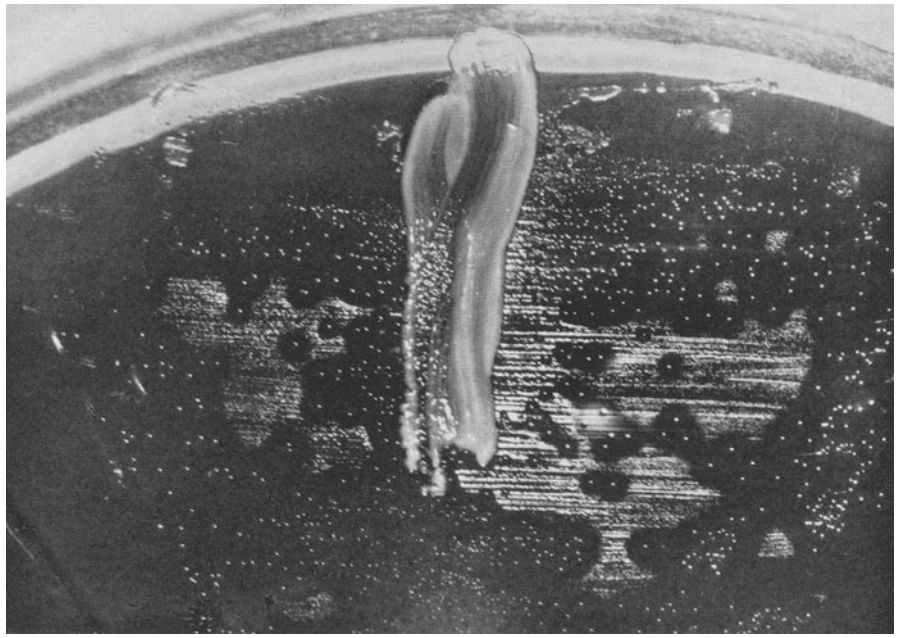

Fig. 5. Inhibition of *H. influenzae* by small pneumococcus colonies in the vicinity of streak inoculation with a Staphylococcus aureus; autoclaved chocolate agar, heavy inoculum of *H. influenzae* to give confluent growth, small inoculum of pneumococcus to give isolated colonies

of *H. influenzae* types b and d. Serum of immunized rabbits afforded passive immunity to rabbits against injection of toxin or live cultures.

In vitro antagonism between *H. influenzae* and other microorganisms common in the respiratory tract. There are occasional hints in the literature to the effect that the growth of *H. influenzae* can be inhibited by other microorganisms (JOCHMANN and KRAUSE 1901; JOCHMANN 103; RIVERS and POOLE 1921; KRISTENSEN 1922; Leading Article 1956). The first convincing account of the inhibiting power of pneumococci on *H. influenzae* was published in 1957 by SCHÄFER. He described the phenomenon, which was repeatable in his hands, without offering an explanation of the "antibiotic" effect. Similar observations had been made in Leeds (ZINNEMANN, unpublished) with mixed non-capsulated *H. influenzae* and pneumococcus cultures growing on autoclaved chocolate agar. Zones of inhibition were seen around single pneumococcus colonies within a confluent growth of *H. influenzae* close to streak inoculation of a *Staphylococcus aureus* (Fig. 5). These zones of inhibition can be seen on chocolate, but

not on fresh blood agar plates and their width coincides roughly with that of the yellow discoloration of the medium due to hydrogen peroxide production by growing pneumococci (McLeod and Gordon 1922). The zone of inhibition of *H. influenzae* together with the zone of discoloration due to hydrogen peroxide does not form if catalase is spread on the chocolate agar before inoculation with *H. influenzae* and the pneumococcus (Cooper 1959; see Fig. 6). The available evidence suggests, therefore, that the inhibitory effect of pneumococci on

Fig. 6. Inhibition of growth of a *H. influenzae* strain around streaks of two different pneumococci on the untreated upper half of a chocolate agar plate. No inhibition of growth around streaks of the same pneumococci occurs on the lower half of the plate which was previously treated with catalase. (From Cooper 1959)

H. influenzae is due to hydrogen peroxide production. To a small extent this inhibitory action can be counteracted by the catalase in *H. influenzae* cultures, but *H. influenzae* is not a strong catalase producer and amounts of catalase seem to vary with the strain of *H. influenzae* used (Cooper 1959).

II. The sensitivity of *H. influenzae* to antibacterial drugs

The realisation that *H. influenzae* is a pathogenic microorganism of some potency in conditions other than epidemic influenza led to investigations into the inhibition of its growth by antibacterial drugs.

Specific *H. influenzae* antiserum. Before the present era of chemotherapy in infections had got into its full stride attempts were confined to the production of an effective antiserum against type b capsulated strains of *H. influenzae* which had first been recognised as the cause of death in a considerable proportion of cases of meningitis in children in the U.S.A. (NEAL, JACKSON and APPELBAUM 1934; LINDSAY, RICE and SELINGER 1940). The first antisera were produced in horses (PITTMAN 1933; WILKES-WEISS and HUNTINGTON 1936; FOTHERGILL 1937) and were used clinically without much striking success. In 1936 HORSFALL, GOODNER and MACLEOD showed that, owing to the smaller size of the pneumococcus antibody molecule of rabbit serum its diffusion into the infected foci, when injected for treatment, takes place more readily and thus combines more effectively with the antigen than that of pneumococcus type-specific horse serum. Since there are close analogies in the antigenic structure of pneumococci and capsulated *H. influenzae* (PITTMAN 1931), *H. influenzae* type b rabbit antiserum was manufactured and its use reported on by PITTMAN (1933) and ALEXANDER (1939). ALEXANDER in particular showed experimentally that 0.6 ml. of a 1 in 100 dilution of such antiserum protects mice against 28875 LD 50 doses of *H. influenzae* type b culture. The protective effect of rabbit antiserum is enhanced by combining it with a suitable sulphonamide. Clinical application of this principle confirmed the protective power of antiserum combined with sulphadiazine in children. The therapeutic effect was believed to be due to neutralisation of the presumably toxic capsular polysaccharides of *H. influenzae* type b [ALEXANDER 1943 (1), (2)].

Inhibition of *H. influenzae* by sulphonamides. Even before the potentiating effect of sulphonamides on type-specific rabbit serum was known, the attention of investigators was drawn to the action of sulphonamides on *H. influenzae* strains, perhaps because supplies of rabbit antiserum were short and the preparation was rather expensive. The first report in this field was published by PITTMAN (1939), in the year in which *H. influenzae* type-specific rabbit antiserum became available for clinical use. By 1942, when some more active and less toxic sulphonamide compounds had been produced PITTMAN established that both sulphathiazole and sulphadiazine in concentrations of 10 mg./100 ml. are efficient inhibiting agents for *H. influenzae*. Six laboratory strains of the b type were more efficiently inhibited by these two compounds, both in in vitro and in vivo experiments, than by sulphanilamide, sulphapyridine, sulphanilyl sulphanilamide or para-nitrobenzoic acid. In mouse protection experiments the animals were challenged with 1000000 minimum fatal doses of a culture suspended in a 3.5—5.0% solution of mucin. Almost simultaneously ALEXANDER, ELLIS and LEIDY (1942) reported similar findings. GUYTON (1940) had earlier investigated a few *H. influenzae* strains in this respect and soon others followed (MCINTOSH and DRYSDALE 1945; DRYSDALE, MCINTOSH and BRODIE 1946; THOMSON, BRUCE and GREEN 1947; ENGBAEK 1948; MARTIN and SUREAU 1948). MØRCH-LUND (1949) found that 56 out of 59 strains of *H. influenzae* from cerebrospinal fluid and otitic pus were sensitive to the high concentration of 500 mg. per 100 ml. of sulphathiazole. Such concentrations cannot be obtained in human tissues and body fluids except perhaps in the urine and consequently it was difficult to interpret the therapeutic value of this sulphonamide in relation

to *H. influenzae* infections. The position was clarified when in 1950 ZINNEMANN tested the sensitivity of 50 capsulated and 50 non-capsulated *H. influenzae* strains on LEVINTHAL agar plates containing, either incorporated in the medium or in solution in holes punched into the agar, 10 mg. per 100 ml. of sulphanilamide, sulphapyridine, sulphathiazole, sulphadiazine, sulphadimidine, sulphamerazine, maphenide and sulphatriad. The concentration of 10 mg. per 100 ml. corresponds to the average blood level obtained with average doses of sulphonamides. Care was taken to neutralize the inhibiting effect of peptones on sulphonamides by using horse blood media, the Harper-Cawston effect as some call it (HARPER and CAWSTON 1945; WALKER, PHILIP, SMYTH and MCLEOD 1947). Both the punch plate method and incorporation of the sulphonamides in LEVINTHAL agar gave rather similar results. Sulphathiazole was the most effective single drug in vitro, almost equalled by sulphadiazine, sulphadimidine and sulphamerazine. A combination of sulphathiazole, sulphadiazine and sulphamerazine in the proportion of 37:37:26, as recommended by FRISK, HAGERMAN, HELANDER and SJÖGREN (1947), LEHR (1947) and MARTIN and SUREAU (1948) and commercially available as "sulphatriad", was at least equally and probably more inhibitory than any of its three constituents alone. In all experiments more non-capsulated than capsulated strains were sensitive to the test concentration of the sulphonamides. Table 3 summarises the sensitivity tests with sulphatriad.

Table 3. *Inhibition by 10 mg. per 100 ml. concentration of sulphatriad, of H. influenzae strains growing on Levinthal agar; punch plate method*

Degree of sensitivity	No. of capsulated *H. influenzae* strains	No. of non-capsulated *H. influenzae* strains
Insensitive	3	0
Marked inhibition or stunted growth	19	35
Complete inhibition .	28	15

Carefully prepared heated horse blood (chocolate) agar plates give equally good readings. This medium has been used in an investigation of some of the many sulphonamides that have come on the market since 1950, using the replica technique (COOPER 1959). This is a two-stage method. The first stage is the "initial punch-hole plate" as used by ZINNEMANN (1950). After the results have been read a replica is made on another plate by transferring growth from the initial plate by means of a velvet disk mounted on a circular wooden block (LEDERBERG and LEDERBERG 1952; ELEK and HILSON 1954). The initial results are revised in the light of the absence or presence of growth within the inhibition zones corresponding to those of the initial plate. This technique makes it possible to distinguish between the bacterial and/or bacteriostatic action of a drug on a given microorganism at selected concentrations. COOPER'S findings are shown in Tables 4 and 5.

When tested in this way capsulated strains are more sensitive than non-capsulated strains. Sulphathiazole and sulphatriad are still the most effective sulphonamides followed by tibatin, gantrisin, elkosin and supronal. It is noteworthy that compared with ZINNEMANN'S results in 1950 a higher proportion of non-capsulated, but not of capsulated strains are insensitive to sulphathiazole and sulphatriad. Most of COOPER'S non-capsulated strains were isolated from bronchitics treated over long periods with terramycin and sulphatriad and an

Table 4. *In-vitro sensitivity of capsulated H. influenzae strains to 9 sulphonamides; replica plate technique*

Sulphonamide	No. and % of strains inhibited by 10 mg. per 100 ml.						Total No. of strains tested
	bactericidal		bacteriostatic		no inhibition		
	No.	%	No.	%	No.	%	
sulphatriad .	10	50	8	40	2	10	20
sulphathiazole	10	50	8	40	2	10	20
gantrisin . .	8	40	9	45	3	15	20
elkosin . . .	8	40	6	30	6	30	20
badional. . .	2	10	5	25	13	65	20
marbadal . .	1	5	5	25	14	70	20
supronal. . .	6	30	6	30	8	40	20
tibatin . . .	8	40	10	50	2	10	20
kynex. . . .	2	22.2	3	23.3	4	44.5	9

Table 5. *In-vitro sensitivity of non-capsulated H. influenzae strains to 9 sulphonamides; replica plate technique*

Sulphonamide	No. and % of strains inhibited by 10 mg. per 100 ml.						Total No. of strains tested
	bactericidal		bacteriostatic		no inhibition		
	No.	%	No.	%	No.	%	
sulphatriad .	43		28		29		100
sulphathiazole	42		27		31		100
gantrisin . .	36		24		40		100
elkosin . . .	30		21		49		100
badional. . .	7		11		82		100
marbadal . .	5		7		88		100
supronal. . .	23		21		56		100
tibatin . . .	36		28		36		100
kynex. . . .	6	21.4	7	25	15	53	28

increase of the percentage of resistant strains under the influence of this treatment is a possible explanation of this discrepancy.

Inhibition of *H. influenzae* strains by antibiotics. *Penicillin.* After the publication of FLEMING'S original paper on the antibacterial action of penicillin (1929) it had been accepted generally that *H. influenzae* belongs to the Gram negative group of organisms on which penicillin has no effect and this view was upheld in subsequent papers (1932, 1937). Although there had been two reports of two meningeal strains being sensitive to penicillin (STRAKER 1945; FORGACS, HUTCHINSON and REWELL 1945) this fact was regarded as an exceptional finding until GORDON and ZINNEMANN (1945) showed that 43 strains of *H. influenzae* isolated from the respiratory tract and 18 strains from cerebrospinal fluid were inhibited by concentrations of penicillin in LEVINTHAL agar ranging from 0.25 to 5.0 units per ml.; the majority of strains were sensitive to 2.5 units per ml. or less. Twelve of the 18 cerebrospinal strains were of type b. Thus the penicillin sensitivity of *H. influenzae* had been established on a broad basis and this fact soon found confirmation by HEWITT and PITTMAN (1946).

Streptomycin. In the same paper HEWITT and PITTMAN recorded the sensitivity of the tested strains to streptomycin which was found to be within the limits of 1.25—10.0 μg. per ml. of fluid medium. SMYTHE published similar results

with strains isolated from cases of meningitis in the London area (1948). Others provided further confirmation of the sensitivity of *H. influenzae* to streptomycin, penicillin or mixtures of the two, and advocated the clinical use of these antibiotics in infections due to *H. influenzae* (ALEXANDER and LEIDY 1946; FOLEY, SHWACHMAN, MCGARRY and WINTER 1949; MØRCH-LUND 1949; OUNSTED 1949; APPELBAUM and NELSON 1950). On the whole it appeared that non-capsulated strains were slightly more sensitive to both penicillin and streptomycin (GORDON and ZINNEMANN 1945; FOLEY, SHWACHMAN, MCGARRY and WINTER 1949) than capsulated ones.

Broad spectrum antibiotics. Then followed the period of rapid progress in the discovery and commercial production of several antibiotics active against both Gram positive and Gram negative bacteria as well as against *Rickettsiae* and the larger viruses. From about 1950 onwards publications concerning the sensitivity of *H. influenzae* strains include those to the tetracyclines (aureomycin, terramycin, tetracycline), chloramphenicol (chloromycetin), bacitracin, the polymyxins B and E, aerosporin, and after 1953 also erythromycin, neomycin, carbomycin, oleandomycin, novobiocin and vancomycin. The latter 10 antibiotics are, in fact, mainly active against either the Gram positive or the Gram negative groups of organisms but will be considered here together with the broad spectrum antibiotics. When summarizing the numerous papers in this field (FINLAND and WILCOX 1950; CHANDLER and HODES 1950, MCCRUMB, HALL, IMBURG, MERIDETH, HELMHOLD, BASORA and WOODWARD 1951; TUNEVALL 1951; SCHOENBACH, SPENCER and MONNIER 1952; MULDER, GOSLINGS, VAN DER PLAS and CARDOZO 1952; FRANKLIN and GARROD 1953; ZINNEMANN 1953, 1957 in STUART-HARRIS and HANLEY's "Chronic bronchitis"; DEL LOVE and FINLAND 1954; TUNEVALL and HEDENIUS 1954; JOHNSTONE, SOLOMON and VOGEL 1955; FAIRBROTHER and WILLIAMS 1956; GOODIER and PARRY 1959; COOPER 1959) it becomes clear that on a weight for weight basis penicillin is the most active antibiotic against *H. influenzae*, closely followed by chloramphenicol, then erythromycin, oxytetracycline, tetracycline and novobiocin. The susceptibility of *H. influenzae* to streptomycin, chlortetracycline, the polymyxins and neomycin is of nearly the same order whilst oleandomycin, carbomycin, vancomycin and bacitracin are not inhibitory in therapeutic concentrations (ALEXANDER, LEIDY and REDMAN 1949; SWIFT and BUSHBY 1951; DEL LOVE and FINLAND 1954; COOPER 1959). On the whole mouse protection tests have shown the in vitro inhibitory action of antibiotics to be borne out in vivo (HEWITT and PITTMAN 1946; ALEXANDER and LEIDY 1946; CHANDLER and HODES 1950; MCCRUMB, HALL, IMBURG, MERIDETH, HELMHOLD, BASORA and WOODWARD 1951). The position was re-assessed with the replica plate technique by COOPER (1959) and the values thus obtained for the bactericidal and bacteriostatic effects of the antibiotics alter the order of merit to some extent if bactericidal action alone is considered, though previous statements in the literature remain valid if bactericidal and bacteriostatic action together are considered. COOPER's findings are reproduced in Tables 6 and 7, together with ZINNEMANN's values (1957) which correspond to COOPER's initial plate readings (Table 8).

It can be seen from Tables 6 and 7 that the replica plate technique substantially confirms the complete inhibition values of the readings obtained with the initial

Table 6. *In vitro sensitivity of capsulated H. influenzae strains to 4 different concentrations of 13 different antibiotics; replica plate technique*

Antibiotic	Strains killed by units per ml.								Strains partially inhibited by 1—10 u/ml.		Strains not inhibited by 10 u/ml.		Total of strains tested
	1 u/ml.		2 u/ml.		5 u/ml.		10 u/ml.						
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
penicillin . . .	1	5	5	25	11	55	14	70	4	20	2	10	20
streptomycin .	0	0	6	30	13	65	15	75	5	25	0	0	20
chlortetracycline	3	15	5	25	10	50	12	60	5	25	3	15	20
chloramphenicol	4	20	16	80	20	100	—	—	—	—	0	0	20
oxytetracycline	2	10	4	20	8	40	13	65	7	35	0	0	20
erythromycin .	7	35	17	85	19	95	—	—	1	5	0	0	20
tetracycline . .	1	5	—	—	7	35	15	75	5	25	0	0	20
sigmamycin . .	0	0	0	0	1	5	5	25	6	30	9	45	20
oleandomycin .	0	0	1	5	4	20	5	25	4	20	11	55	20
novobiocin . .	4	20	9	45	16	80	17	85	2	10	1	5	20
vancomycin . .	0	0	0	0	0	0	0	0	3	15.8	16	84.2	19
PA 114	0	0	0	0	1	10	—	—	1	10	8	80	10
ristocetin . . .	0	0	0	0	0	0	0	0	2	20	8	80	10

Units: Oxford units for penicillin and μg. for other antibiotics; u/ml.: units per ml.

Table 7. *In vitro sensitivity of non-capsulated H. influenzae strains to 4 different concentrations of 13 different antibiotics; replica plate technique*

Antibiotic	Strains killed by units per ml.								Strains partially inhibited by 1—10 u/ml.		Strains not inhibited by 10 u/ml.		Total of strains tested
	1 u/ml.		2 u/ml.		5 u/ml.		10 u/ml.						
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
penicillin . . .	3		10		12		43		28		29		100
streptomycin .	9		37		72		86		12		2		100
chlortetracycline	7		22		39		53		31		16		100
chloramphenicol	39		82		96		99		1		0		100
oxytetracycline	11		19		31		52		29		19		100
erythromycin .	31		67		87		92		8		0		100
tetracycline . .	3		14		27		48		34		18		100
sigmamycin . .	1	1.5	2	3	5	7.5	12	12.6	35	36.8	48	50.5	95
oleandomycin .	2		4		11		18		33		49		100
novobiocin . .	20		34		67		83		10		7		100
vancomycin . .	1	1.5	—		2	3	3	4.5	12	12.6	80	84	95
PA 114	1	3	—		—		—		4	15.1	28	84.9	33
ristocetin . . .	1	3	—		—		—		1	3	31	94	33

Units: Oxford units for penicillin and μg. for other antibiotics; u/ml.: units per ml.

Table 8. *In vitro sensitivity of non-capsulated H. influenzae strains to 4 different concentrations of seven different antibiotics; direct punch plate method*

Antibiotic	Percentage of strains completely inhibited by				Total No. of strains tested
	1 u/ml.	2 u/ml.	5 u/ml.	10 u/ml.	
penicillin . . .	6	12	70	98	50
streptomycin .	2	16	68	88	50
chlortetracycline	4	6	40	88	50
chloramphenicol	40	84	100	—	50
oxytetracycline	2	8	74	100	50
tetracycline . .	9	54.5	81.8	95.5	22
erythromycin .	5.7	34.3	60.0	100	35

Units: Oxford units for penicillin and μg. for other antibiotics; u/ml.: units per ml.

plate in the case of streptomycin and chloromycetin, both for capsulated and non-capsulated strains. As was to be expected, values for penicillin, aureomycin and terramycin are about 30% lower when the bactericidal effect alone is considered. When the values for bactericidal and bacteriostatic effects are added up the inhibition levels approximate to those given by ZINNEMANN (1953) for partial inhibition. In COOPER's experiments capsulated strains of *H. influenzae* are noticeably more sensitive to all antibiotics than non-capsulated ones.

