

Shortly after her out-patient attendance on January 7, 1956 (five months after starting carbimazole), she first noticed a few spots on her chest, but unfortunately did not bother to report them; on January 18 a definite rash appeared on her arms and legs, and bruises developed at the right elbow, wrist, and ankle, and on both thighs. She then stopped drug therapy, but she still did not report until her routine out-patient appointment on January 21, when she was admitted at once. On admission her temperature was 99° F. (37.2° C.), and she had marked pallor of the skin and mucous membranes. A purpuric rash was present, mainly around the suprasternal notch, the nipples, the dorsal aspect of both elbows, and on both hands. Haematomata were present, as mentioned earlier, and below the right lower eyelid, while petechiae were present in the mouth and right conjunctiva. The fundi were clear. The Hess test was strongly positive with grossly prolonged bleeding and clotting times. There were no other abnormal clinical findings. Table II shows the blood picture on admission and thereafter. Sternal puncture on January 24 showed an aplastic marrow.

TABLE II.—Haematological Findings

Date	Hb	P.C.V.	White Blood Cells			Platelets (per c.mm.)
			Total (per c.mm.)	Differential		
				Polys	Lymphs	
21/1/56	54%	25%	1,500	7%	93%	2,000
23/1/56	67%					
24/1/56	65%	28%	1,700	2%	98%	1,500
26/1/56	59%	25%	3,700		100%	1,400

Chlortetracycline was started with a loading dose of 1 g. followed by 500 mg. every six hours, together with prednisolone, 15 mg. six-hourly. One pint (570 ml.) of blood was transfused on January 22, by which time she was febrile—102° F. (38.9° C.)—and had a sore throat. By the 25th there was some minor clinical improvement, but during that evening further petechiae developed. In the early hours of the 26th a left hemiplegia developed and she died a few hours later.

Post-mortem examination revealed large haemorrhages into the skin, and petechiae on both pleural surfaces, pericardium, and peritoneum were noted. There were free haemorrhages into the lungs and submucosal haemorrhages into the alimentary canal. The immediate cause of death was a massive haemorrhage into the right cerebral hemisphere. Histological examination of ileum, sternum, femur, and vertebral body showed "virtually complete aplasia involving almost all marrow cells with replacement by a few foci of lymphocytes."

### Comment