Antibiotic levels in cerebrospinal fluid and in sputum. It is generally assumed that antibiotic levels in body tissues correspond to the blood level. For the rational treatment of *H. influenzae* infections the level of antibiotics in the cerebrospinal fluid and in the sputum is of considerable importance and is not necessarily close to the blood level. The blood-brain barrier is more easily permeable to some antibiotics than to others. Thus chloramphenicol reaches the cerebrospinal fluid in higher concentrations than most other antibiotic drugs (ANDERSON and JEWELL 1945; ROSS, BISCHOFF, PREISSER and ORR 1949; SCHOENBACH, SPENCER and MONNIER 1952; ROY, KRIEGER, CRAIG, COHEN, MCNAUGHTON and SILVERTHORNE 1952). Unfortunately, the relatively good in vitro activity of penicillin is not borne out by its performance in vivo because blood levels therapeutically efficaceous against *H. influenzae* are not easy to reach or to maintain with ordinary doses. Also, cerebrospinal fluid and sputum levels are a small fraction of the corresponding blood levels. Similarly chlortetracycline (aureomycin) and oxytetracycline (terramycin) give low levels in the cerebrospinal fluid (ROSS 1955). Very little is known about antibiotic levels in the sputum and most authors have confined themselves to determining the sputum levels of one or two antibiotics only. Table 9 summarizes the findings reported in the literature.

It is difficult to judge how far the values obtained with different techniques by the various authors are comparable. For this reason the levels of a number of antibiotics were determined in the sputum of bronchiectatic children who underwent prolonged treatment with large doses of these drugs (see ALLIBONE, ALLISON and ZINNEMANN 1956). The values obtained were not included in that report, but they are given in Table 10.

By the time this work was carried out it had been realized that chloramphenicol, when given over long periods, may lead to blood dyscrasias and for this reason this antibiotic could not be included.

In addition to the minimum and maximum assay values obtained the average sputum level for each antibiotic is shown. It is clear from these figures that roughly similar average sputum concentrations are obtained with streptomycin, chlortetracycline, oxytetracycline and erythromycin while penicillin gives a level approximately six times lower and tetracycline one almost three times higher. The low penicillin levels were quite consistent and agree well with the findings of other inverstigators shown in Table 9. The high average level of tetracycline was due to two of a total of seven patients who were able to concentrate this drug particularly well to a level of between 4 and 10 μg. per ml. of sputum. The average level of the remaining five patients was 1.17 μg. per ml. which is shown in brackets in Table 10. This level is much the same as that of the other effective antibiotics though in individual cases tetracycline may prove to be more useful when the patient is a good "sputum concentrator". If one compares the average,

Table 9. *Levels of antibiotics in sputum and bronchial aspirate as reported in the literature*

Authors	Year of publication	Antibiotic levels in sputum or bronchial aspirates in patients treated with					
		penicillin	estopen	streptomycin	chloramphenicol	oxytetracycline	erythromycin
JENSEN et al.	1950	0.06—0.56	0.14—1.25	—	—	—	—
BARACH et al.	1952	—	—	—	—	1—2	—
MULDER et al.	1952	0.06—0.28	—	2—5	—	—	—
GRIGSBY et al.	1952	0.002—0.05	0.05—0.1	—	—	—	—
GOSLINGS and HERS .	1953	0.01—2.2	0.02—3.1	—	—	—	—
FRANKLIN and GARROD	1953	—	—	—	<2—8	—	—
MAY	1955	0.03—0.09	0.5—3.0	—	—	—	—
LOPEZ-BELIO et al. . .	1956	—	—	—	—	—	1.1—2.4

The figures indicate Oxford units per ml. for penicillin and estopen and μg. per ml. for all other antibiotics.

Table 10. *Range of assay results and average level of antibiotic activity (units per ml.) in sputum of bronchiectatic children*

Antibiotic	No. of assays done	Range of results	Average sputum level
penicillin . . .	9	0.075—0.25	0.16
streptomycin .	10	0.5—2.0	1.0
chlortetracycline	37	0.25—5.0	1.1
oxytetracycline	152	0.25—8.0	0.93
tetracycline . .	34	0.25—10.0	2.78 (1.17)
erythromycin .	156	0.075—4.0	0.99

The figures indicate Oxford units per ml. for penicillin and μg. per ml. for all other antibiotics.

and in many cases also the maximum, levels with the values for complete inhibition of *H. influenzae* obtained with comparable laboratory techniques and shown in Tables 7 and 8 it can be seen that the average sputum levels are too low for the complete inhibition of *H. influenzae* in the sputum. This situation was in fact confirmed clinically as the sputa of these bronchiectatic children became free of *H. influenzae* only when the maximal doses of these antibiotics were reinforced with an equal amount of the triple sulphonamide sulphatriad. On the other hand, chloramphenicol, which is one of the most active antibiotics against *H. influenzae* in vitro and gives entirely adequate sputum levels in FRANKLIN and GARROD'S hands (see Table 9), was able to achieve complete elimination of *H. influenzae* from the sputum without the addition of a sulphonamide (FRANKLIN and GARROD 1953; ALLIBONE, ALLISON and ZINNEMANN 1956).

Since the apparently greater sensitivity of *H. influenzae* to a combination of an antibiotic and a sulphonamide has been mentioned, consideration of the action of combined drugs is required at this point.

III. The action of combinations of substances inhibitory to *H. influenzae*

The idea of combining two antibacterial substances appears to have its origin in the desperate search for effective treatment of *H. influenzae* meningitis of children which had a 90—100% fatality rate before 1937 (WILKES-WEISS and

HUNTINGTON 1936; MUTCH 1941). There was only a small reduction of this high fatality rate when sulphonamides or antiserum were used separately as therapeutic measures.

Synergism. POVITZKY (1937) first combined prontosil and antiserum and observed that more mice infected with *H. influenzae* type b survived when treated with the two drugs than with either alone. When more powerful sulphonamides became available first PITTMAN (1942) and then ALEXANDER [1943 (1), (2)] and ALEXANDER and LEIDY (1943) repeated and amplified these mouse protection experiments and could show again that there is a marked synergistic effect of sulphonamides when combined with *H. influenzae* type b rabbit antiserum. Then the numerous inquiries into the antibacterial effect of sulphonamides and antibiotics led some workers to study the action of a combination of these two types of drugs on sensitive strains of bacteria (UNGAR 1943; THOMAS and HAYES 1947; KOLMER 1948; DOMAGK 1952); it was observed generally that there was an increase in the degree of sensitivity amounting to synergism, even if the strain was resistant to one of the drugs (CHAIN and DUTHIE 1945). By that time development of resistance to antibiotics began to play a more prominent part, particularly when it became known that bacterial populations may develop resistance of a very high order during a 24 hour exposure to streptomycin (MURRAY, KILHAM, WILCOX and FINLAND 1946; BIRMINGHAM, KAYE and SMITH 1946; *Report* 1946; ALEXANDER, RAKE and DONOVICK 1946; WILSON 1948; SMYTHE 1948; ROSCOE and GLEESON-WHITE 1948). In 1948 Demerec explained this disconcerting phenomenon on genetical grounds and stated: "Theoretically, the most effective way of preventing the origin of resistant strains of bacteria is the use in clinical treatment of a mixture of two antibiotics, when such are available, that affect the same pathogen but are independent in their actions. The evidence of independence is that bacterial strains that have developed resistance to one antibiotic are still sensitive to the other, and vice versa. If such a mixture of two antibiotics is used, then only bacteria that are resistant to both can survive the treatment and form . . . resistant strains. Such bacteria would be exceedingly rare." This principle of DEMEREC'S had been shown experimentally to be correct in respect of streptomycin and sulphonamides by KLEIN and KIMMELMAN (1947); in addition to this these authors reported that the drugs reinforce each other.

Interference. Combinations of antibiotics have to be chosen with some care to avoid interference which occurs not infrequently when the broad spectrum antibiotics and penicillin are used together (JAWETZ, GUNNISON and COLEMAN 1950; GUNNISON, COLEMAN and JAWETZ 1950) and also when they are combined with streptomycin (JAWETZ, GUNNISON and SPECK 1951). Later the same group of authors postulated that combinations of bactericidal antibiotics such as penicillin and streptomycin are often synergic, combinations of broad spectrum antibiotics, in bacteriostatic concentrations, such as chloramphenicol and the tetracyclines, are additive only while combinations between the bactericidal and bacteriostatic groups of drugs are likely to show interference (JAWETZ and GUNNISON 1952). The question of combined chemotherapy has been reviewed by GARROD (1953), who pointed out that laboratory tests of synergism and interference based on viable counts are complicated and hardly a practical routine proposition. The situation was eased considerably by the adaptation of the

LEDERBERGS' replica plating technique (1952) to the requirements of routine bacteriology by ELEK, HILSON and JEWELL (1953) and ELEK and HILSON (1954). It has since been shown to be applicable to the testing for synergism between sulphatriad and antibiotics in relation to capsulated and non-capsulated *H. influenzae* strains (COOPER 1959; see Tables 11 and 12).

Table 11. *Evidence of synergism between sulphatriad and each of 13 antibiotics on capsulated H. influenzae strains tested in vitro*

Antibiotic	No. and percentage of strains showing synergism of 10 mg. per 100 ml. S 3 with antibiotic at concentration								No. synergism		Total of strains tested
	1 u/ml.		2 u/ml.		5 u/ml.		10 u/ml.				
	No.	%	No.	%	No.	%	No.	%	No.	%	
penicillin . . .	5	25	6	30	88	40	10	50	10	50	20
streptomycin .	7	35	11	55	14	70	15	75	5	25	20
chlortetracycline	8	40	9	45	10	50	—	—	10	50	20
chloramphenicol	10	50	14	70	17	85	—	—	3	15	20
oxytetracycline	4	20	8	30	—	—	9	45	11	55	20
tetracycline . .	2	10	—	—	6	30	9	45	11	55	20
erythromycin .	9	45	14	70	16	80	17	85	3	15	20
sigmamycin . .	0	0	1	5	2	10	6	30	14	70	20
oleandomycin .	2	10	4	20	—	—	6	30	14	70	20
novobiocin . .	3	15	4	20	5	25	8	40	12	60	20
vancomycin . .	1	5.3	—	—	2	10.6	—	—	17	89.4	20
PA 114	1	10	—	—	2	20	—	—	8	80	10
ristocetin . . .	0	0	0	0	0	0	0	0	10	100	10

S3 sulphatriad; PA 114 laboratory designation of a Pfizer antibiotic under investigation. Units: Oxford units for penicillin and μg. for other antibiotics; u/ml.: units per ml.

Table 12. *Evidence of synergism between sulphatriad and each of 13 antibiotics on non-capsulated H. influenzae strains tested in vitro*

Antibiotic	No. and percentage of strains showing synergism of 10 mg. per ml. S 3 with antibiotic at concentration								No synergism		Total of strains tested
	1 u/ml.		2 u/ml.		5 u/ml.		10 u/ml.				
	No.	%	No.	%	No.	%	No.	%	No.	%	
penicillin . . .	18		22		29		32		68		100
streptomycin .	23		36		53		61		39		100
chlortetracycline	24		30		39		40		60		100
chloramphenicol	47		57		64		70		30		100
oxytetracycline	24		31		40		42		58		100
tetracycline . .	25		32		34		37		63		100
erythromycin .	42		59		68		73		27		100
sigmamycin . .	16	16.8	19	20	24	25.3	27	28.4	68	71.6	95
oleandomycin .	16		17		20		27		73		100
novobiocin . .	17		23		27		31		69		100
vancomycin . .	3	4.5	5	7.5	—		6	9	89	91	95
PA 114	1	3	—		—		—		32	97	33
ristocetin . . .	1	3	—		—		2	6	31	94	33

S3 sulphatriad; PA 114 laboratory designation of a Pfizer antibiotic under investigation Units: Oxford units for penicillin and μg. for other antibiotics; u/ml.; units per ml.

On the basis of COOPER'S in vitro results the greatest chance of enhanced inhibition of capsulated and non-capsulated *H. influenzae* strains can be expected from a combination of sulphatriad with chloramphenicol, erythromycin and also

streptomycin. It is noteworthy that the action of novobiocin, which with the replica plate technique shows so much promise as an antibiotic highly active against *H. influenzae* (see Tables 6 and 7), is unlikely to be more effective when combined with sulphatriad though such a combination might be useful in preventing the emergence of resistant strains. With all antibiotics synergism occurs more frequently when acting on capsulated strains.

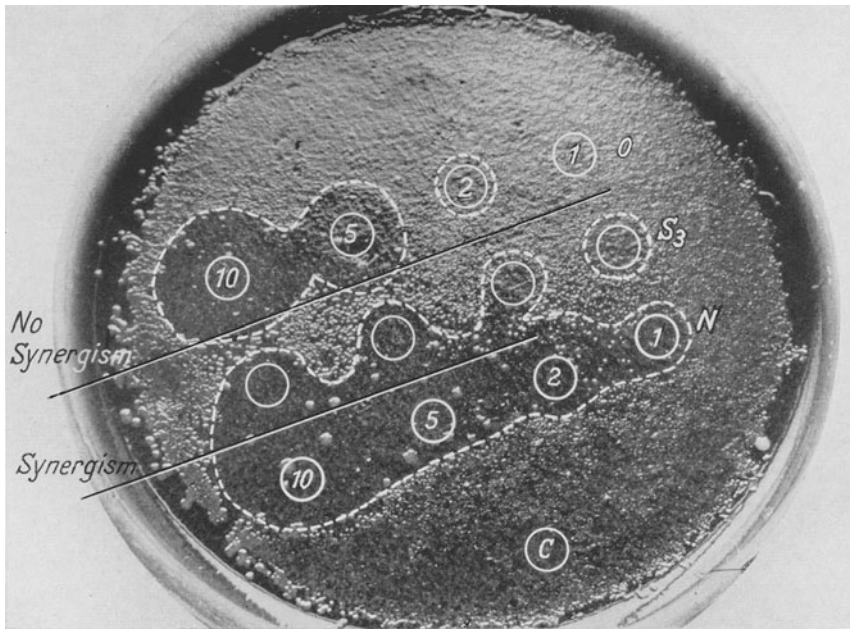

Fig. 7. Replica plate showing the imprints, from right to left of: in the middle row four punch holes filled with a constant concentration of sulphatriad (10 mg./100 ml.), below a row of four punch holes filled with increasing concentrations of novobiocin (*1*, *2*, *5* and *10* μg/ml) and above a row of four punch holes with the same concentrations of oleandomycin. On the primary plate there were inhibition zones around all punch holes except the two containing 1 μg/ml novobiocin and oleandomycin. Note indication of bacteriostatic effect of sulphatriad, 2 μg./ml. novobiocin and oleandomycin, the presence of synergism between sulphatriad and novobiocin at 5 and 10 μg./ml. concentrations and the complete absence of synergism between 5 and 10 μg./ml. of oleandomycin and sulphatriad. Inoculum of initial plate was *H. influenzae*. (From COOPER 1959.)

○ position of punch hole on the initial plate; ◌ zone of inhibition of growth of *H. influenzae*; *C* = control hole; *O* = oleandomycin; S_3 sulphatriad; *N* = novobiocin. Figures indicate units of antibiotic per ml. Constant concentration of S_3 10 mg./100 ml.

Techniques. ELEK and HILSON (1954) described five different laboratory methods for the determination of the presence or absence of synergism and interference and elaborated further on the method using the convenient filter paper disks impregnated with antibiotic solutions (ELEK, HILSON and JEWELL 1953). Some laboratories may prefer to use instead the punch hole technique as they feel that diffusion is better if fluids are employed (COOPER 1959).

On primary chocolate agar plates inoculated evenly with *H. influenzae*, filter paper disks are placed at three equidistant points, one disk with antibiotic A, one with antibiotic B and on the third point a disk each with antibiotic A and B are superimposed. If growth occurs underneath the paper disks deposited on point three, interference has taken place. In testing for synergism the two antibiotic disks are not superimposed, but are placed separately, yet close enough

together to produce overlapping or touching of the zones of inhibition. Synergism is shown by flattening of the contour where the zone edges meet and by extension of the inhibition zones beyond their expected radius on the sides facing or overlapping each other. Such growth patterns do not necessarily indicate whether the inhibition of growth is due to bacteriostatic or bactericidal action, but this can be determined by transferring the growth pattern from the primary plates, just described, to the surface of a fresh medium—the replica plate—free from drugs. The transfer is effected by means of a sterile stamp which consists of a cylindrical block of wood to one circular surface of which a disk of velvet or velveteen is glued. Replica plating reveals bacteriostatic inhibition when growth around any filter paper disk appears to be completely inhibited on the primary plate, yet growth is present in the corresponding area of the replica plate. Interference resulting in bacteriostatic action of two drugs of which one alone produces bactericidal action can only be revealed on the replica plate. The method also decides whether synergism apparent on the primary plates is due to bactericidal or to bacteriostatic effects. If more than one concentration is to be tested a linear arrangement on one plate of several disks or cups of each drug, all equidistant, will usually provide the sought for answer (see Fig. 7).

Since two antibiotics may display synergism after 24 hours though they have shown interference during the first 12 hours of incubation (JAWETZ, GUNNISON and SPECK 1951), the incubation period before replica plating may be important and for practical purposes "replication" is best carried out after at least 18 hours incubation.

IV. Pathological conditions due to infection with *H. influenzae*

A. Meningitis

Meningitis due to the influenza bacillus was first described by SLAWYK (1899). As already stated when discussing the capsulated form of *H. influenzae*, a number of careful observers considered the infective agent to differ from the influenza bacillus in the respiratory tract. Finally it was established that most strains from cases of meningitis are capsulated and of type b, infrequently of type a and still more rarely of other types, or non-capsulated (PITTMAN 1931; MULDER 1939; GORDON, WOODCOCK and ZINNEMANN 1944). Mention is made in the literature of isolated cases of meningitis due to *H. influenzae* type f [ALEXANDER 1943 (1); MARTINEZ and JUAN 1948; ROSENBLATT and ZWEIFLER 1951]. CAYTON and ZINNEMANN (unpublished) isolated *H. influenzae* type e1e2 from a boy who previously had had repeated attacks of meningitis, mostly caused by pneumococci.

The literature on *H. influenzae* menigitis, before specific treatment became available, has been reviewed by RIVERS [1922 (1)], WILKES-WEISS and HUNTINGTON (1936) and MUTCH (1941). Judging by the number of publications from, and the number of cases reported in, the U.S.A. the infection must be more frequent there than in Europe. During some periods it was the commonest form of meningitis of children under the age of two in some regions of the U.S.A. (FOTHERGILL 1937; LINDSAY, RICE and SELINGER 1940). Also, about 100 cases have occurred annually in Australia in Melbourne and Syndney alone (RUBBO, personal communication). *H. influenzae* meningitis is most common in childhood

although occasionally an adult case is seen. A fall, a knock on the head or an upper respiratory infection figures frequently in clinical histories. Thus, direct spread from the nasopharynx through a fissure in the cribriform plate is a possible pathway of infection. However, 70% of meningitis cases may also occur together with, or following the course of, *H. influenzae* septicaemia (FOTHERGILL 1937). Often, particularly in small infants, the infection starts quite suddenly without any warning symptoms. Without specific treatment the case fatality rate is between 90 and 100% (WILKES-WEISS and HUNTINGTON 1936; MUTCH 1941). Clinically no certain distinction can be made between meningitis due to meningococci, pneumococci or other agents of acute purulent meningitis on the one hand and *H. influenzae* on the other. Yet, an exact bacteriological diagnosis is important because *H. influenzae* is relatively less sensitive to sulphonamides or antibiotics, or mixtures of both, than most of the other organisms and meningitis due to it requires a considerably more energetic treatment.

Laboratory diagnosis. This can be made by microscopical examination of a Gram stained smear of the sediment of the spinal fluid, together with the direct Neufeld reaction with type b antiserum in a hanging drop preparation from the spinal fluid; by culture, slide or tube agglutination, or both, and Neufeld reaction with a young subculture; by the ring precipitation test directly with the cerebrospinal fluid or with a broth culture and by a haemagglutination test described by WARBURTON, KEOGH and WILLIAMS (1949). However, there does not seem to be any particular advantage in the haemagglutination test which is more complicated than the conventional ones.

Treatment. This should be started as soon as possible with the highest possible doses of antibacterial drugs. After the encouraging, though by present standards modest successes of treatment with a sulphonamide or antiserum alone (see GORDON, WOODCOCK and ZINNEMANN 1944) the combination of sulphadiazine and type-specific rabbit antiserum gave a recovery rate of 78% (ALEXANDER 1944) and even of 93% (SMITH, WILSON and HODES 1946). However, from Australia there were reports of serious haemolytic anaemia following serum treatment (SCHIAVONE and RUBBO 1953; MARGOLIS 1955). With the advent of powerful antibiotics treatment with the expensive and bulky type specific rabbit antiserum, with its inherent risk of haemolytic and serum reactions, was soon superseded and the same fate befell it as the serum treatment of lobar pneumonia after the discovery of the action of sulphonamides. After the in vitro sensitivity of *H. influenzae* to penicillin had been firmly established (GORDON and ZINNEMANN 1945) this antibiotic, mainly combined with a sulphonamide, was used with considerable success, particularly in countries where neither type specific rabbit antiserum nor streptomycin nor the broad spectrum antibiotics were available at first (MCINTOSH and DRYSDALE 1945; DRYSDALE, MCINTOSH and BRODIE 1946; ZINNEMANN 1946; TURNER 1947; THOMSON, BRUCE and GREEN 1947; GOTTLIEB, FORSYTH and ALLOTT 1947; MARTIN and SUREAU 1948; BRAID and MEYER 1949; SANDILANDS 1950). Meanwhile streptomycin had been used with great success in the U.S.A. (ALEXANDER, RAKE and DONOVICK 1946; BIRMINGHAM, KAYE and SMITH 1946; WEINSTEIN 1946; PAINE, MURRAY, HARRIS and FINLAND 1947) and after an interval similarly favourable reports from European countries were published (ROSCOE and GLEESON-WHITE 1948; WILSON 1948;

SMYTHE 1948; ENGBAEK 1948; MARTIN and SUREAU 1948; OUNSTED 1949). Before long streptomycin was combined with a sulphonamide to prevent or delay the emergence of resistant strains (ENGBAEK 1948; YAMPOLSKY and JONES 1949; APPELBAUM and NELSON 1950; ALLIBONE, PICKUP and ZINNEMANN 1951). With this treatment APPELBAUM and NELSON were able to reduce their case fatality rate in 90 patients to 3.4%, but had to admit that nine of their survivors had severe sequelae of which only nerve deafness could be attributed to the use of streptomycin; hydrocephalus, defective vision and blindness, mental impairment and hemiparesis were due to residual damage caused by the infective process. Most of the streptomycin-treated cases had received, at least for some time of their illness, intrathecal injections of penicillin or streptomycin. It was left to HOYNE and BROWN (1948) to show that, more often than not, intrathecal treatment is not required. Systemic treatment alone is highly effective if it consists of a combination of penicillin and streptomycin (ALLIBONE and ZINNEMANN, unpublished).

Within a short space of time three broad spectrum antibiotics, i.e. chloramphenicol (chloromycetin), chlortetracycline (aureomycin) and oxytetracycline (terramycin) were discovered, manufactured and marketed in the U.S.A. so that reports on their efficacy in systemic treatment of *H. influenzae* meningitis appeared almost simultaneously, much to the confusion of those who had to deal with clinical cases (CARABELLE, MITCHELL and SALMON 1950; PRATHER and SMITH 1950; GREEN, MANKIKAR and MILLET 1950; DRAKE, BRADLEY, IMBURG, MCCRUMB and WOODWARD 1950; CHANDLER and HODES 1950; MCCRUMB, HALL, IMBURG, MERIDETH, HELMHOLD, BASORA and WOODWARD 1951; HOYNE and RIFF 1951; SCOTT and WALCHER 1952; SCHOENBACH, SPENCER and MONNIER 1952). At about the same time polymyxin B and E, with their effectiveness restricted mainly to the Gram negative range of microorganisms, were also reported to have had a curative effect in 8 cases (SWIFT and BUSHBY 1951). Some authors could not find any difference between treatment with one or the other antibiotic (SCHOENBACH, SPENCER and MONNIER 1952), others thought that chlortetracycline alone gives equally good results as the same antibiotic combined with streptomycin and sulphafurazole (gantrisin) (LEPPER, WEHRLE and BLATT 1952). Yet another group of observers adopted a combination of oxytetracycline, streptomycin and a sulphonamide as standard treatment and achieved a fatality rate as low 3.2% in 62 patients (KOCH and CARSON 1955). It was pointed out at about that time that with the eight drugs then available—*H. influenzae* type b specific rabbit antiserum, sulphonamides, penicillin, streptomycin, polymyxin, chloramphenicol, chlor- and oxytetracycline—225 combinations are possible and that, in view of the interference phenomenon, this embarras de richesse is not without danger (Leading Article 1952). Since then three more antibiotics with powerful inhibitory effects on *H. influenzae* have been marketed, i.e. tetracycline, erythromycin and novobiocin. This increases the number of possible combinations to 2047 if from one to eleven of these drugs are to be combined. There seems to be an almost inexhaustible field for clinical inquiry, but in view of the reported fatality rates below four per cent it would be futile to expect significantly better results with any of the many as yet untried combinations. Early diagnosis and prompt treatment in adequate doses are as much

likely to improve fatality rates as any new drug or new combination. This seems to be generally realised for, while the medical journals for 10 years prior to 1952 very frequently published papers on the treatment of *H. influenzae* meningitis they now do so only rarely. *H. influenzae* meningitis is the infection par excellence demonstrating, if any such demonstration is needed, how much modern chemotherapy has changed the prognosis for the patient. Within twenty years the clinician's attitude of helplesseness when faced with a near 100% case fatality rate has been altered to one of self reproach if this figure is more than one or two per cent above that reported by APPELBAUM and NEAL, or KOCH and CARSON.

Remaining problems. Yet, with all these successes on the credit side there are still some unsolved aspects of *H. influenzae* meningitis. As regards the epidemiology of the infection, there is no report of epidemic *H. influenzae* menigitis. In fact, the occurrence of more than one case in one and the same family is such a rarity that it has been deemed worthy of special comment or chosen as the subject of a report in a medical journal (HERTZOG, CAMERON and KARLSTROM 1944; CARABELLE, MITCHELL and SALMON 1950). On the other hand, OUNSTED (1950) found that *H. influenzae* meningitis occurs predominantly in families with two or more elder siblings under the age of 12 whilst the majority of cases with meningococcal meningitis and of 100 control cases of children in hospital for complaints other than meningitis had no or only one elder sibling. On the basis of these findings OUNSTED thought that *H. influenzae* may have to pass through one or two partially immune contacts before the strain acquires the capacity to pass through the meningeal barrier. There is some support for OUNSTED's hypothesis in the findings of BOISVERT and his co-workers (GOOD, FOUSEK, GROSSMAN and BOISVERT 1943; BOISVERT 1948), who isolated *H. influenzae* type b from other members of the families of cases with *H. influenzae* meningitis.

The incidence of capsulated *H. influenzae* strains in the child population with respiratory infections was nearly five per cent in two independent surveys, one in New York [ALEXANDER 1943 (1)] and the other in Leeds (DAWSON and ZINNEMANN 1952). More than three quarters of all typable strains isolated in New York were of type b, whilst in Leeds this type represented less than a fifth of the total number of 32 strains, with type f constituting nearly one third and type e one fourth of the total. During the same period all meningitis cases caused by *H. influenzae* in the Leeds area were of type b. DAWSON and ZINNEMANN concluded at the time that these findings suggested that type b is more virulent for man than the other five known *H. influenzae* types. Since then the transformation experiments, referred to in the paragraph on the complexity of the *H. influenzae* capsule, have made another interpretation of the findings possible, i.e. change of type according to the matrix on which growth takes place.

B. Acute obstruction of the respiratory tract (acute epiglottitis)

Non-diphtherial obstruction of either the larynx, with or without gross signs of inflammation, or of other parts of the respiratory tract below the pharynx, particularly in children, is often due to infection with *H. influenzae* type b and still carries a remarkably high case fatality rate (JONES and CAMPS 1957). LEMIERRE, MEYER and LAPLANE (1936) first implicated *H. influenzae* as the infective agent

in this condition. In 1941 SINCLAIR described 10 cases in the U.S.A., DE NAVASQUEZ (1942) reported the first recognized case in Great Britain and in 1943 ALEXANDER (1) contributed another 13 cases. Small series of cases were published by DUBOIS and ALDRICH (1943), STIEGLER (1946), DAVIS (1947), MILLER (1948), RABE (1948) and CAMPS (1953). JONES and CAMPS (1957) drew attention to the frequent occurrence of this condition as a problem in forensic medicine. The largest series of cases published, together with an excellent description of the condition, is that of BERENBERG and KEVY (1958). CAMPS (1953) tabulated the clinical differences between acute epiglottitis and acute laryngotracheobronchitis, based on NEFFSON'S monograph (1949) on the latter condition, though MILLER (1948) seems to have been the first to attempt the differential diagnosis of the two infections. Both ALEXANDER [1943 (1)] and NEFFSON (1949) seem to regard acute epiglottitis and acute laryngotracheobronchitis as closely related. ALEXANDER [1943 (1)] saw extension of the inflammatory process into the trachea and bronchi only on rare occasions. All authors agree on the following points:—that the children are in a state of shock out of all proportion to the severity of the infective lesion, that *H. influenzae* type b can be grown from blood cultures in most cases, and that intubation may be dangerous but that tracheotomy together with antibacterial therapy will save life in a high proportion of the affected children when supported by adequate chemotherapy. The accompanying bacteraemia may cause complications such as pneumonia, empyema, adenitis, periarticular abscesses, pharyngeal cellulitis and meningitis [ALEXANDER 1943 (1)].

Laboratory diagnosis. This rests mainly on the isolation of *H. influenzae* type b from a laryngeal swab and from a blood culture. Frequently the organism is absent or present only in small numbers in the naso-pharynx. Thus culturing from a throat swab, or a direct NEUFELD reaction with mucus from this site does not necessarily reveal *H. influenzae* type b. In severe cases, however, capsular antigen may be present in the patient's serum and urine and can be demonstrated by the precipitin test. Cultural methods are slow and waiting for their results should not delay therapeutic decisions. As with all infections, specimens for bacteriological examinations should be collected before antibacterial therapy is started. It is advisable to aspirate some tracheal mucus through the tracheotomy tube in case *H. influenzae* cannot be isolated from the other samples collected as it is important to establish the sensitivity pattern of each strain.

Therapy. Apart from tracheotomy the same principles of antibacterial therapy as those applied in meningitis should be observed.

C. Oto-rhinological infections

The paediatrician is likely to consult his colleagues from the ear, nose and throat department not only with a view to carrying out a tracheotomy in acute epiglottitis, but also in cases of paranasal sinusitis and otitis whether acute, chronic or relapsing which may be due to *H. influenzae*. HIRSCH (1912) was one of the first to draw attention to the comparatively frequent occurrence of this organism in otitis and PRYM (1919) and CROWE and THACKER-NEVILLE (1919) in paranasal sinusitis. Scandinavian workers have lately confirmed these reports. NIELSEN (1945) found *H. influenzae* in 20% of 802 cases of acute otitis

media; in 14.6% *H. influenzae* was the only pathogenic organism isolated. URDAL and BERGDAL (1949) examined the bacterial flora of 81 cases of acute and chronic maxillary sinusitis. *H. influenzae* was present in 31% as compared with pneumococci in 40% and haemolytic streptococci in 6%; the aspirate was sterile in 20%. In 14 cases (17%) *H. influenzae* was the only pathogenic organism isolated. In the light of the knowledge of *H. influenzae* acquired since 1931 TUNEVALL and his clinical colleagues reassessed its role in these sites [BJUGGREN and TUNEVALL 1950; BJUGGREN, KRAEPELIN, LIND and TUNEVALL 1952; TUNEVALL 1952 (1), (2); BJUGGREN and TUNEVALL 1952]. Their investigations were not confined merely to the isolation and serological typing of *H. influenzae* strains, but extended to the identification of other common pathogens such as the pneumococcus, streptococcus and *Staphylococcus aureus*; they correlated isolation rates in pathological oto-rhinological conditions with carrier rates in apparently healthy children; lastly they looked for complement fixing antibodies against *H. influenzae*, using a one per cent sodium carbonate extract of *H. influenzae* as antigen. In otitis of children *H. influenzae* was responsible for the infection in 7.3% of a total of 178 children as compared with 48% for haemolytic streptococci, 21% for pneumococci, 12% for *Staphylococcus aureus*; in 11% no pathogen could be isolated. The observations were made during a period when penicillin with or without a sulphonamide was given therapeutically and the relatively low sensitivity of *H. influenzae* to these two antibacterial drugs may be the reason why the Swedish workers found the relapse rate of *H. influenzae* infections higher than that of infections caused by any of the other pathogenic microorganisms. In 108 apparently normal children *H. influenzae* was found 14 times in aspirates from the maxillary sinus, pneumococci 24 times and *Staphylococcus aureus* 8 times. The corresponding figures for nasal carriage were 20, 57 and 75. Although beta-haemolytic streptococci were never isolated from antral aspirates they were found in the noses of 29 children. It is evident, therefore, that although *Staphylococcus aureus* and beta-haemolytic streptococci are frequent inhabitants of the apparently normal nasal mucosa they are the least common pathogens in sinusitis of children. On the other hand, *H. influenzae* with the lowest incidence on the nasal mucosa is the second most frequent pathogen in antral discharge. A clinical point made by the Swedish authors is that the children investigated appeared healthy, yet discharges, many purulent and due to bacterial pathogens were often found. On these grounds they introduced the conception of "occult sinusitis" in children. *H. influenzae* sinusitis is characterised by a peculiar, copious, sticky mucus (BJUGGREN 1950) which is also seen in *H. influenzae* otitis of children and it is thought that in these cases the middle ear infection has its origin in an occult *H. influenzae* sinusitis. Capsulated *H. influenzae* strains were encountered relatively frequently in otitis—in 8 out of 34 children—and 4 strains belonged to type b. In occult maxillary sinusitis capsulated strains were found in only 2 out of 38 children. Antibodies against *H. influenzae* and pneumococci were present in a significantly higher percentage of children who were either nasal or sinus carriers whilst the differences in respect of staphylococcal antibody as between carriers and non-carriers were not significant.

Antibody levels against pneumococci and *Staphylococcus aureus*, and occasionally against beta-haemolytic streptococci, were found to be elevated

during the first years of life of children with otitis whilst *H. influenzae* complement fixing antibody could not be demonstrated until the fourth year. Relapses of otitis due to pneumococci or *H. influenzae* usually coincided with periods when antibody formation against these pathogens was poor or absent.

The bacteriological diagnosis on material collected on swabs should not present any difficulties.

Therapy. Chloramphenicol is favoured as the antibiotic of choice in oto-rhinological infections due to *H. influenzae* (BJUGGREN and TUNEVALL 1952), but others report good results with streptomycin (LUNDGREN 1950) or oxytetracycline (NIELSEN 1951).

D. Pneumonia and empyema

Occasionally *H. influenzae* plays a part as secondary invader of lung tissue in bronchopneumonia (WILSON and MILES in TOPLEY and WILSON 1955, pp. 1878 seq.). In the common cold, which is so often a precursor, the role of *H. influenzae* as secondary invader is regarded by many as not established (Report 1930, Report 1939) and others associate it in a number of cases with this condition (HOYLE 1932). The fact that *H. influenzae* can be isolated from the nasopharynx of normal people in a higher proportion than from those with coryza throws some doubt on the validity of the claims of investigators who regard *H. influenzae* as a primary pathogen in that infection (Report 1930).

There is no doubt, however, that pneumonia with *H. influenzae* as primary pathogen does occur though it appears that such pneumonic consolidations are not seen very often in Europe, so much so that MULDER, with his vast experience in the field of *H. influenzae* infections, stated that "*H. influenzae* pneumonia of the American textbooks does not exist, or is exceedingly rare" (1956). The condition does occur in Europe, though, and one of the first fully documented reports of a larger series of cases appeared in Switzerland (HEGGLIN and GRUMBACH 1941). It described 18 cases seen within 2 years, 9 of which occurred as an epidemic outbreak in an army battalion. All cases were characterized by a peculiar, subacute and atypical form of pneumonia together with diffuse bronchitis, no temperature or only subfebrile elevation of temperature with an occasional rise, radiodiagnostically with irregular, marked, cloudy lung infiltrations and strongly positive WASSERMANN, KAHN and cytochol reactions. The serum reactions became negative with the disappearance of the pneumonic infiltrations. *H. influenzae* was seen in great numbers in direct films of sputa and was isolated in abundance from them. The serum of previously WASSERMANN-negative rabbits, after having been immunized with killed suspensions of the isolated *H. influenzae* strains, became WASSERMANN-positive after two injections. Similar clinical and serological observations, though without the appropriate bacteriological investigations, had been made previously by FANCONI (1936) and LINDAU (1939) on four cases each.

According to ALEXANDER [1943 (1)] pneumonia due to *H. influenzae* in infants under one year of age is usually due to the b type and is associated with empyema and bacteriaemia, not infrequently followed by meningitis. The outlook is unfavourable unless rigorous therapeutic measures are adopted. No absolute or relative figures of cases seen or treated are given by ALEXANDER.

It appears then that *H. influenzae* pneumonia may occur in adults as well as in infants and children. This situation has been confirmed by a number of subsequent reports of well documented cases, although each of these reports concerns no more than five patients. Some of the authors have tried to establish the aetiological role of *H. influenzae* in their cases of pneumonia beyond any reasonable doubt by isolation of the organism from the blood, from pleural fluid or from the lung tissue itself at post mortem examination (KEEFER and RAMMELKAMP 1942; LANDAU 1945; FISHER and SHAW 1947; HIRSCH and NASSAU 1947; CROWELL and LOUBE 1954; NASOU, ROMANSKY and BARR 1954; NYHAN, RECTANUS and FOUSEK 1955). Four cases of pulmonary infections due to *H. influenzae* are included in KEEFER's report (1946) on the therapeutic use of streptomycin, without data as to age of the patients or the site from which the strains were isolated. DURANT, SOKALCHUK, NORRIS and BROWN (1946) described three cases of acute pulmonary disease associated with bronchiectasis. *H. influenzae* was isolated from bronchial aspirates from each of these. Only one of the strains was capsulated and of type b. Regarding DURANT et al.'s report it has to be remembered that *H. influenzae* is found consistently in the bronchial secretions of bronchiectatics, as will be seen under **F.**, and that therefore the bacterial flora in bronchial aspirates of such patients need not necessarily be identical with that of a complicating acute pneumonic infiltration.

In all, the publications since 1945 concern 12 adults, 8 infants under 1 year of age and 1 child of five years. Thus the conception that *H. influenzae* pneumonia, if it occurs at all, is an infection of early childhood has to be abandoned.

Another misconception continued in many textbooks (WRIGHT 1953; NELSON 1954; WILSON and MILES 1955) is that *H. influenzae* is primarily associated with bronchopneumonia. Of 9 cases described in detail in the papers of CROWELL and LOUBE, and of NYHAN, RECTANUS and FOUSEK, 8 were radiologically pneumonias of the lobar or segmental type, both in children and adults, with post-mortem confirmation in one case.

Apart from the 2 cases of DURANT, SOKALCHUK, NORRIS and BROWN the *H. influenzae* strains isolated since 1945 from pulmonary consolidations were capsulated and of type b with the exception of one strain from a case of CROWELL and LOUBE which could not be typed.

Laboratory diagnosis. Pleural aspirates are handled in much the same way as specimens of cerebrospinal fluid. The techniques useful in the examination of sputum specimens are discussed under **F.**

Therapy. The prognosis of *H. influenzae* pneumonia and empyema has been improved markedly by modern chemotherapy. Of the 21 cases quoted from the literature since 1945 only one died, and this child did not have any antibacterial treatment. Such treatment follows the lines established for *H. influenzae* meningitis. NYHAN et al. stress that "a combination of 2 antibacterial agents to which the organism isolated is found susceptible be used and that one of these be chloramphenicol or streptomycin", but successful treatment with one effective antibiotic is not unusual (NASOU et al.).

E. Conjunctivitis

Though perhaps they do not come frequently to the notice of bacteriologists, members of the *Haemophilus* group are one of the commonest causes of acute conjunctivitis (PITTMAN and DAVIS 1950; WILSON and MILES 1955). This type of conjunctivitis is of considerable interest historically, for the causative haemophilic bacilli were seen in 1883 by KOCH in Egypt in the pus from benign conjunctivitis and described in greater detail by WEEKS in 1886 and 1887, i.e. several years before PFEIFFER discovered the influenza bacillus. Almost simultaneously with WEEKS, KARTULIS (1887) published his description of a similar, but not identical organism, and early classifications of bacteria used KARTULIS' published details as a basis. Owing to the confusion arising from these variations the KOCH-WEEKS bacillus was not selected as the *Haemophilus* type species. Also, until 1950 most workers in the *Haemophilus* field believed the KOCH-WEEKS bacillus and the influenza bacillus to be identical. Thus *H. influenzae* now occupies a position which by rights and usage should belong to the KOCH-WEEKS bacillus or *H. aegyptius*. The differences of opinion as to the identity or otherwise of the KOCH-WEEKS and the influenza bacillus began soon after PFEIFFER's description of the influenza bacillus. Ophthalmologists appear to have regarded the KOCH-WEEKS bacillus as different and as their very own and were supported by a number of bacteriologists (MORAX and BEACH 1896; AXENFELD 1908; PESCH 1921; HAMMERSCHMIDT 1921; ALI 1926) while the opposition to that idea grew with the increase of knowledge of the growth requirements of the *Haemophilus* group [NOGUCHI and COHEN 1915; FILDES 1923/24, KNORR 1924 (1), (2), (3); SCOTT 1929; GUYTON 1940]. Finally PITTMAN and DAVIS (1950) and DAVIS, PITTMAN and GRIFFITS (1950) succeeded in separating the two species by means other than their growth requirements and on the basis of two other properties characteristic for *H. aegyptius*. It appears, however, that both *H. influenzae* and *H. aegyptius* can occur in one and the same outbreak of acute benign conjunctivitis in warm climates (PITTMAN and DAVIS 1950).

Experimental conjunctivitis in human volunteers caused by *H. aegyptius* is more severe than that caused by *H. influenzae*; neither organism produced conjunctivitis when instilled into the eyes of conventional experimental animals, including monkeys and chickens (DAVIS and PITTMAN 1949). Because of the different severity it is, therefore, of some epidemiological importance to distinguish between *H. aegyptius* and *H. influenzae*.

Laboratory diagnosis. Eye swabs should be plated out as soon as possible after they have been taken from the conjunctiva and they must not be allowed to dry out.

H. aegyptius may conveniently be differentiated from *H. influenzae* by its capacity to agglutinate human red blood corpuscles of any blood group. Convenient volumes of fluid *H. aegyptius* cultures or saline suspensions of cultures from solid media are mixed with an equal volume of a 0.5% suspension of washed human red blood corpuscles and left at room temperature. Usually gross clumping occurs almost immediately. In addition to this most useful and simple method, growing the isolated strains in a shake culture in a semi-solid agar medium suitable for haemophili, for the characteristic comet-like colony formation of *H. aegyptius*, may be helpful.

Treatment. Topical application of adequate concentrations of any of the antibacterial drugs effective against *Haemophilus* species should eliminate both *H. influenzae* and *H. aegyptius* from the conjunctival sac and its adnexa. In human volunteers infected with *H. aegyptius* streptomycin was used successfully in this way (DAVIS and PITTMAN 1949). Since then equally and more effective antibiotics have become available and may be preferable to streptomycin on account of the latter drug's capacity to sensitize the skin.

F. Bronchiectasis and chronic bronchitis

PFEIFFER, in his first detailed paper on the influenza bacillus (1893), stated that this microorganism occurs in great numbers in the sputum of bronchitics and bronchiectatics who have suffered from an attack of influenza, and that during non-epidemic periods their sputum is free from the influenza bacillus. The terms bronchitis and bronchiectasis were not easily definable in those days; even to-day there is no unanimity about their meaning and definition. For instance MULDER has consistently avoided the term bronchiectasis which condition he seems to regard as one of the last stages in the natural history of chronic bronchitis (1937, 1938, 1940, 1956).

1. Bronchiectasis

It is perhaps unfortunate that the name bronchiectasis focuses attention on the bronchial dilatation rather than on the infective factor and the destructive inflammation of the bronchial walls. These may in places lead to dense infiltration inside and around the bronchial walls with hyperplasia of the mucosa causing constriction and obstruction demonstrable radiologically. There is extensive destruction of the bronchial elastic laminae and unstriped muscle, endarteritis of the bronchial vessels, degeneration of cartilage, and fibrosis of lung tissue. Only after the normal supports of the bronchi have been thus ravaged and the mechanisms for bronchial drainage shattered can the normal pressure differences on the two sides of the bronchial wall produce dilatation (ALLISON, GORDON and ZINNEMANN 1943). Analogous, though not identical pathological stages have been demonstrated experimentally after ligation of the left bronchus of rats (CHENG 1954). It is convenient to use the term bronchiectasis and to separate the condition from chronic bronchitis, thus indicating the radiological demonstration of saccular or fusiform dilatation of the larger bronchi.

After PFEIFFER's remarks there is no mention in the literature of a definite association of *H. influenzae* with bronchiectasis until 1905, when BOGGS reported the presence of the organism in the sputum of five cases, and LORD could isolate it repeatedly during a 17-months' period from the sputum of a case of diffuse bronchiectasis confirmed at post-mortem examination, and in eight others in which bronchiectasis was strongly suspected. VOGT (1911) found *H. influenzae* in pure culture or as the predominating organism in five children with the clinical diagnosis of bronchiectasis, which was confirmed radiologically in two. A year later VOGT had collected more such cases and together with BRÜCKNER and GAEHTGENS reported a total of 14 cases of bronchiectasis, in 12 of which *H. influenzae* was found in abundance. LUETSCHER (1915) described *H. influenzae*

as the second most important infectious agent in chronic non-tuberculous respiratory infections. Following the great influenza pandemic of 1918/19 BOSSERT and LEICHTENTRITT (1921) described more than 30 cases of post-influenzal bronchiectasis associated with *H. influenzae*. They were convinced that the organism was of aetiological importance in bronchiectasis. There followed a number of reports which stated either that *H. influenzae* did not occur any more frequently than other bacteria in bronchiectasis (OPIE, BLAKE, SMALL and RIVERS 1921; OPIE 1928; GREEY 1932) or that the authors could not isolate it at all (ERMATINGER 1928; BALLON, SINGER and GRAHAM 1931/32); this was probably due to inadequate technique. On the basis of his bacteriological experiences during and after the influenza pandemic ELKELES was convinced of the predominant role of the influenza bacillus in bronchiectasis (1922) and continued his observations for a decade, but unfortunately described cases of progressive bronchiectasis as "chronic influenza" (1931). This terminology of his had its foundation in the belief that *H. influenzae* was the causative agent of clinical influenzae, hence a condition in the respiratory tract with a preponderance of *H. influenzae* must be "chronic influenza". However, by 1937 he had ceased to use this terminology and described five more cases of diffuse bronchiectasis, this time in Argentina, with a predominance of *H. influenzae* in the sputum. At that time ELKELES advocated the use of bronchoscopy combined with bacteriological investigations in the hope that this technique would lead to a more general appreciation of the role of *H. influenzae* in bronchiectasis. ELKELES' findings were confirmed in 1943 when ALLISON, GORDON and ZINNEMANN demonstrated that non-capsulated *H. influenzae* was present in 63% of bronchial secretions collected with all necessary sterile precautions through the bronchoscope from 100 consecutive adult cases of bronchiectasis. In other lung conditions *H. influenzae* was isolated in a significantly lower proportion of cases and none of the other possible respiratory pathogens appeared to play an important role. These findings were confirmed seven years later by BENSTEAD (1950). The observations of VOGT and his associates in bronchiectatic children were also confirmed by FRANKLIN and GARROD (1953) who found *H. influenzae* either alone or together with pneumococci and other respiratory organisms in the sputum of 35 out of 36 patients. If any further proof was needed that *H. influenzae* is not merely a contaminating microorganism from the upper respiratory tract, this was supplied by ALLIBONE, ALLISON and ZINNEMANN (1957). They isolated non-capsulated *H. influenzae* in profusion from the bronchial aspirates of 27 out of 32 children (84.4%) suffering from radiologically proven bronchiectasis. At the same time *H. influenzae* was present in the maxillary sinuses of 60% of the patients. Over periods of observation extending from several months to several years it was seen that the bacteriological findings in the sputum of these patients remained fairly constant, always provided that such sputa were not coughed up in the ordinary way but were obtained by postural drainage together with massage of the back.

Both FRANKLIN and GARROD and ALLIBONE, ALLISON and ZINNEMANN observed that adequate antibiotic therapy could eliminate *H. influenzae* from the sputum of these children. Simultaneously with the disappearance of *H. influenzae* the sputa became mucoid. After cessation of treatment *H. influenzae* and pus

usually reappeared together (Fig. 8). Phagocytosis of *H. influenzae* can be seen easily in such bronchiectatic sputa (Fig. 9).

ALLIBONE, ALLISON and ZINNEMANN agree with MULDER's dictum (MULDER, GOSLINGS, VAN DER PLAS and CARDOZO 1952): "In our opinion, the above observations lead with certainty to the conclusion that the group of non-encapsulated

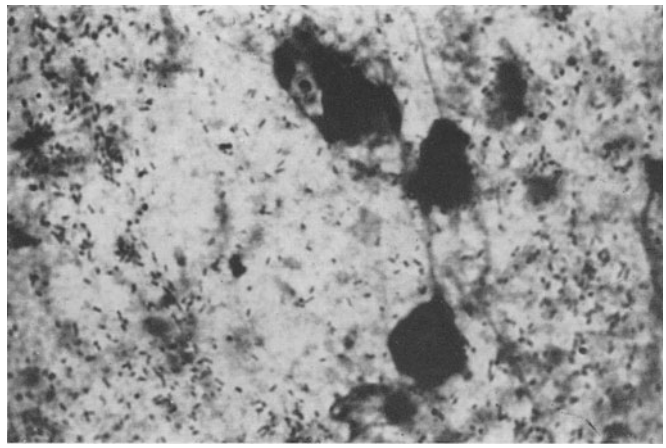

Fig. 8. *H. influenzae* in average, purulent bronchiectatic sputum. Gram stain. Note abundance of *H. influenzae*

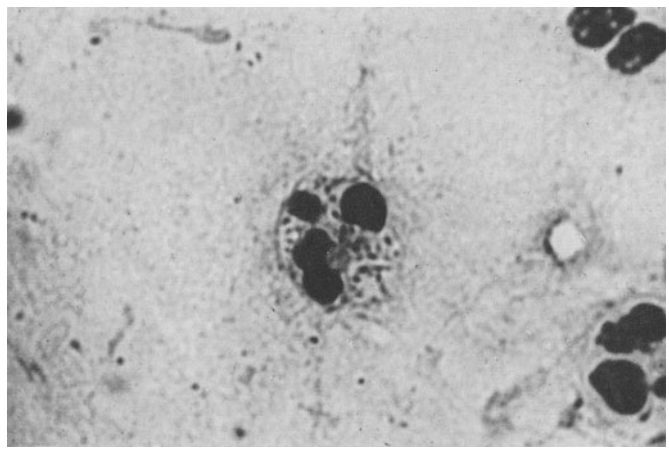

Fig. 9. Phagocytosis of *H. influenzae* in bronchiectatic sputum. Leishman stain

Hemophilus (influenzae) is a pathogen and not a saprophyte of the mucous membrane of the bronchial tree . . . we are entitled to say that the aetiology of bronchitis and that of bronchiectasis cannot possibly be understood if the part played by *Hemophilus* infection is overlooked."

Laboratory diagnosis. This may have its difficulties, particularly when *H. influenzae* is not the only or predominant organism, but is present together with the pneumococcus and other antagonistic bacteria. It depends to a great deal on good inoculation technique and correct preparation of culture media.

Most of the points of importance will be discussed when considering bronchitis, where the same difficulties exist in a somewhat exaggerated from.

Treatment. Therapeutic efforts cannot hope to repair the damage to the bronchi. If the pathological process is a circumscribed one and restricted to a lobe of one or both lungs, resection may have to be attempted in an effort to prevent spread of the infection. The best that can be expected from internal medication is clearance of pus and *H. influenzae* from the sputum and perhaps in children arrest of deterioration. Attempts to influence the propagation of *H. influenzae* in man were made very early by PFEIFFER (1893) and some of those who followed in his footsteps. PFEIFFER admitted that so far killed vaccines of the influenza bacillus did not seem to prevent attacks of clinical influenza. The idea turned up again when JACKSON and JACKSON (1934) and ELKELES (1937) reported successes in the treatment of bronchiectasis with autogenous *H. influenzae* vaccines. WYNN-WILLIAMS and MOYES (1951) first reported an average reduction by 83% of the amount of sputum in adult bronchiectasis with three daily doses of 0.5 g. chloramphenicol; penicillin, streptomycin or both together reduced the quantity of sputum in the same patients by only 58%. Unfortunately the bacteriology of this investigation, though apparently carried out, was not reported. Similarly, no bacteriological results were included when HARRIS, GORNELL, SHORE and LOVITZ (1952) published their successful treatment with chloramphenicol, at first with continuous and subsequently with intermittent medication of 36 bronchiectatics. MULDER, GOSLINGS, VAN DER PLAS and CARDOZO (1952) confirmed these findings and added bacteriological data showing that pus and *H. influenzae* as a rule, disappeared together during antibiotic therapy. FRANKLIN and GARROD (1953) saw almost dramatic reduction of sputum quantity and purulence, as well as disappearance of *H. influenzae* in 36 bronchiectatic children treated with 14—66 mg. chloramphenicol per kg. bodyweight per day for one to two weeks. They had to discontinue this type of treatment after one of the patients died of aplastic anaemia. ALLIBONE, ALLISON and ZINNEMANN (1957) showed that no antibiotic on its own, except chloramphenicol, is able to eliminate pus and *H. influenzae* from the sputum of bronchiectatic children. However, if large doses (two to four grams daily) of oxytetracycline, tetracycline or erythromycin are combined with an equal amount of a triple sulphonamide the result achieved is as good as that obtained with chloramphenicol. The time required for sputum conversion may vary from one to twelve weeks (see Table 13).

Table 13. *Response of bronchiectatic children to antibacterial treatment as assessed by conversion of purulent to mucoid sputum and disappearance of H. influenzae*

Response within	No. of patients
1 week	17 (62.9%)
2 weeks	6 (22.2%)
3—4 weeks	1
5—6 weeks	1
12 weeks	1
no response	1
Total	27

In 1957 DOUGLAS, SOMNER, MARKS and GRANT again confirmed that in adults the response to chloramphenicol is better than to either penicillin or oxytetracycline. All authors agree that in severe cases relapse occurs rapidly, often within a week after cessation of treatment. The question has not been resolved yet whether to maintain bronchiectatic patients, once freed from pus and *H. influenzae* by massive dosage, on continuous doses of antibacterial drugs in small or moderate

amounts (HELM, MAY and LIVINGSTONE 1954, 1956; *Report* 1957; CHERNIAK, VOSTI, DOWLING, LEPPER and JACKSON 1959) or to employ intermittent therapy with the highest possible doses as did HARRIS, GORNELL, SHORE and LOVITZ (1952) and ALLIBONE, ALLISON and ZINNEMANN (1957). Table 14 summarizes the therapeutic results with antibacterial therapy, as judged by clearance of sputum from pus and pathogens, thus far reported in the literature, but not all papers quoted in the text provide data in a suitable form for this evaluation.

Table 14. *Therapeutic results with antibacterial drugs in bronchiectasis as judged by clearance of sputum from pus and pathogens; all authors*

Authors	Year	No. of cases	Improved	No change	Drug(s) used
WYNN-WILLIAMS and MOYES .	1951	15	15	0	C
MULDER, GOSLINGS et al. . . .	1952	59	58	1	P, P + S, T
HARRIS, GORNELL et al. . . .	1952	38	36	2	C
FRANKLIN and GARROD. . . .	1953	36	34	2	C
HELM, MAY and LIVINGSTONE .	1954	8	7	1	T
ALLIBONE, ALLISON and ZINNEMANN	1956	27	26	1	C, T + S3, TC + S3, E + S3
DOUGLAS, SOMNER et al. . . .	1957	42	35	7	C, T, P
Total.		225	211 (93.6%)	14 (6.2%)	

P penicillin; S streptomycin; C chloramphenicol; T oxytetracycline; TC tetracycline; E erythromycin; S3 triple sulphonamide (sulphatriad).

2. Chronic bronchitis

Chronic, as distinct from acute bronchitis is a clinical entity which, according to the literature, occurs frequently in Great Britain and the Netherlands, but either does not exist to any great extent or is not recognised elsewhere. In the mind of some workers chronic bronchitis seems to be equated with bronchial asthma, in that of others with bronchiectasis. Although mixed forms of asthma, chronic bronchitis and bronchiectasis exist, British authors are agreed that these are three separate clinical entities to which these labels are justifiably attached. The definition of chronic bronchitis usually accepted in Great Britain is as follows.

Cough, expectoration and inspiratory but not expiratory dyspnoea, causing some degree of disability; usually with febrile exacerbations during the winter months after which the dyspnoea becomes progressively worse; sputum may be either mucoid or purulent; bronchographically beading and irregularities of the small bronchi, projections and pouching, with pool formation in bronchioles, but no localised bronchial dilatation (see L. REID 1956, SIMON 1958). Pathologically there is hypertrophy of the mucus secreting elements (L. REID 1954, 1956) and secondary, centrilobular emphysema (LEOPOLD and GOUGH 1957). Of the factors suspected as the cause of chronic bronchitis—atmospheric pollution, heredity, smoking and socio-economic conditions (JOULES 1954, 1956; PEMBERTON and GOLDBERG 1954; WALLER and LAWTHER 1955; D. D. REID 1956, 1958; D. D. REID and FAIRBAIRN 1958; STOCKS 1959) — infection is the one factor most easily amenable to investigation. With treatment in mind the infective factor, therefore, has been the subject of much recent work.

MULDER was the first to draw attention to the predominant part of *H. influenzae* in acute and chronic purulent bronchitis, both in tropical and temperate climates (1937, 1938, 1940, 1956). STUART-HARRIS, POWNALL, SCOTHORNE and FRANKS (1953) concentrated their attention on the pneumococcus and found all types of pneumococci as the predominant organism in chronic bronchitis, but on their own admission did not use media favouring the growth of *H. influenzae*. On the other hand they used mouse inoculations for the isolation of pneumococci from sputum and in this way possibly overemphasized with this hypersensitive technique the part played by the pneumococcus. A little later, in a different place and under different conditions, using improved methods for the isolation of *H. influenzae*, STUART-HARRIS and his collaborators (BROWN, COLEMAN, ALLEY, STRANAHAN and STUART-HARRIS 1954) increased the isolation rate of *H. influenzae* from 15.3 to 50.6% and that of the pneumococcus from 50.5 to 73.5%. MAY confirmed that *H. influenzae* and the pneumococcus are the two organisms most commonly implicated in chronic bronchitis, and that *H. influenzae* is more prominent [1952, 1953 (1)]. In this context MAY introduced the concept of the "potential pathogens", referring to *H. influenzae*, the pneumococcus, *Staphylococcus aureus*, *Kl. pneumoniae* and beta-haemolytic streptococci. In another paper MAY (2) in 1953 established that pus and whatever potential pathogen was present, disappeared during antibacterial chemotherapy and reappeared together on its cessation (the so-called therapeutic test). The reports of a group of workers at the Hammersmith Hospital, London, gave further support to the conception of *H. influenzae* as the principal pathogen in chronic bronchitis (ELMES, KNOX and FLETCHER 1953; KNOX, ELMES and FLETCHER 1955; ELMES, FLETCHER and DUTTON 1957). EDWARDS, BUCKLEY, FEAR, WILLIAMSON and ZINNEMANN (1957) showed that the incidence of non-capsulated *H. influenzae* was twice as high in purulent as in mucoid bronchitic sputa while the incidence of the pneumococcus was unrelated to sputum purulence.

MAY (1) pointed out that single specimens of sputum may fail to reveal the pathogenic microorganisms in chronic bronchitis (1953) and suggested that at least two and preferably five samples should be examined, or that culture plates should be inoculated with sputum homogenized by RAWLINS' method (1953) with a 1% pancreatin solution. However, if a ball of sputum the size of a pea is used as inoculum, isolation rates of the same order as those of MAY's are obtained (EDWARDS, BUCKLEY, FEAR, WILLIAMSON and ZINNEMANN 1957). Swedish workers have recently expressed doubts about the accuracy of the information on the bronchial flora gleaned with these methods and have shown considerable discrepancies to exist between the culture results of sputum, washed or unwashed, and bronchial aspirates in patients with asthma and chronic bronchitis (BERGMAN, COLLDAHL and NILSSON 1955; TUNEVALL and WASSERMANN 1956). The Continental and American schools have long practised the three times repeated washing of sputum flakes in physiological saline to free the material of the superficially adhering contaminants from the upper respiratory tract (KITASATO 1892; BOGGS 1905; LUETSCHER 1915; BOECKER and KAUFFMANN 1931; MULDER 1938, 1956). However, as is to be expected, this washing technique is not entirely foolproof (BERGMAN and COLLDAHL 1956). BRUMFITT, WILLOUGHBY and BROMLEY (1957) compared cultures from bronchial swabs collected through

the bronchoscope with cultures taken on the same day from sputum and throat swabs from the same patients. They found that growth obtained from sputum samples was representative of the bronchial flora if the same organisms were absent in the throat, but any bacteria present in the throat were liable to be present in the sputum also and were thus apt to give a wrong impression of the bacteriological state of the bronchi. In healthy subjects the bronchi are sterile when swabbed in this way. BRUMFITT et al. emphasize that *H. influenzae* is commonly present in the bronchi in chronic, mucopurulent bronchitis, with or without bronchiectasis, and they stress further that pneumococci are rarely found in the bronchi of such patients. This combined sputum and throat culture technique, practised on 37 cases of chronic bronchitis, showed the predominant role of *H. influenzae*, but, because of the simultaneous occurrence in throat and sputum cultures, left the question of its significance in 12 of these 37 cases unanswered (BRUMFITT and WILLOUGHBY 1958). There were only 14 patients with purulent bronchitis whose bronchial secretions were obtained by bronchoscopy (BRUMFITT, personal communication) and this illustrates well the difficulties under which investigators in the field of chronic bronchitis labour. For a number of reasons medical ethics does not justify bronchoscopic examination of bronchitics unless haemoptysis is one of the symptoms. Thus, clear cut evidence on a numerically adequate scale is difficult to obtain. Yet, it is clear that bacteriological findings in bronchoscopic aspirates must mirror the true state of the bronchial flora more accurately than sputum cultures. The relevant figures have been culled from those reports in the literature that give precise figures, and are presented in Tables 15 to 19. These tables compare the evidence for the role of *H. influenzae* and the pneumoccus in both bronchiectasis and bronchitis, based on bacteriological examinations of sputum and of bronchial aspirates.

Table 15. *Sputum bacteriology in bronchiectasis; all authors*

Authors	Year	No. of cases	Per cent of cases in which	
			H. influenzae present	Pneumococcus present
STUART-HARRIS et al. .	1953	27	22.2	58.0
FRANKLIN and GARROD	1953	36	97.2	2.7
MULDER et al.	1956	363	84.0	—
ALLIBONE et al.	1956	32	100.0	—
DOUGLAS et al.	1957	13	53.8	61.5

— no observation.

Table 16. *Bacteriology of bronchial aspirates in bronchiectasis; all authors*

Authors	Year	No. of cases	Per cent of cases in which	
			H. influenzae present	Pneumococcus present
ALLISON et al.	1943	100	63.0	14.0
BENSTEAD	1950	30	40.0	33.3
ALLIBONE et al.	1956	32	84.0	—
BRUMFITT and WILLOUGHBY	1958	5	100.0	—

— no observation.

Table 17. *Sputum bacteriology in chronic bronchitis; all authors*

Authors	Year	No. of cases	Per cent of cases in which			
			H. influenzae present when sputum			pneumococcus present
			purulent		mucoid	
STUART-HARRIS et al. .	1953	113	-----	- 15.3 -	-----	50.5
BROWN et al.	1954	20	-----	- 50.6 -	-----	73.5
MAY	1954	85	64.7		10.3	20.0—71.0
MULDER	1956	315	83.0		—	—
DOUGLAS et al.	1957	25	-----	- 56.0 -	-----	56.0
EDWARDS et al.	1957	320	65.4		34.1	39.7
ELMES et al.	1957	88	—		63.0	13.0
BRUMFITT and WILLOUGHBY	1958	37	51.4		—	16.2

- - - no distinction made between mucoid and purulent sputum; — no observation.

Table 18. *Bacteriology of bronchial secretions in chronic bronchitis; all authors*

Authors	Year	No. of cases	Per cent of cases in which		
			H. influenzae present when sputum		pneumococcus present
			purulent	mucoid	
ALLISON et al.	1943	26	—	0.0	?
BENSTEAD	1946	11	—	9.0	0.0
BRUMFITT and WILLOUGHBY	1958	14	92.8	—	—

— no observation; ? some observations made but unsuitable for this table.

Table 19. *Summary of tables 15—18; incidence of H. influenzae and the pneumococcus in sputum and bronchial aspirate in bronchiectasis and bronchitis; average per cent of all authors*

Disease	Specimen	No. of cases	Percentage of all cases in which		
			H. influenzae present when sputum		pneumococcus present
			purulent	mucoid	
bronchiectasis	sputum	471	59.6	—	24.4
	bronchial secretion	167	71.0	—	?
chr. bronchitis	sputum	803	55.2	43.9	42.2
	bronchial secretion	51	92.8	4.5	0.0

— no observation; ? some observations made but unsuitable for this table.

It is evident from Tables 15 to 19 that in bronchiectasis the incidence of *H. influenzae* in the sputum is confirmed by, and is in fact substantially higher in, bronchoscopic samples. Precise figures regarding the pneumococcus cannot be culled from the literature and there is scope for further investigations in this respect. The numerical confirmation available for bronchiectasis is lacking in purulent bronchitis where only the 14 bronchoscopic examinations of BRUMFITT and his colleagues have been carried out. In mucoid bronchitis the isolation rate of *H. influenzae* from bronchial secretions in 37 cases is only one tenth that of its isolation rate from sputum. If these figures were confirmed by a greater number of similar investigations then it would appear that *H. influenzae* does not play as important a part in mucoid bronchitis as findings in mucoid bronchitic sputa would lead us to believe. Further, it would seem that the incidence of the pneumococcus in over 42% of bronchitic sputa is equally misleading and

that its presence in such specimens is probably due to admixture of secretion from the upper respiratory tract and the throat. This gap in our knowledge can be filled only if more bronchoscopic examinations are carried out in purulent and mucoid bronchitis and if these findings are published[1].

In vivo antagonism between the pneumococcus and *H. influenzae* as related to chronic bronchitis. With the more intensive investigation of the significance of *H. influenzae* in chronic bronchitis the antagonism of pneumococci to *H. influenzae* described under the general heading of "The genus *Haemophilus*" has been noticed by some observers in vivo in man. ELMES, KNOX and FLETCHER (1953) and DOUGLAS, SOMNER, MARKS and GRANT (1957) reported that when they

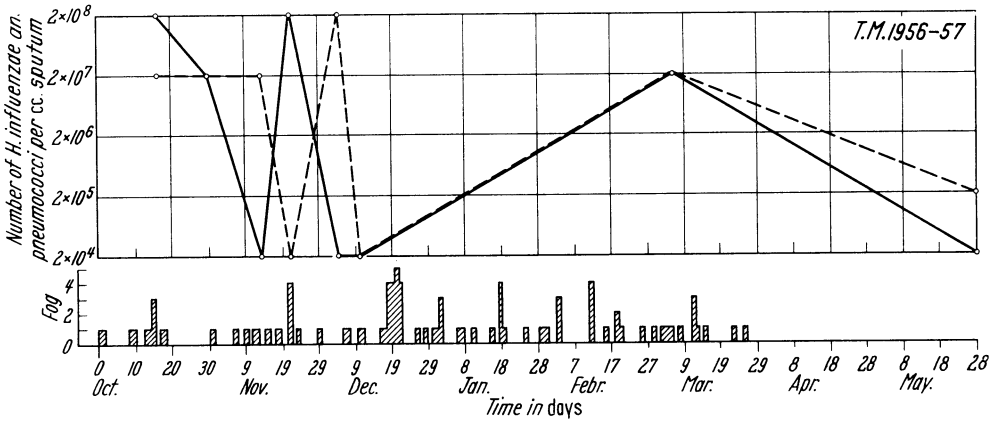

Fig. 10. Diagram of the viable *H. influenzae* and pneumococcus counts in the sputum of patient T. M., October 1956 to May 1957. o— —o Number of pneumococci per ml. of sputum. o——o Number of *H. influenzae* per ml. of sputum. ▨ Fog: *1* Hazy or misty; *2* Very hazy; *3* Slight fog or for part of day only; *4* Fog; *5* Thick fog

Figs. 10—12. Diagrams of viable *H. influenzae* and pneumococuss counts of three patients during the winter months. (COOPER 1959.)

eliminated the pneumococcus from the sputum of chronic bronchitics by penicillin therapy *H. influenzae* proliferated profusely with deterioration of the state of the sputum inspite of continued penicillin therapy. MAY (1954) on the basis of observations on the same patients which continued for two years, stated that *H. influenzae* and the pneumococcus are "clearly more or less complementary to each other; and, when the incidence of pneumococci decreased, that of *Haemophilus influenzae* increased proportionately". This in vivo relationship of the two most frequently occurring "potential pathogens" in chronic bronchitis is of considerable importance for, if according to BRUMFITT, WILLOUGHBY and BROMLEY (1957) the pneumococcus is rarely present in the bronchi of bronchitics, it seems that on occasions *H. influenzae* can be replaced by the pneumococcus in this site. COOPER (1959) looked into this relationship by counting the number of viable organisms per ml. of each of the two species in homogenized sputa of chronic bronchitics, known to have harboured *H. influenzae* for some years. Counts of pneumococci were carried out on chocolate agar plates containing 2 μg. of streptomycin per ml. and counts of *H. influenzae* on chocolate agar plates

[1] After the final proofs of this review had gone to press A. W. LEES and W. MCNAUGHT published the results of their bronchoscopic examinations in 28 bronchitics and 14 controls, confirming the predominant role of *H. influenzae* and the secondary importance of the pneumococcus. They made no distinction between purulent and mucoid bronchitis. Lancet **1959 II**, 1112.

containing 0.5 unit penicillin per ml. The observations were made during two consecutive winters on two groups of patients, the first group receiving treatment with terramycin and sulphatriad and the second group identical looking dummy capsules and tablets. When the numbers counted were plotted some rather

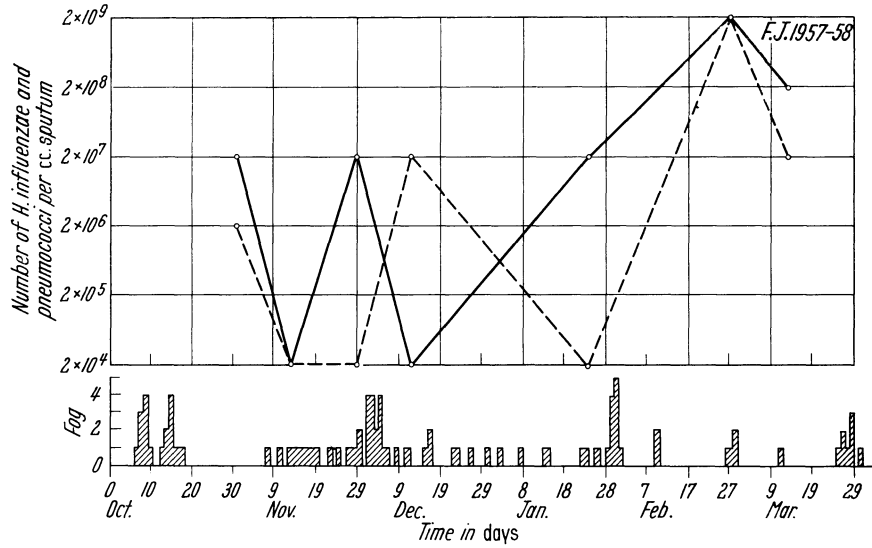

Fig. 11. Diagram of the viable *H. influenzae* and pneumococcus counts in the sputum of patient F. J., November 1957 to March 1958. Explanation of signs see Fig. 10

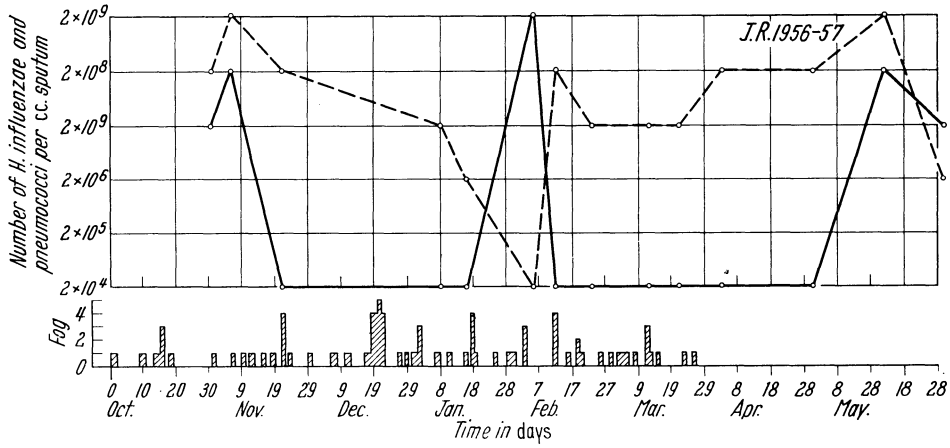

Fig. 12. Diagram of the viable *H. influenzae* and pneumococcus counts in the sputum of patient J. R., November 1956 to May 1957. Explanation of signs see Fig. 10

characteristic graphs resulted which show that generally pneumococcus counts are low when *H. influenzae* counts are high and vice versa (see Figs. 10 to 12). As a rule the numbers of *H. influenzae* per ml. of sputum were considerably higher than those of pneumococci, independent of antibacterial treatment. There was no definite correlation of high pneumococcus or *H. influenzae* counts with exacerbations, though some high *H. influenzae* counts seemed connected with the incidence of thick fog. The connection between *H. influenzae* and fog has also

been commented on by ELMES, KNOX and FLETCHER (1953). The relationship of *H. influenzae* and the pneumococcus in chronic bronchitis is apt to complicate the task of the clinician and the bacteriologist as regards the choice of effective antibacterial therapy, but further and more detailed observations are required before the phenomenon can be understood fully and its significance assessed in the natural history and clinical picture of chronic bronchitis.

***H. influenzae* strain identity in chronic bronchitis.** The tactics of antibacterial therapy may be influenced appreciably if it were established that elimination of one strain of *H. influenzae* in a bronchitic were soon followed by reinfection with another strain. COOPER (1959) attempted to identify the non-capsulated

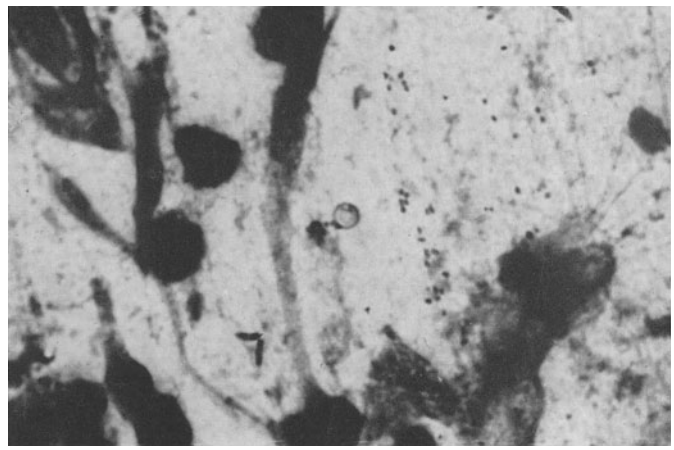

Fig. 13. *H. influenzae* in average, purulent bronchitie sputum. Gram stain. Note relative paucity of *H. influenzae*

H. influenzae strains in such patients by means of agglutination with the patients' own sera and by determining their sensitivity patterns to a wide choice of antibiotics. It could be shown that in a number of cases, but by no means in all, the strains present originally had been replaced by different non-capsulated strains during a six months' period.

Laboratory diagnosis. The bacteriology of chronic bronchitis is at present in an exploratory stage and as a consequence no hard and fast rules for the bacteriological diagnosis of microorganisms associated with the condition can be given, but the various points concerning sampling and culturing, discussed earlier in this chapter, should be borne in mind. Because of the exacting nature of *H. influenzae* and the antagonism of microorganisms occurring in sputum much time and effort may have to be spent on details. These difficulties have induced some workers to rely solely on microscopic examinations of stained sputum flakes (see Fig. 13).

It has been shown, however, that in only 81% of 158 sputum specimens from bronchitics could *H. influenzae* be recognised microscopically by workers with many years of experience in this field (EDWARDS et al. 1957). MULDER is reported to have stated that *H. influenzae* can be identified in Gram stained sputum smears with almost 100% certainty (Leading article 1956). Gram stained films are of little value in recognising the presence of pneumococci, since these cannot

be readily distinguished from other Gram positive cocci unless capsulation is clearly evident, which is not often the case.

Treatment. EDWARDS, BUCKLEY, FEAR, WILLIAMSON and ZINNEMANN (1957) tried autogenous *H. influenzae* vaccines in chronic bronchitics without observing any clinical improvement or any difference in the bacterial flora of the sputum.

Table 20. *Therapeutic results with antibacterial drugs in chronic bronchitis as juged by clearance of sputum from pus and/or pathogens; all authors*

Authors	Year	No. of cases	No. improved	No. without change	Drug(s) used
MULDER et al. . . .	1952	18	13	5	P, P + S, T
HARRIS et al. . . .	1952	13	7	6	C
HELM et al.	1954	17	15	2	T
EDWARDS et al. . .	1957	29	21	8	T, T + S3
DOUGLAS et al. . .	1957	89	60	29	P
MOYES and KERSHAW	1957	52	33	19	TC
MAY	1958	37	26	11	T
Total		255	175	80	

P penicillin; C chloramphenicol; S streptomycin; T terramycin (oxytetracycline); TC tetracycline; S3 sulphatriad (triple sulphonamide).

A trial of a commercial *H. influenzae* vaccine met with similar failure (BROWN and WILSON 1959).

With the advent of streptomycin the burden of tuberculosis on chest physicians has been eased considerably and has enabled them to devote more time to non-tuberculous chronic infections of the chest. In Great Britain chronic bronchitis has received the lion's share of this attention, particularly since oral antibiotics have been marketed. The results of such therapeutic efforts are shown in table 20 in which only publications permitting judgement by sputum clearance are included.

Table 21. *Therapeutic results with antibacterial treatment in bronchiectasis and chronic bronchitis compared; summary of Tables 14 and 20*

Disease	No. of cases	No. improved	No. without change
Bronchiectasis. .	225	211 93.6%	14 6.2%
Chronic bronchitis	255	175 68.6%	80 31.4%

Table 21 summarizes and compares the contents of Tables 14 and 20.

It can be seen that there is a more than 90% success rate in bronchiectasis while this figure is 25% lower in bronchitis. The overall failure rate in bronchitis is about five times as high as in bronchiectasis. In part this difference may be due to the somewhat smaller dosage schedules often employed in bronchitis. On the other hand MAY [1953 (2)] stated that "patients expectorating only muccoid sputum do not seem to benefit by chemotherapy, no matter what bacteria are present", and "the removal of 'pathogens' from patients producing purely mucoid sputum apparently does not affect the amount expectorated." Possibly these patients with mucoid sputa constitute the 30% failure rate in chronic bronchitis, but when considering this possibility one would have to make a clear distinction between mucoid bronchitis and bronchial asthma, which is not apparent in all the papers referred to here. All authors stress the temporary nature of improve-

ment if antibacterial treatment is withdrawn. HELM, MAY and LIVINGSTONE (1954, 1956), OSWALD and MAY (1956), EDWARDS, BUCKLEY, FEAR, WILLIAMSON and ZINNEMANN (1957) and BUCHANAN, BUCHANAN, MELROSE, MCGUINESS and PRICE (1958) report marked benefit from long-term chemotherapy extending for periods of up to nearly three years. The considerable expense of continuous treatment with broad spectrum antibiotics caused ELMES, FLETCHER and DUTTON (1957) to use oxytetracycline for one week only at the onset of exacerbations while a control group of patients received undistinguishable dummy capsules under similar circumstances. The treated group lost half as much time from work with each exacerbation than the control group, but this difference was not significant statistically as it could have occurred by chance once in ten times. Economic considerations such as prompted the investigation of ELMES, FLETCHER and DUTTON (1957) are a questionable basis for therapeutic efforts. In this particular instance they appear rather misplaced since MOYES and KALINOWSKI (1959) were able to show that long-continued chemotherapy gives markedly greater benefit to bronchitics than either intermittent dosage or five-day courses of antibiotics at the onset of each exacerbation.

Only EDWARDS, BUCKLEY, FEAR, WILLIAMSON and ZINNEMANN (1957) combined oxytetracycline with a triple sulphonamide and found this combination more effective than either drug alone. Clearly, chronic bronchitics might obtain much relief if an antibiotic as effective against *H. influenzae* as chloramphenicol, but as cheap and as comparatively free from side effects as penicillin, were found. At present the tetracyclines, and on its in vitro performance possibly erythromycin, when combined with a sulphonamide, are best able to fill that role, but cannot claim to be the ideal drugs for the purpose.

The efforts made in Great Britain at the present time to increase our knowledge of chronic bronchitis and to improve the lot of those who suffer from it are underscored by the publication, within the space of a few months, of three monographs on the subject, to which those interested in chronic bronchitis are referred (OGILVIE and NEWELL 1957; STUART-HARRIS and HANLEY 1957; OSWALD 1958).

G. Miscellaneous infections due to *H. influenzae*

If looked for, *H. influenzae* can be isolated from a number of unusual sites, particularly in children. The origin of these infections is not always clear, but it has to be remembered, as shown in parts **A**, **B** and **D** of this review, that *H. influenzae* not infrequently causes a septicaemia. After invasion of the blood stream a microscopic bacterial embolus may find suitable conditions for propagation in any tissue of the human body and thus give rise to acute or chronic inflammatory processes. Bacteriaemia of a transient nature may occur after tonsillectomy. CLARKE (1912) first reported severe *H. influenzae* septicaemia following tonsillectomy. ROGERS and ZINNEMANN (1958 and unpublished) found *H. influenzae* in blood cultures taken within five minutes of tonsillotomy or tonsillectomy in 5 out of 38 children thus investigated.

Reports of *H. influenzae* septicaemia with and without complications can be found scattered in the literature (MEUNIER 1897; THURSFIELD 1910; REISS and GINS 1911; LEICHTENTRITT and SCHOBER 1924; KLINKE 1924; CABOT and

PAINTER 1929; SOMERFORD 1929; BENJAMIN 1931; KOCH 1932). There are also accounts of cases of *H. influenzae* endocarditis with septicaemia and it is reasonable to assume that in such cases bacteriaemia or septicaemia was the primary event (FIESSINGER 1922; LECHNER and BOETZEL 1927; FRANK 1931; FIESSINGER and ARNANDET 1932; FIESSINGER and ALBEAUX-FERNET 1933). A clear distinction between *H. influenzae* and other haemophili is not always drawn in these primarily clinical papers. Other lesions which most likely arise as a result of haematogenous spread are pericarditis (KRESKY 1943), pyarthrosis and osteomyelitis (WEAVER and SHERWOOD 1938; ROGERS and ZINNEMANN 1958 and unpublished), skin infection and exanthema (LEICHTENTRITT and SCHOBER 1924; ROGERS and ZINNEMANN, unpublished), perianal abscess, infected thyroglossal cyst (ROGERS and ZINNEMANN, unpublished), brain abscess (ZINNEMANN 1946). Two reports state that symptoms of pharyngitis, conjunctivitis or croup—presumably glottitis or epiglottitis—preceded septicaemia or endocarditis (LEICHTENTRITT and SCHOBER 1924; KRESKY 1943). Meningitis can follow the initial septicaemia (LEICHTENTRITT and SCHOBER 1924; CABOT and PAINTER 1929; BENJAMIN 1931). It appears, therefore, that the sequence of pathological events is often first an upper respiratory tract infection with accompanying septicaemia and in the second instance localisation elsewhere, possibly at points of low tissue resistance or injury. Some of the reports quoted refer specifically to *H. influenzae* type b, but others do not mention whether capsulated or non-capsulated *H. influenzae* strains were involved.

In rare cases *H. influenzae* can be isolated from the urine of children or the urethra of adults (ROGERS and ZINNEMANN 1958 and unpublished). These young patients had either a recent, violent injury, congenital abnormalities of the urinary tract or a calculus in the pelvis of a kidney. It seems reasonable to assume that in these cases too *H. influenzae* reached the urinary system by way of the blood stream.

This explanation cannot be adduced when *H. influenzae* is isolated from the lumen of appendices removed at operation. ROGERS and ZINNEMANN (1958 and unpublished) found non-capsulated *H. influenzae* in 11 out of 327 appendices (3.4%) of children. It seems likely that *H. influenzae*, well enveloped in nasopharyngeal mucus, may have survived the passage through the acid contents of the gastric cavity to find a suitable environment for propagation in the lymphatic tissue of the appendix. This seems all the more likely because pneumococci were often the only other organism isolated together with *H. influenzae* from the appendices. It does not seem impossible that under these circumstances *H. influenzae* can be the causative agent of appendicitis. More detailed work is required to settle this point.

At the conclusion of this review it is perhaps easier to understand the life-long fascination *H. influenzae* has had for ROSHER, and to which reference was made in the introduction. From the last authoritative account of the role of *H. influenzae* in pandemic influenza (PFEIFFER 1931) which was published shortly before the isolation of the influenza virus in 1933 (SMITH, ANDREWES and LAIDLAW) it appears that *H. influenzae* does not play the same part in epidemic influenza as we have known it since 1918/19. None of the waves of epidemic influenza, including the pandemic of Asian influenza of 1957/58, has had a fatality rate

approaching that of 1918/19. It is difficult to believe that so many experienced investigators who then found *H. influenzae* in bronchial and lung lesions, should have been entirely wrong. It has been mooted that no impressive reports on the isolation of *H. influenzae* during the pandemic of Asian influenza were published because, owing to chemotherapy *H. influenzae* had no chance to flourish as a secondary invader. As we have seen in part **F** it is not easy to eliminate *H. influenzae* from the respiratory tract even with rather high doses of antibiotics combined with sulphonamides. It is rather unlikely, therefore, that usual doses of antibacterial drugs given to sufferers from Asian influenza prevented pulmonary complications caused by *H. influenzae*. Reliable investigators have implicated penicillin resistant strains of *Staphylococcus aureus* in the majority of fatal cases of that epidemic (HERS, GOSLINGS, MASUREL and MULDER 1957; GILES and SHUTTLEWORTH 1957; ROBERTS 1957; OSWALD, SHOOTER and CURWEN 1958). The wisest attitude to adopt, in view of all the evidence accumulated during the last three decades, is to withhold final judgement on the role of *H. influenzae* in pandemic influenza while bearing in mind that the association of one of the influenza viruses with one of the forms of haemophilic bacilli may constitute a particular risk for man (Leading article 1958). The therapeutic means at our disposal may minimise that risk, if it should be proved to exist, by the rational use of large doses of combinations of antibacterial drugs effective against *H. influenzae*.

References

ALEXANDER, H. E.: Type b anti-influenzal rabbit serum for therapeutic purposes. Proc. Soc. exp. Biol. (N.Y.) **40**, 313 (1939).

— (1) Experimental basis for the treatment of Haemophilus influenzae infections. Amer. J. Dis. Child. **66**, 160 (1943).

— (2) Experimental investigations as a basis for treatment of type b Hemophilus influenzae meningitis in infants and children. J. Pediat. **23**, 640 (1943).

— Treatment of type b Hemophilus influenzae meningitis. J. Pediat. **25**, 517 (1944).

— The Hemophilus group. In: Bacterial and mycotic infections of man (R. Dubos), p. 484. Philadelphia: J. B. Lippincott Company 1948.

— C. ELLIS and G. LEIDY: Treatment of type-specific Hemophilus influenzae infections in infancy and childhood. J. Pediat. **20**, 673 (1942).

—, and M. HEIDELBERGER: Chemical studies on bacterial agglutination. V. Agglutinin and precipitin content of antisera to Hemophilus influenzae type b. J. exp. Med. **71**, 1 (1940).

—, and G. LEIDY: Experimental investigations as a basis for treatment of type b Hemophilus influenzae meningitis in infants and children. J. Pediat. **23**, 640 (1943).

— — Influence of streptomycin on type b Hemophilus influenzae. Science **104**, 101 (1946).

— — Transformation type specificity of H. influenzae. Proc. Soc. exp. Biol. (N.Y.) **73**, 485 (1950).

— — Determination of inherited traits of H. influenzae by desoxyribonucleic acid fractions isolated from type-specific cells. J. exp. Med. **93**, 345 (1951).

— — and C. MACPHERSON: Production of types A, B, C, D, E and F H. influenzae antibody for diagnostic and therapeutic purposes. J. Immunol. **54**, 207 (1946).

— — and W. REDMAN: Comparison of the action of streptomycin, polymyxin B, aureomycin and chloromycetin on H. pertussis, H. parapertussis, H. influenzae and five enteric strains of Gram-negative bacilli. J. clin. Invest. **28**, 867 (1949).

— G. RAKE and R. DONOVICK: Hemophilus influenzae meningitis treated with streptomycin. J. Amer. med. Ass. **132**, 434 (1946).

ALI, M.: B. aegyptius (Koch-Weeks' bacillus). Its cultural characters and morphology. J. roy. micr. Soc. Ser. II **46**, 112 (1926).

ALLIBONE, E. C., P. R. ALLISON and K. ZINNEMANN: Significance of H. influenzae in bronchiectasis of children. Brit. med. J. **1956 I**, 1457.
— J. D. PICKUP, and K. ZINNEMANN: Haemophilus influenzae meningitis treated with streptomycin. Difficulties in diagnosis. Lancet **1951 I**, 610.
ALLISON, P. R., J. GORDON and K. ZINNEMANN: The incidence and significance of H. influenzae in chronic bronchiectasis. J. Path. Bact. **55**, 465 (1943).
ANDERSON, D. G., and M. JEWELL: The absorption, excretion and toxicity of streptomycin in man. New Engl. J. Med. **233**, 485 (1945).
APPELBAUM, E., and J. NELSON: Streptomycin in the treatment of influenzal meningitis. J. Amer. med. Ass. **143**, 715 (1950).
AXENFELD, T.: Bacteriology of the eye, pp. 133—157. Transl. by A. Macnab. London: Baillière, Tindall and Cox 1908.
BALLON, H., J. J. SINGER and E. A. GRAHAM: Bronchiectasis. J. thorac. Surg. **1**, 154, 296, 397, 502 (1931/32).
BARACH, A. L., H. A. BICKERMAN and G. J. BECK: Antibiotic therapy in infections of the respiratory tract. Arch. intern. Med. **90**, 808 (1952).
BENJAMIN, E. L.: Case of influenzal bacteremia in child eight months old. Arch. Pediat. **48**, 340 (1931).
BENSTEAD, J. G.: The flora of 100 bronchial secretions. Lancet **1950 I**, 206.
BERENBERG, W., and S. KEVY: Acute epiglottitis in childhood. A serious emergency readily recognized at the bedside. New Engl. J. Med. **258**, 870 (1958).
BERGMAN, S., and H. COLLDAHL: Sputumdiagnosis in asthma bronchiale with special reference to the occurrence of a complicating bronchial infection. Acta allerg. (Kbh.) **10**, 63 (1956).
— — and E. NILSSON: Bronchial infection and B.S.R. in asthma with a description of a method for sterile removal of bronchial secretion. Acta allerg. (Kbh.) 8, 163 (1955).
BIRMINGHAM, J. R., R. KAYE and M. H. D. SMITH: Streptomycin in the treatment of influenzal meningitis. J. Pediat. **29**, 1 (1946).
BJUGGREN, G.: Some factors affecting duration and relapse rate in otitis and sinusitis in children. Acta oto-laryng. (Stockh.) Suppl. **95** (1950).
— S. KRAEPELIN, J. LIND and G. TUNEVALL: Occult sinusitis in children. Acta oto-laryng. (Stockh.) **42**, 287 (1952).
—, and G. TUNEVALL: The Pfeiffer bacillus in otitis in children. Acta oto-laryng. (Stockh.) **38**, 130 (1950).
— — Otitis in childhood. A clinical and serobacteriological study with special reference to the significance of Haemophilus influenzae in relapses. Acta oto-laryng. (Stockh.) **42**, 311 (1952).
BLAŠKOWIČ, D.: Über experimentelle Möglichkeiten mit dem H. influenzae Pfeiffer b Typus. Zbl. Bakt., I. Abt. Orig. **149**, 202 (1942).
BOECKER, E., and F. KAUFFMANN: Bakteriologische Diagnostik, p. 149. Berlin: Springer 1931.
BOGGS, T. R.: The influenza bacillus in bronchiectasis. Amer. J. med. Sci. **130**, 902 (1905).
BOISVERT, P. L.: Familial epidemiology of Haemophilus influenzae, type b, infections. Amer. J. Dis. Child. **75**, 427 (1948).
BOSSERT, O., und B. LEICHTENTRITT: Influenzabazillus und chronische Lungenerkrankungen im Kindesalter. Dtsch. med. Wschr. **47**, 150 (1921).
BRADFORD, J. R., E. F. BASHFORD and J. A. WILSON: The filter passing virus of influenza, together with an appendix of clinical notes on the cases of influenza from which the virus is recovered (by F. CLAYTON). Quart. J. Med. **12**, 259 (1918/19).
BRAID, F., and R. B. MEYER: Penicillin in the treatment of influenzal meningitis. Brit. med. J. **1949 II**, 11.
BROWN, C. C., M. B. COLEMAN, R. D. ALLEY, A. STRANAHAN and C. H. STUART-HARRIS: Chronic bronchitis and emphysema: Significance of bacterial flora in the sputum. Amer. J. Med. **17**, 478 (1954).
— H. M., and R. N. WILSON: Chronic bronchitis in industry. An account of a trial of H. influenzae vaccine. Brit. med. J. **1959 I**, 263.
BRÜCKNER, G., W. GAEHTGENS u. H. VOGT: Zur Bakteriologie der Respirationserkrankungen im Kindesalter. II. Jb. Kinderheilk. **76**, 417 (1912).

Brumfitt, W., and M. L. N. Willoughby: Laboratory differentiation of chronic bronchial disease. Lancet **1958 I**, 132.

— — and Bromley: An evaluation of sputum examination in chronic bronchitis. Lancet **1957 II**, 1306.

Buchanan, J., W. W. Buchanan, A. G. Melrose, J. B. McGuiness and A. U. Price: Long-term prophylactic administration of tetracycline to chronic bronchitics. Lancet **1958 II**, 719.

Cabot, R. C., and F. M. Painter: Septic fever and secondary anaemia. New Engl. J. Med. **200**, 832 (1929).

Camps, F. E.: Acute epiglottitis (acute supraglottitis). Proc. roy. Soc. Med. **46**, 281 (1953).

Carabelle, R. W., D. D. Mitchell and G. W. Salmon: Influenzal meningitis treated with chloromycetin. J. Pediat. **37**, 37 (1950).

Chain, E., and E. S. Duthie: Bactericidal and bacteriolytic action of penicillin on the staphylococcus. Lancet **1945 I**, 652.

Chandler, C. A., and H. L. Hodes: Aureomycin in the treatment of experimental and clinical infections with H. influenzae, type b. Pediatrics **5**, 267 (1950).

Chapman, O. D., and W. Osborne: Serological relationships between Diplococcus pneumoniae and Hemophilus influenzae. J. Bact. **44**, 620 (1942).

Cheng, K.-K.: The experimental production of bronchiectasis in rats. J. Path. Bact. **67**, 89 (1954).

Cherniak, N. S., K. L. Vosti, H. F. Dowling, M. H. Lepper and G. G. Jackson: Long-term treatment of bronchiectasis and chronic bronchitis. Arch. intern. Med. **103**, 345 (1959).

Clarke, J. M.: A case of general infection by the influenza bacillus. Lancet **1912 I**, 1465.

Cohen: La méningite cérébro-spinale septicémique. Ann. Inst. Pasteur **23**, 273 (1909).

Cooper, A. W.: Some observations on the infective factor in chronic, non-specific respiratory disease of adults, with special reference to Haemophilus influenzae. Ph. D. thesis, The University of Leeds 1959.

Crowe, S. J., and W. S. Thacker-Neville: The influenza bacillus in paranasal sinus infections. Bull. Johns. Hopk. Hosp. **30**, 322 (1919).

Crowell, J., and S. D. Loube: Primary Haemophilus influenzae pneumonia. Report of four cases. Arch. intern. Med. **93**, 921 (1954).

Cummins, S. L.: Introduction. In: Studies of influenza in hospitals of the British armies in France, 1918, pp. 6—17. Special Report Series No 36. Medical Research Committee. London: H. M. Stationery Office 1919.

Davis, D. J.: Food accessory factors (vitamins) in bacterial culture with special reference to haemophilic bacilli. I. J. infect. Dis. **21**, 392 (1917).

— (1) Food accessory factors in bacterial growth. III. Further observations on the growth of Pfeiffer's bacillus (B. influenzae). J. infect. Dis. **29**, 171 (1921).

— (2) The accessory factors in bacterial growth. IV. The satellite or symbiosis phenomenon of Pfeiffer's bacillus (B. influenzae). J. infect. Dis. **29**, 178 (1921).

— (3) The accessory factors in bacterial growth. V. The value of the satellite (or symbiosis) phenomenon for the classification of hemophilic bacteria. J. infect. Dis. **29**, 187 (1921).

—, and M. Pittman: Effectiveness of streptomycin in treatment of experimental conjunctivitis caused by Haemophilus sp. Amer. J. Ophthal. **32**, part II, 111 (1949).

— — and J. J. Griffits: Hemagglutination by the Koch-Weeks bacillus (H. aegyptius). J. Bact. **59**, 427 (1950).

— H. V.: Obstructive laryngitis in children caused by Hemophilus influenzae bacillus type b. J. Kans. med. Soc. **48**, 105 (1947).

Dawson, B., and K. Zinnemann: Incidence and type distribution of capsulated H. influenzae strains. Brit. med. J. **1952 I**, 740.

Demerec, M.: Origin of bacterial resistance to antibiotics. J. Bact. **56**, 63 (1948).

Domagk, G.: Die experimentellen Grundlagen für eine kombinierte Anwendung von Sulfonamiden und Penicillin. Dtsch. med. Wschr. **77**, 1591 (1952).

Douglas, A. C., A. R. Somner, B. L. Marks and I. W. B. Grant: Effect of antibiotics on purulent sputum in chronic bronchitis and bronchiectasis. Lancet **1957 II**, 214.

Drake, M. E., J. E. Bradley, J. Imburg, F. R. McCrumb and T. E. Woodward: Aureomycin in the treatment of influenzal meningitis. J. Amer. med. Ass. **142**, 463 (1950).

DRYSDALE, C. F., D. G. MCINTOSH and J. BRODIE: Meningitis due to a penicillin-sensitive, sulphonamide-insensitive Pittman b strain of H. influenzae: Recovery. Brit. med. J. **1946 II**, 223.

DUBOIS, P. G., and C. A. ALDRICH: Hemophilus influenzae type b laryngitis with bacteremia. J. Pediat. **23**, 184 (1943).

DUBOS, J. R.: A soluble toxin produced by Hemophilus influenzae. J. Bact. **43**, 77 (1942).

DUCREY, A.: Recherches expérimentales sur la nature intime du principe contagieux du chancre mou. Ann. Derm. Syph. (Paris), III. sér., **1**, 56 (1890).

DURANT, T. M., A. J. SOKALCHUK, C. M. NORRIS and C. L. BROWN: Streptomycin therapy in Hemophilus influenzae pulmonary infections. J. Amer. med. Ass. **131**, 194 (1946).

DUVAL, C. V., and W. H. HARRIS: The antigenic property of the Pfeiffer bacillus as related to its value in the prophylaxis of epidemic influenza. J. infect. Dis. **25**, 384 (1919).

EATON, F. D., E. C. GERWE and G. F. LEONARD: Production of anti-haemophilus influenzae type b rabbit serum. J. Immunol. **59**, 165 (1948).

EDWARDS, G., A. R. BUCKLEY, E. C. FEAR, G. M. WILLIAMSON and K. ZINNEMANN: Adult chronic bronchitis — the infective factor and its treatment. Brit. med. J. **1957 II**, 259.

ELEK, S. D., and G. R. F. HILSON: Combined agar diffusion and replica plating techniques in the study of antibacterial substances. J. clin. Path. **7**, 37 (1954).

— — and P. JEWELL: Laboratory aspects of combined antibiotic treatment. Brit. med. J. **1953 II**, 1298.

ELKELES, G.: Zur Influenzafrage. (Mit besonderer Berücksichtigung des Verhältnisses zur croupösen Pneumonie). Z. Hyg. Infekt.-Kr. **98**, 135 (1922).

— Die Bedeutung neuzeitiger bakteriologischer Untersuchungsverfahren für die ärztliche Praxis: Lungenkrankheiten. Schweiz. med. Wschr. **61**, 677 (1931).

— Die ätiologische Bedeutung der Influenzabazillen für die Entstehung von Bronchiektasien. Schweiz. med. Wschr. **67**, 356 (1937).

ELMES, P. C., C. M. FLETCHER and A. A. C. DUTTON: Prophylactic use of oxytetracycline for exacerbations of chronic bronchitis. Brit. med. J. **1957 II**, 1272.

— K. KNOX and C. M. FLETCHER: Sputum in chronic bronchitis. Effects of antibiotics. Lancet **1953 II**, 903.

ENGBAEK, H. C.: Pfeiffer meningitis treated with streptomycin, sulphonamide and type-specific rabbit immune serum. Acta med. scand. **131**, 146 (1948).

— Undersøgelser over Pfeiffers bacil. Thesis Copenhagen 1949; quoted from G. TUNEVALL(3) 1952.

— (1) Pfeiffer's bacillus. Serological studies with special reference to capsular antigens. Acta path. microbiol. scand. **27**, 378 (1950).

— (2) The phenomenon of iridescence in bacterial cultures, with particular reference to Pfeiffer's bacillus. Acta path. microbiol. scand. **27**, 388 (1950).

ENGERING, P.: Meningitis durch influenzaähnliche Bazillen. Zbl. Bakt., I. Abt. Orig. **90**, 21 (1923).

ERMATINGER, L. H.: Microorganisms of lung abscess and bronchiectasis. J. infect. Dis. **43**, 391 (1928).

EVERALL, P. H.: A plate method for demonstrating the growth factor requirements of the genus Haemophilus. J. med. Lab. Technol. **11**, 181 (1953).

FAIRBROTHER, R. W., and B. L. WILLIAMS: Two new antibiotics. Antibacterial activity of novobiocin and vancomycin. Lancet **1956 II**, 1177.

FANCONI, G.: Die pseudoluetische, subakute hilifugale Bronchopneumonie des heruntergekommenen Kindes. Schweiz. med. Wschr. **66**, 821 (1936).

FIESSINGER, N.: Les infections dues au coccobacille de Pfeiffer. In: Nouveau traité de Médecine et de Thérapeutique, p. 337. Paris: Baillière & Fils 1922.

—, et M. ALBEAUX-FERNET: Deux nouveaux cas d'endocardite maligne bute à bacille de Pfeiffer. Bull. Soc. méd. Hôp. Paris **49**, 391 (1933).

—, et A. ARNANDET: Endocardite maligne à forme lente à bacille de Pfeiffer. Bull. Soc. méd. Hôp. Paris **48**, 629 (1932).

FILDES, P.: New medium for growth of B. influenzae. Brit. J. exp. Path. **1**, 129 (1920).

— The nature of the effect of blood-pigment upon the growth of B. influenzae. Brit. J. exp. Path. **2**, 16 (1921).

FILDES, P.: The nature of the action of potato upon the growth of B. influenzae. Brit. J. exp. Path. **3**, 210 (1922/23).
— The classification of haemoglobinophilic bacteria, based upon their relation to blood-pigment and to the „vitamine" factor. Brit. J. exp. Path. **4**, 265 (1923/24).
— The growth requirements of haemolytic influenza bacilli and the bearing of these upon the classification of related organisms. Brit. J. exp. Path. **5**, 69 (1924/25).
FINLAND, M., and C. WILCOX: In vitro susceptibility of Hemophilus influenzae to seven antibiotics. Amer. J. clin. Path. **20**, 335 (1950).
FISHER, A. J., and E. B. SHAW: Streptomycin treatment of empyema caused by H. influenzae. Amer. J. Dis. Child. **74**, 468 (1947).
FLEMING, A.: On the antibacterial action of cultures of penicillium with special reference to their use in the isolation of B. influenzae. Brit. J. exp. Path. **10**, 226 (1929).
— On specific antibacterial properties of penicillin and potassium tellurite; incorporating method of demonstrating some bacterial antagonisms. J. Path. Bact. **35**, 831 (1932).
— Selective bacteriostasis. Report Proc. 2nd Internat. Congr. Microbiol., London 1937, p. 33.
—, and I. H. MACLEAN: On the occurrence of influenza bacilli in the mouths of normal people. Brit. J. exp. Path. **11**, 127 (1930).
FOLEY, G. E., H. SHWACHMAN, M. MCGARRY and W. D. WINTER: Morphologic characteristics and antibiotic resistance of Haemophilus influenzae. Amer. J. Dis. Child. **78**, 659 (1949).
FORGACS, P., I. R. HUTCHINSON and R. E. REWELL: Penicillin sensitivity of Haemophilus influenzae. Two sensitive pathogenic strains. Lancet **1945 I**, 785.
FOTHERGILL, L. D.: Haemophilus influenzae (Pfeiffer bacillus) meningitis and its specific treatment. New Engl. J. Med. **216**, 587 (1937).
—, and J. WRIGHT: Influenzal meningitis. Relation of age incidence to bactericidal power of blood against causal organism. J. Immunol. **24**, 273 (1933).
FRÄNKEL, E.: Bakteriologische Befunde bei Grippe. Dtsch. med. Wschr. **44**, 1422 (1918).
FRANK, H.: Influenzabazillen-Endocarditis unter dem Bilde der Endocarditis lenta. Münch. med. Wschr. **78**, 1509 (1931).
FRANKLIN, A. W., and L. P. GARROD: Chloramphenicol treatment of bronchiectasis in children. Brit. med. J. **1953 II**, 1067.
FRIEDBERGER, E.: Über ein neues zur Gruppe des Influenzabacillus gehöriges hämoglobinophiles Bakterium (Bacillus haemoglobinophilus canis). Zbl. Bakt., I. Abt. Orig. **33**, 401 (1902/03).
FRISK, A. R., G. HAGERMAN, S. HELANDER and B. SJÖGREN: „Sulpha-combination" — a new chemotherapeutic principle. Brit. med. J. **1947 I**, 7.
GARDNER, H. L., and C. D. DUKES: Haemophilus vaginalis vaginitis. Amer. J. Obstet. Gynec. **69**, 962 (1955).
GARROD, L. P.: Combined chemotherapy in bacterial infections. Brit. med. J. **1953 I**, 953.
GIBSON, H. G., F. B. BOWMAN and J. I. CONNOR: The etiology of influenza: A filtrable virus as the cause. With some notes on the culture of the virus by the method of Noguchi, pp. 19—36. Special Report Series No 36, Medical Research Committee. London: H. M. Stationery Office 1919.
GILDER, H., and S. GRANICK: Studies on hemophilus group of organisms; quantitative aspects of growth on various porphyrin compounds. J. gen. Physiol. **31**, 103 (1947).
GILES, C., and E. M. SHUTTLEWORTH: Post-mortem findings in 46 influenza deaths. Lancet **1957 II**, 1224.
GOOD, P. G., M. D. FOUSEK, M. F. GROSSMAN and P. L. BOISVERT: Study of familial spread of Hemophilus influenzae, type B. Yale J. Biol. Med. **15**, 913 (1943).
GOODIER, T. E. W., and W. R. PARRY: Sensitivity of clinically important bacteria to six common antibacterial substances. Lancet **1959 I**, 356.
GORDON, J., H. E. DE C. WOODCOCK and K. ZINNEMANN: Meningitis due to Pittman and non-Pittman strains of H. influenzae. Brit. med. J. **1944 I**, 779.
— M., and K. ZINNEMANN: The in-vitro sensitivity of H. influenzae to penicillin with special reference to meningeal strains of Pittman's type b. Brit. med. J. **1945 II**, 795.
GOSLINGS, W. R. D., and J. F. P. HERS: Studies on the action of the hydroiodide of diethylamino-ethylester of benzylpenicillin. Acta med. scand. **146**, 127 (1953).

GOTTLIEB, B., C. C. FORSYTH and E. N. ALLOTT: H. influenzae meningitis in infants treated with penicillin and sulphonamides. Lancet **1947 II**, 164.

GRASSBERGER, R.: Beiträge zur Bakteriologie der Influenza. Z. Hyg. Infekt.-Kr. **25**, 453 (1897).

GREEN, R., D. S. MANKIKAR and J. S. MILLETT: Influenzal meningitis treated with chloramphenicol. Brit. med. J. **1950 II**, 1154.

GREEY, P. H.: The bacteriology of bronchiectasis. An analysis based on nine cases in which lobectomy was done. J. infect. Dis. **50**, 203 (1932).

GREKOWITZ, G.: Über ein gramnegatives Stäbchen aus der Gruppe der hämoglobinophilen Bakterien (Bacterium haemophilum mucosum). Zbl. Bakt., I. Abt. Orig. **112**, 143 (1929).

GRIGSBY, M. E., T. H. HAIGHT and M. FINLAND: Clinical and laboratory observations on the hydriodide of diethylaminoethylester of penicillin G. New Engl. J. Med. **247**, 807 (1952).

GUNNISON, J. B., V. R. COLEMAN and E. JAWETZ: Interference of aureomycin and of terramycin with the action of penicillin in vitro. Proc. Soc. exp. Biol. (N.Y.) **75**, 549 (1950).

GUYTON, J. S.: Effects of sulfanilamide and sulfapyridine on the Koch-Weeks bacillus (Haemophilus influenzae). Arch. Ophthal. (Chicago) **23**, 1243 (1940).

HAMMERSCHMIDT, J.: Über den Erreger der Koch-Weeksschen Konjunktivitis. Münch. med. Wschr. **68**, 1246 (1921).

HARPER, G. J., and W. C. CAWSTON: The in-vitro determination of the sulphonamide sensitivity of bacteria. J. Path. Bact. **57**, 59 (1945).

HARRIS, M. S., L. GORNELL, C. SHORE and H. LOVITZ: Chloramphenicol in the control of bronchopulmonary suppuration. Dis. Chest. **21**, 450 (1952).

HEGGLIN, R., and A. GRUMBACH: The Wassermann positive infiltration of the lungs. Schweiz. med. Wschr. **71**, 578 (1941).

HELM, W. H., J. R. MAY and J. L. LIVINGSTONE: Long-term oxytetracycline (terramycin) therapy in advanced chronic respiratory infections. Lancet **1954 II**, 630.

— — — Long-term oxytetracycline (terramycin) therapy in advanced chronic respiratory infections. Lancet **1956 I**, 775.

HENRY, H.: On meningitis due to haemophilic organisms, so called influenzal meningitis. J. Path. Bact. **17**, 174 (1912/13).

HERS, J. F. P., W. R. O. GOSLINGS, N. MASUREL and J. MULDER: Death from Asiatic influenza in the Netherlands. Lancet **1957 II**, 1164.

HERTZOG, A. J., I. L. CAMERON and A. E. KARLSTROM: Influenzal meningitis in brothers. J. Amer. med. Ass. **124**, 502 (1944).

HEWITT, W. L., and M. PITTMAN: Antibacterial action of penicillin, penicillin X and streptomycin on Hemophilus influenzae. Pbl. Hlth Rep. (Wash.) **61**, 768 (1946).

HIRSCH, C.: Influenzabazillen bei Erkrankungen des Ohres. Z. Ohrenheilk. **66**, 193 (1912).

— W., u. E. NASSAU: Influenza-Bazillen-Infektionen und Penicillin (Meningitis, Empyema, Osteomyelitis). Ann. paediat. (Basel) **168**, 289 (1947).

HOLT, L. E.: The bacteriology of acute infections of the respiratory tract in children, with especial reference to influenza. Arch. intern. Med. **5**, 449 (1910).

HORSFALL, F. L., K. GOODNER and C. M. MACLEOD: Type-specific antipneumococcus rabbit serum. Science **84**, 579 (1936).

HOYLE, L.: The mouse-pathogenic bacteria (pneumococcus, B. influenzae etc.) of the upper respiratory tract: Their relation to acute infectious coryza. J. Path. Bact. **35**, 817 (1932).

HOYNE, A. L., and R. H. BROWN: Intrathecal therapy not required for H. influenzae meningitis. A report of 28 cases. J. Amer. med. Ass. **136**, 597 (1948).

—, and E. R. RIFF: Terramycin therapy for meningitis. J. Pediat. **39**, 151 (1951).

IVANOVICS, G., u. C. IVANOVICS: Beiträge zur Kenntnis des „Bacterium influenzae murium" (Kairies and Schwartzer). Zbl. Bakt., I. Abt. Orig. **139**, 184 (1937).

JACKSON, C., and C. L. JACKSON: Bronchiectatic septic tank; its prophylaxis and treatment. Amer. Rev. Tuberc. **30**, 599 (1934).

JAWETZ, E., and J. B. GUNNISON: Studies on antibiotic synergism and antagonism: Scheme of combined antibiotic action. Antibiot. and Chemother. **2**, 243 (1952).

— — and V. R. COLEMAN: Combined action of penicillin with streptomycin or chloromycetin on enterococci in vitro. Science **111**, 254 (1950).

JAWETZ, E., J. B. GUNNISON and R. S. SPECK: Studies on antibiotic synergism and antagonism: The interference of aureomycin, chloramphenicol and terramycin with the action of streptomycin. Amer. J. med. Sci. **222**, 404 (1951).

JENSEN, K. A., P. J. DRAGSTED and I. KIAER: Undersøgelser af penicillinpraeparater og dos erringsmäder — II. Ugeskr. Laeg. **112**, 1075 (1950).

JOCHMANN, G.: Über das fast constante Vorkommen influenzaähnlicher Bacillen im Keuchhustensputum. Z. Hyg. Infekt.-Kr. **44**, 498 (1903).

—, u. P. KRAUSE: Zur Ätiologie des Keuchhustens. Z. Hyg. Infekt.-Kr. **36**, 193 (1901).

JOHNSTONE, B., S. SOLOMON and J. M. VOGEL: The antibacterial range of erythromycin. N.Y. St. J. Med. **55**, 3457 (1955).

JONES, H. M., and F. E. CAMPS: Acute epiglottitis: Supraglottitis. Practitioner **178**, 223 (1957).

JOULES, H.: A preventive approach to common diseases of the lung. Brit. med. J. **1954 II**, 1259.

— Symposium: Chronic bronchitis. Proc. roy. Soc. Med. **49**, 779 (1956).

KAIRIES, A., und K. SCHWARTZER: Studien zu einer bakteriellen Influenza der Mäuse und Beschreibung eines „Bacterium influenzae murium". Zbl. Bakt., I. Abt. Orig. **137**, 351 (1936).

KARTULIS, A.: Zur Ätiologie der ägyptischen catarrhalischen Conjunctivitis. Zbl. Bakt. **1**, 289 (1887).

KEEFER, C. S.: Streptomycin in the treatment of infections. J. Amer. med. Ass. **132**, 4 (1946).

—, and C. H. RAMMELKAMP: Hemophilus influenzae bacteremia. Report of two cases. Ann. intern. Med. **16**, 1221 (1942).

KHAIRAT, O.: Endocarditis due to new species of Haemophilus. J. Path. Bact. **50**, 497 (1940).

KITASATO, S.: Über den Influenzabazillus und sein Culturverfahren. Dtsch. med. Wschr. **18**, 28 (1892).

KLEIN, M., and L. J. KIMMELMAN: Correlation between inhibition of drug resistance and synergism in streptomycin and penicillin. J. Bact. **54**, 363 (1947).

KLIENEBERGER, C.: Über hämophile Bacillen. Dtsch. med. Wschr. **31**, 575 (1905).

KLINKE, K.: Influenzabazilleninfektion auf dem Blutwege. Dtsch. med. Wschr. **50**, 37 (1924).

KNORR, M.: (1) Untersuchungen über den Erreger der ägyptischen Augenentzündung (Koch-Weekssches Bakterium) und seine Beziehungen zum Pfeifferschen Influenzabazillus. I. Mitteilung. Zbl. Bakt., I. Abt. Orig. **92**, 371 (1924).

— (2) Untersuchungen über den Erreger der ägyptischen Augenentzündung (Koch-Weekssches Bakterium) und seine Beziehungen zum Pfeifferschen Influenzabazillus. II. Ansprüche an Nährböden. Zbl. Bakt., I. Abt. Orig. **92**, 385 (1924).

— (3) Das Koch-Weekssche Bakterium und der Pfeiffersche Influenzabazillus. Ergebn. Hyg. Bakt. **6**, 350 (1924).

KNOX, K., P. C. ELMES and C. M. FLETCHER: Chronic bronchitis. An attempt to control infection with Haemophilus influenzae by aerosol therapy. Lancet **1955 I**, 120.

KOCH, F. E.: Influenzabakterien-Sepsis. Münch. med. Wschr. **79**, 706 (1932).

— R.: Bericht über die Thätigkeit der deutschen Cholerakommission in Ägypten und Ostindien. Wien. med. Wschr. **33**, 1548 (1883).

—, and M. J. CARSON: Management of Hemophilus influenzae, type b, meningitis. J. Pediat. **46**, 18 (1955).

KOLMER, J. A.: Synergistic or additive activity of chemotherapeutic compounds. Amer. J. med. Sci. **215**, 136 (1948).

KRESKY, P. J.: Suppurative pericarditis due to Haemophilus influenzae, type b; characteristic syndrome ushered in by symptoms of croup. Amer. J. Dis. Child. **65**, 305 (1943).

KRISTENSEN, M.: Investigations into the occurrence and classification of the haemoglobinophilic bacteria, pp. 272 seq. Copenhagen: Levin & Munksgaard 1922.

LANDAU: Clinicopathological conference: Willard Parker Hospital, New York. Arch. Pediat. **62**, 282 (1945).

Leading article: Haemophilus meningitis. Lancet **1952 I**, 1003.

— Aetiology of chronic bronchitis. Brit. med. J. **1956 I**, 906.

— Influenza 1889 and 1957. Lancet **1958 I**, 833.

LECHNER, A., u. A. BOETZEL: Über Influenzabazillensepsis. Münch. med. Wschr. **74**, 1182 (1927).

LEDERBERG, J., and E. M. LEDERBERG: Replica plating and indirect selection of bacterial mutants. J. Bact. **63**, 399 (1952).

LEHR, D.: Prevention of renal damage by the use of mixtures of sulphonamides; animal-experimental and clinical studies. Brit. med. J. **1947 II**, 943.

LEICHTENTRITT, B., u. H. SCHOBER: Influenzabazillensepsis mit rezidivierendem Exanthem. Klin. Wschr. **3**, 1092 (1924).

LEIDY, G., E. HAHN and H. E. ALEXANDER: In-vitro production of new types of Hemophilus influenzae. J. exp. Med. **97**, 467 (1953).

LEMIERRE, A., A. MEYER et R. LAPLANE: Les septicémies à bacille de Pfeiffer. Ann. Méd. **39**, 97 (1936).

LEOPOLD, J. G., and J. GOUGH: The centrilobular form of hypertrophic emphysema and its relation to chronic bronchitis. Thorax **12**, 219 (1957).

LEPPER, M. H., P. F. WEHRLE and N. BLATT: Treatment of Hemophilus influenzae meningitis. Comparison of aureomycin alone versus aureomycin, streptomycin and gantrisin. Amer. J. Dis. Child. **83**, 763 (1952).

LEVINTHAL, W.: Bakteriologische und serologische Influenzastudien. Z. Hyg. Infekt.-Kr. **86**, 1 (1918).

— u. H. FERNBACH: Morphologische Studien an Influenzabazillen und das ätiologische Grippeproblem. Z. Hyg. Infekt.-Kr. **96**, 456 (1922).

LEWIS, P. A., and R. E. SHOPE: Swine influenza. II. A haemophilic bacillus from the respiratory tract of infected swine. J. exp. Med. **54**, 361 (1931).

LINDAU, A.: Transitory, non-specific, strong Wassermann-Kahn reactions; report of occurrence in 4 members of same family. Acta chir. scand. **82**, 355 (1939).

LINDSAY, J. W., E. C. RICE and M. A. SELINGER: Treatment of meningitis due to Hemophilus influenzae (Pfeiffer's bacillus); review of 108 cases. J. Pediat. **17**, 220 (1940).

LISTER, F. S.: The bacteriology of epidemic influenza on the Witwatersrand. Med. J. S. Afr. **14**, 290 (1918).

—, and E. TAYLOR: Experimental investigation of epidemic influenza at Durban. Publ. S. Afr. Inst. med. Res. No **12**, 9 (1919).

LOPEZ-BELIO, M., Y. TAKIMURA, E. J. FORNATTO and P. H. HOLINGER: Erythromycin in the bronchial tree after oral, intravenous, and aerosol administration. Antibiot. Ann. **1956/57**, 152.

LORD, F. T.: Infections of the respiratory tract with influenza bacilli and other organisms, their clinical and pathological similarity, and confusion with tuberculosis. Boston med. surg. J. **152**, 537, 574 (1905).

LOVE, B. DEL, and M. FINLAND: Susceptibility of recently isolated strains of Hemophilus influenzae to eleven antibiotics in vitro. J. Pediat. **45**, 531 (1954).

LUETSCHER, J.: A bacteriological and clinical investigation of the non-tuberculous infections of the respiratory tract, with special reference to sputum cultures as a means of diagnosis. Arch. intern. Med. **16**, 657 (1915).

LUNDGREN, N.: Comments during discussion. Acta oto-laryng. (Stockh.) Suppl. **95**, 210 (1950).

LWOFF, A.: Revision et démembrement des Haemophilae. Le genre Moraxella nov. gen. Ann. Inst. Pasteur **62**, 168 (1939).

—, and M. LWOFF: (1) Studies on codehydrogenase. Nature of growth factor „V". Proc. roy. Soc. B **122**, 253 (1937).

— — (2) Studies on codehydrogenase: Physiological function of growth factor „V". Proc. roy. Soc. B **122**, 360 (1937).

— — (3) Rôle physiologique de l'hémine pour Haemophilus influenzae Pfeiffer. Ann. Inst. Pasteur **59**, 129 (1937).

MACKIE, T. J., C. E. VAN ROOYEN and E. GILROY: An epizootic disease occurring in a breeding stock of mice. Bacteriological and experimental observations. Brit. J. exp. Path. **14**, 132 (1933).

MACPHERSON, C. F. C., M. HEIDELBERGER, H. E. ALEXANDER and G. LEIDY: The specific polysaccharides of Haemophilus influenzae types a, b, c, d and f. J. Immunol. **52**, 207 (1946).

MARGOLIS, J.: Haemolytic anaemia in Haemophilus influenzae meningitis treated with specific rabbit antiserum. Med. J. Aust. **1955 I**, 912.

MARTIN, R., et B. SUREAU: Traitement moderne des méningites purulentes aiguës, p. 86. Paris: Flammarion 1948.

MARTINEZ, C., and A. JUAN: Bacteriologic study of 340 cases of purulent meningitis of childhood. Rev. cubana Lab. clin. **2**, 257 (1948).

MAY, J. R.: The bacteriology of chronic bronchitis. Lancet **1952 II**, 1206.

— (1) The bacteriology of chronic bronchitis. Lancet **1953 II**, 534.

— (2) Antibiotics in chronic bronchitis. Lancet **1953 II**, 899.

— Pathogenic bacteria in chronic bronchitis. Lancet **1954 II**, 839.

— The laboratory background to the use of penicillin in chronic bronchitis and bronchiectasis. Brit. J. Tuberc. **49**, 166 (1955).

— Applied bacteriology. In: Recent trends in chronic bronchitis by N. C. OSWALD, pp. 167 to 180. London: Lloyd-Luke 1958.

MCCRUMB, F. R., H. E. HALL, J. IMBURG, A. MERIDETH, R. HELMHOLD, J. y D. BASORA and T. E. WOODWARD: Treatment of Haemophilus influenzae meningitis with chloramphenicol and other antibiotics. J. Amer. med. Ass. **145**, 469 (1951).

MCINTOSH, D. G., and C. F. DRYSDALE: Meningitis due to a penicillin- and sulphonamide-sensitive Pittman b strain of H. influenzae: Recovery. Brit. med. J. **1945 II**, 796.

— J.: Studies in the aetiology of epidemic influenza. Special Report Series No. 63; Medical Research Council. London: H. M. Stationery Office 1922.

MCLEOD, J. W., and J. GORDON: Production of hydrogen peroxide by bacteria. Biochem. J. **16**, 499 (1922).

— A. G. RITCHIE and C. A. DOTTRIDGE: Incidence of infections with Pfeiffer's bacillus before, during and after the 1918 epidemic. Quart. J. Med. **14**, 327 (1921).

MEUNIER, H.: Dix cas de bronchopneumonies infantiles dues au bacille de Pfeiffer (Influenza-bacillus). Arch. gén. Méd. **1**, 129 (1897).

MILLER, A. H.: Hemophilus influenzae type b epiglottitis or acute supraglottic laryngitis in children. Laryngoscope (S. Louis) **58**, 514 (1948).

MORAX, V., and G. W. BEACH: The bacteriology of the different varieties of acute conjunctivitis in general, and of acute contagious conjunctivitis in particular. Arch. Ophthal. (Chicago) (old ser.) **25**, 54 (1896).

MØRCH-LUND, E.: Determination of bacterial resistance to penicillin, sulfathiazole and streptomycin. Acta path. microbiol. scand. **26**, 821 (1949).

MOYES, E. N., and S. Z. KALINOWSKI: Prophylactic chemotherapy in chronic bronchitis. Tubercle (Lond.) **40**, 112 (1959).

—, and R. A. KERSHAW: Long-continued treatment with tetracycline and prednisolone in chronic bronchitis. Lancet **1957 II**, 1187.

MULDER, J.: Haemophilus „influenzae" (Pfeiffer) als ubiquitaire verwekker van acute en chronische etterige bronchitis. Assen: Van Gorcum 1937.

— Haemophilus influenzae (Pfeiffer) as an ubiquitous cause of common acute and chronical purulent bronchitis. Acta med. scand. **94**, 98 (1938).

— Haemophilus influenzae of the respiratory type as a cause of purulent meningitis. J. Path. Bact. **48**, 175 (1939).

— Haemophilus influenzae and influenza virus in relation to bronchitis. J. Path. Bact. **50**, 317 (1940).

— Bacteriology of bronchitis. Proc. roy Soc. Med. **49**, 773 (1956).

— W. R. O. GOSLINGS, M. C. VAN DER PLAS and P. L. CARDOZO: Studies on the treatment with antibacterial drugs of acute and chronic mucopurulent bronchitis caused by Hemophilus influenzae. Acta med. scand. **143**, 32 (1952).

MURRAY, R., L. KILHAM, C. WILCOX and M. FINLAND: Development of streptomycin resistance of Gram-negative bacilli in vitro and during treatment. Proc. Soc. exp. Biol. (N.Y.) **63**, 470 (1946).

MUTCH, N.: Pfeiffer's bacillus (influenzal) meningitis. Lancet **1941 II**, 751.

NASOU, J. R., M. J. ROMANSKY and J. F. BARR: The treatment of Haemophilus influenzae, type b, bacteraemia, and pneumonia with erythromycin. Antibiotics Annual, Medical Encyclopedia, p. 470. New York 1953/54.

NAVASQUEZ, S. DE: Acute laryngitis and septicaemia due to Haemophilus influenzae type b. Brit. med. J. **1942 II**, 187.

NEAL, J. B., H. W. JACKSON and E. APPELBAUM: Meningitis due to influenza bacillus of Pfeiffer (Hemophilus influenzae); study of 111 cases with four recoveries. J. Amer. med. Ass. **102**, 513 (1934).

NEFFSON, A. H.: Acute laryngotracheobronchitis. New York: Grune & Stratton 1949.

NELSON, W. E.: Textbook of pediatrics. Philadelphia & London: W. B. Saunders Company 1954.

NETER, E.: Antigenic relationship between H. influenzae type b and pneumococcus type VI. Proc. Soc. exp. Biol. (N.Y.) **52**, 289 (1943).

— Yeast autolysate: A culture medium for Haemophilus influenzae. Science **106**, 350 (1947).

NIELSEN, J. C.: Studies of the aetiology of acute otitis media. Thesis, Copenhagen 1945; quoted from K. URDAL and P. BERDAL 1949.

— Terramycinbehandling af otitis media. Ugeskr. Laeg. **113**, 1546 (1951).

NOGUCHI, H., and M. COHEN: Bacteriological and clinical studies of an epidemic of Koch-Weeks bacillus conjunctivitis associated with cell inclusion conjunctivitis. J. exp. Med. **22**, 304 (1915).

NYHAN, W. L., D. R. RECTANUS and M. D. FOUSEK: Hemophilus influenzae type b pneumonia. Pediatrics **16**, 31 (1955).

OGILVIE, A. G., and D. J. NEWELL: Chronic bronchitis in Newcastle-on-Tyne. Edinburgh & London: Livingstone 1957.

OLITZKI, A. L., and A. SULITZEANU: Antigen structures of Haemophilus aegyptius and H. influenzae demonstrated by gel precipitation technique of Ouchterlony. Abstr. VIIth Internat. Congr. Microbiol. Stockh., p. 161. Uppsala: Almqvist & Wiksells 1958.

OLSON, O.: Zur Bakteriologie der Influenza. Münch. med. Wschr. **66**, 231 (1919).

— Untersuchungen über den Pfeifferschen Influenzabacillus während der Grippepandemie 1918—1920. Zbl. Bakt., I. Abt. Orig. **84**, 497 (1920).

OPIE, E. L.: Pathological anatomy of influenza. Based chiefly on American and British sources. Arch. Path. (Chicago) **5**, 285 (1928).

— F. G. BLAKE, J. C. SMALL and T. M. RIVERS: Epidemic respiratory disease. The pneumonias and other infections of the respiratory tract accompanying influenza and measles. London: Kimpton 1921.

OSWALD, N., R. A. SHOOTER and M. P. CURWEN: Pneumonia complicating Asian influenza. Brit. med. J. **1958 II**, 1305.

— N. C.: Recent trends in chronic bronchitis. London: Lloyd-Luke 1958.

—, and J. R. MAY: Long-term chemotherapy in chronic bronchitis. Lancet **1956 II**, 814.

OUNSTED, C.: Haemophilus influenzae meningitis treated with streptomycin. Lancet **1949 II**, 639.

— Haemophilus influenzae meningitis. A possible ecological factor. Lancet **1950 I**, 161.

PAINE, T. F., R. MURRAY, H. W. HARRIS and M. FINLAND: Streptomycin in the treatment of certain Gram-negative bacillus infections of the central nervous system. Amer. J. med. Sci. **213**, 676 (1947).

PARK, W. H., and A. W. WILLIAMS: Studies on the aetiology of the pandemic of 1918. Amer. J. publ. Hlth **9**, 45 (1919).

— — and G. COOPER: The result of the use of absorption of agglutinin on the identification of strains of influenza bacilli. Proc. Soc. exp. Biol. (N.Y.) **16**, 120 (1918 to 1919).

PEMBERTON, J., and C. GOLDBERG: Air pollution and bronchitis. Brit. med. J. **1954 II**, 567.

PESCH, K.: Vergleichende Untersuchungen über den Erreger der Koch-Weeksschen Konjunktivitis und das Pfeiffersche Influenzastäbchen. Münch. med. Wschr. **68**, 390 (1921).

PFEIFFER, R.: Vorläufige Mittheilungen über den Erreger der Influenza. Dtsch. med. Wschr. **18**, 28 (1892).

— Die Ätiologie der Influenza. Z. Hyg. Infekt.-Kr. **13**, 357 (1893).

— Zur Ätiologie der diesjährigen Influenzaepidemie. Berl. klin. Wschr. **56**, 118 (1919).

— Die Ätiologie der Influenza. Zbl. Bakt., I. Abt. Orig. **121**, 241 (1931).

PICKETT, M. J., and R. M. STEWART: Identification of hemophilic bacilli by means of the satellite phenomenon. Amer. J. clin. Path. **23**, 713 (1953).

PITTMAN, M.: Variation and type specificity in the bacterial species Hemophilus influenzae. J. exp. Med. **53**, 471 (1931).
— The action of type-specific Hemophilus influenzae antiserum. J. exp. Med. 58, 683 (1933).
— The interrelation of the amount of V-factor and the amount of air necessary for the growth of Hemophilus influenzae type b in certain media. J. Bact. **30**, 149 (1935).
— The protection of mice against Hemophilus influenzae (non-type specific) with sulphapyridine. Publ. Hlth Rep. (Wash.) **54**, 1769 (1939).
— Antibacterial action of several sulfonamide compounds on Hemophilus influenzae type b. Publ. Hlth. Rep. (Wash.) **57**, 1899 (1942).
— A classification of the hemolytic bacteria of the genus Haemophilus: Haemophilus haemolyticus Bergey et al. and Haemophilus parahaemolyticus nov. spec. J. Bact. **65**, 750 (1953).
— Genus IV. Haemophilus, Family V. Brucellaceae, Nom. Nov. In: BERGEY'S Manual of determinative bacteriology, 7th ed., p. 407. London: Baillière, Tindall & Cox 1957.
—, and D. J. DAVIS: Identification of the Koch-Weeks bacillus (H. aegyptius). J. Bact. **59**, 413 (1950).
POVITZKY, O. R.: Immune serum and prontosyl. Combined treatment for protection of the mouse against fatal dose of Haemophilus influenzae. N.Y. St. J. Med. **37**, 1748 (1937).
PRATHER, G. W., and M. H. D. SMITH: Chloramphenicol in the treatment of Hemophilus influenzae meningitis. J. Amer. med. Ass. **143**, 1405 (1905).
PRITCHETT, I. W., and E. G. STILLMAN: The occurrence of bacillus influenzae in throats and saliva. J. exp. Med. **29**, 259 (1919).
PRYM, P.: Erkrankungen der Nasennebenhöhlen und des Mittelohres bei Influenza. Dtsch. med. Wschr. **45**, 880 (1919).
RABE, F.: Infectious croup. III. Hemophilus influenzae type b croup. Pediatrics **2**, 559 (1948).
RAWLINS, G. A.: Liquefaction of sputum for bacteriological examination. Lancet **1953 II**, 538.
REID, D. D.: General epidemiology of chronic bronchitis. Proc. roy. Soc. Med. **49**, 767 (1956).
— Environmental factors in respiratory disease. Lancet **1958 I**, 1237, 1289.
—, and A. S. FAIRBAIRN: The natural history of chronic bronchitis. Lancet **1958 I**, 1147.
— L.: Pathology of chronic bronchitis. Proc. roy. Soc. Med. **49**, 771 (1956).
— L. MCA.: Pathology of chronic bronchitis. Lancet **1954 I**, 275.
REISS, E., und H. A. GINS: Influenza-Bakteriämie. Münch. med. Wschr. **58**, 2211 (1911).
REITLER, R.: Susceptibility to Haemophilus influenzae infection in relation to the age factor. Experientia (Basel) **10**, 22 (1954).
Report: Report on a study of the nasopharyngeal bacterial flora of a group of the Manchester population during the period July, 1925 to Sept. 1927. Min. Hlth. Rep. on pbl. Hlth. med. Subj. No 58. London: H. M. Stationery Office 1930.
— A study of the nasopharyngeal bacterial flora of different groups of persons observed in London and South-East England during the years 1930 to 1937. Min. Hlth. Rep. on pbl. Hlth. med. Subj. No 90. London: H. M. Stationery Office 1939.
— The bacteriological examination of water supplies. Min. Hlth. Rep. on pbl. Hlth. med. Subj. No 71. London: H. M. Stationery Office 1956.
— Prolonged antibiotic treatment of severe bronchiectasis. A report by a subcommittee of the Antibiotics Clinical Trials (Non-Tuberculous) Committee of the Medical Research Council. Brit. med. J. **1957 II**, 255.
RIVERS, T. M.: (1) Influenzal meningitis. Amer. J. Dis. Child. **24**, 102 (1922).
— (2) Influenza-like bacilli: Growth of influenza-like bacilli on media containing only an autoclave-labile substance as an accessory food factor. Bull. Johns Hopk. Hosp. **33**, 429 (1922).
—, and L. A. KOHN: The biological and serological reactions of influenza bacilli producing meningitis. J. exp. Med. **34**, 477 (1921).
—, and A. K. POOLE: Growth requirements of influenza bacilli. Bull. Johns Hopk. Hosp. **32**, 202 (1921).
ROBERTS, G. B. S.: Fulminating influenza. Lancet **1957 II**, 944.

ROGERS, K. B., and K. ZINNEMANN: The recognition of non-capsulated strains of Haemophilus influenzae in unusual sites. Abstr. VIIth Internat. Congr. Microbiol. Stockholm, p. 324. Uppsala: Almqvist & Wiksells 1958.

ROSCOE, J. D., and M. H. GLEESON-WHITE: Haemophilus influenzae meningitis. Four cases treated with streptomycin. Lancet **1948 II**, 885.

ROSENBLATT, P., and B. M. ZWEIFLER: Type f Haemophilus influenzae meningitis. J. Pediat. **38**, 620 (1951).

ROSHER, A. B.: Haemophilus influenzae and its relation to epidemic influenza. Proc. roy. Soc. Med. **40**, 749 (1947).

ROSS, S.: Treatment of pyogenic meningitis in infants and children. Antibiot. and Chemother. **5**, Suppl. 1, 39 (1955).

— H. BISCHOFF, W. PREISSER and W. ORR: Some observations on the absorption and excretion of aureomycin and chloramphenicol. J. clin. Invest. **28**, 1049 (1949).

ROY, T. E., E. KRIEGER, G. CRAIG, D. COHEN, G. A. MCNAUGHTON and N. SILVERTHORNE: Studies on the absorption of chloramphenicol in normal children in relation to the treatment of meningitis. Antibiot. and Chemother. **2**, 505 (1952).

SACQUÉPÉE, M.: Évolution bactériologique d'une épidémie de grippe. Arch. Méd. exp. **13**, 562 (1901).

SANDILANDS, J.: Haemophilus influenzae meningitis. Report of five cases. Lancet **1950 II**, 52.

SCHÄFER, W.: Über H. influenzae. Zbl. Bakt., I. Abt. Orig. **169**, 471 (1957).

SCHIAVONE, D. J., and S. RUBBO: Anaemia associated with Haemophilus influenzae meningitis. Lancet **1953 II**, 696.

SCHOENBACH, E. B., H. C. SPENCER and J. MONNIER: Treatment of H. influenzae meningitis with aureomycin and chloramphenicol. Experience in thirty consecutive cases. Amer. J. Med. **12**, 263 (1952).

SCOTT, J. R., and D. N. WALCHER: Intravenous chloramphenicol in the treatment of meningitis due to Hemophilus influenzae (type b). J. Pediat. **41**, 442 (1952).

— W. M.: The influenza group of bacteria. In: A system of bacteriology, p. 375. Medical Research Council. London: H. M. Stationery Office 1929.

SIMON, G.: Radiological appearances. In: Recent trends in chronic bronchitis by N. C. OSWALD, pp. 63—87. London: Lloyd-Luke 1958.

SINCLAIR, S. E.: Haemophilus influenzae type b in acute laryngitis with bacteraemia. J. Amer. med. Ass. **117**, 170 (1941).

SLAWYK: Ein Fall von Allgemeininfektion mit Influenzabazillen. Z. Hyg. Infekt.-Kr. **32**, 443 (1899).

SMITH, M. H. D., P. E. WILSON and H. L. HODES: The treatment of influenzal meningitis. J. Amer. med. Ass. **130**, 331 (1946).

— W., C. H. ANDREWES and P. P. LAIDLAW: A virus obtained from influenza patients. Lancet **1933 II**, 66.

SMYTHE, P. M.: Haemophilus influenzae meningitis treated with streptomycin. Lancet **1948 II**, 485.

SOMERFORD, A. E.: Fatal case of influenza septicaemia. Lancet **1929 I**, 821.

STIEGLER, C.: Hemophilus influenzae epiglottitis. Clin. Proc. Child. Hosp. (Wash.) **2**, 185 (1946).

STOCKS, P.: Cancer and bronchitis mortality in relation to athmospheric deposit and smoke. Brit. med. J. **1959 I**, 74.

STRAKER, E. A.: Serological typing of meningeal strains of H. influenzae. Lancet **1945 I**, 817.

STUART-HARRIS, C. H., and T. HANLEY: Chronic bronchitis, emphysema and cor pulmonale. Bristol: Wright 1957.

— M. POWNALL, C. M. SCOTHORNE and Z. FRANKS: The factor of infection in chronic bronchitis. Quart. J. Med., new ser. **22**, 121 (1953).

SWIFT, P. N., and S. R. M. BUSHBY: Haemophilus influenzae meningitis treated with polymyxin. Lancet **1951 II**, 183.

THJØTTA, T.: Studies on bacterial nutrition. I. Growth of bacillus influenzae in hemoglobin-free media. J. exp. Med. **33**, 763 (1921).

THJØTTA, T., and O. T. AVERY: (1) Studies on bacterial nutrition. II. Growth accessory substances in the cultivation of hemophilic bacilli. J. exp. Med. **34**, 97 (1921).
— — (2) Studies on bacterial nutrition. III. Plant tissue, as a source of growth accessory substances, in the cultivation of bacillus influenzae. J. exp. Med. **34**, 455 (1921).
THOMAS, J. C., and W. HAYES: The synergic action of penicillin and sulphathiazole on S. typhi. J. Hyg. (Camb.) **45**, 313 (1947).
THOMSON, J., L. M. BRUCE and M. G. GREEN: H. influenzae meningitis in relation to treatment. A clinical study of four cases. Brit. med. J. **1947II**, 414.
THURSFIELD, H.: Influenzal septicaemia, with a short review of the present status of bacillus influenzae. Quart. J. Med. **4**, 7 (1910).
TUNEVALL, G.: Studies on Haemophilus influenzae. Susceptibility in vitro to antibiotics. Acta path. microbiol. scand. **29**, 203 (1951).
— (1) Sero-bacteriological investigations of sinusitis in children. Acta oto-laryng. (Stockh.) **42**, 298 (1952).
— (2) Oto-rhinological infections in childhood. Acta paediat. (Stockh.) Suppl. **92** (1952).
— (3) Studies on Haemophilus influenzae. Type characteristics. Acta path. microbiol. scand. **30**, 203 (1952).
— (1) Studies on Haemophilus influenzae. A complement fixation test for Haemophilus influenzae antibody. Acta path. microbiol. scand. **32**, 258 (1953).
— (2) Studies on Haemophilus influenzae. Haemophilus influenzae antigens studied by the gel precipitation method. Acta path. microbiol. scand. **32**, 193 (1953).
—, and P. HEDENIUS: Laboratory and clinical studies with erythromycin. Antibiot. and Chemother. **4**, 678 (1954).
—, u. J. WASSERMANN: Betydelsen av bakteriefynd i upphostningsprov. Svenska Läk.-Tidn. **53**, 463 (1956).
TURNER, E. K.: Report of a case of resistant Haemophilus influenzae meningitis responding to penicillin. Med. J. Austr. **1**, 205 (1947).
TYTLER, W. H., R. M. JANES and G. M. DOBBIN: Studies of influenza in hospitals of the British Armies in France, 1918. M.R.C. Special Report Series No 36, p. 77. London: H. M. Stationery Office 1919.
UNGAR, J.: Synergistic effect of para-aminobenzoic acid and sulphapyridine on penicillin. Nature (Lond.) **152**, 245 (1943).
URDAL, K., and P. BERGDAL: The microbial flora in 81 cases of maxillary sinusitis. Acta otolaryng. (Stockh.) **37**, 20 (1949).
VALENTINE, E., and G. M. COOPER: On the existence of a multiplicity of races of B. influenzae as determined by agglutination and agglutination absorption. J. Immunol. **4**, 359 (1919).
VOGT, H.: Zur Bakteriologie der Respirationserkrankungen im Kindesalter. I. Jb. Kinderheilk. **73**, 142 (1911).
WALKER, N., R. PHILIP, M. M. SMYTH and J. W. MCLEOD: Observations on the prevention of bacterial growth by sulphonamides, with special reference to the Harper and Cawston effect. J. Path. Bact. **59**, 631 (1947).
WALLER, R. E., and P. J. LAWTHER: Some observations on London fog. Brit. med. J. **1955II**, 1356.
WARBURTON, M. F., E. V. KEOGH and S. W. WILLIAMS: Haemagglutination test for diagnosis of influenzal meningitis. Med. J. Austr. **1949I**, 135.
WEAVER, J. B., and L. SHERWOOD: Hematogenous pyarthrosis due to Bacillus haemophilus influenzae and Corynebacterium xerosis. Surgery 4, 908 (1938).
WEEKS, J. E.: The bacillus of acute conjunctival catarrh, or „pink eye". Arch. Ophthal. (Chicago) (old ser.) **15**, 441 (1886).
— The pathogenic microbe of "acute catarrhal conjunctivitis". Med. Rec. (N. Y.) **31**, 571 (1887).
WEINSTEIN, L.: The treatment of meningitis due to Haemophilus influenzae with streptomycin. A report of nine cases. New Engl. J. Med. **235**, 101 (1946).
WILKES-WEISS, D., and W. HUNTINGTON: The treatment of influenzal meningitis with immune serum. J. Pediat. **9**, 462 (1936).
WILLIAMSON, G. M., and K. ZINNEMANN: The occurrence of two distinct capsular antigens in H. influenzae type e strains. J. Path. Bact. **63**, 695 (1951).

WILLIAMSON, G. M., and K. ZINNEMANN: The degradation of Haemophilus influenzae type e capsular antigens. J. Path. Bact. **68**, 453 (1954).
WILSON, C.: Streptomycin in non-tuberculous infections; summary of report to Medical Research Council. Lancet **1948 II**, 445.
— G. S., and A. A. MILES: Revision of „Topley and Wilson's principles of bacteriology and immunity", 4th ed., vol 1 and 2, pp. 896, 1630, 1861, 1878. London: Arnold 1955.
WOLLSTEIN, M.: Serum treatment of influenzal meningitis. J. exp. Med. **14**, 73 (1911).
— An immunological study of Bacillus influenzae. J. exp. Med. **22**, 445 (1915).
WRIGHT, F. H.: The lungs. In: Holt's Pediatrics, p. 544. New York: Appleton-Century-Crofts 1953.
WYNN-WILLIAMS, N., and E. N. MOYES: Bronchiectasis treated with chloramphenicol. Edinb. med. J. **58**, 503 (1951).
YAMPOLSKY, J., and J. P. JONES: Present treatment of influenzal meningitis. Amer. J. Dis. Child. **78**, 97 (1949).
ZAMENHOF, S., and G. LEIDY: Further studies on poly-ribophosphate and other polysugar-phosphates. Fed. Proc. **13**, 327 (1954).
— — P. L. FITZGERALD, H. E. ALEXANDER and E. CHARGAFF: Polyribophosphate, the type-specific substance of Hemophilus influenzae, type b. J. biol. Chem. **203**, 695 (1953).
ZEPP, H. D., and H. L. HODES: Antigenic relation of type b H. influenzae to type 29 and type 6 pneumococci. Proc. Soc. exp. Biol. (N.Y.) **52**, 315 (1943).
ZINNEMANN, K.: A survey of the outcome of 20 cases of H. influenzae meningitis related to bacterial type. Brit. med. J. **1946 II**, 931.
— Sulphonamide sensitivity of H. influenzae strains, with special reference to the combined use of antibacterial drugs. Brit. med. J. **1950 II**, 705.
— The in-vitro sensitivity of H. influenzae to five different antibiotics. Brit. med. J. **1953 II**, 1069.
— Inhibition of Haemophilus influenzae by seven antibiotics. Table in: Bronchitis, emphysema and cor pulmonale by STUART-HARRIS, p. 199. Bristol: Wright 1957.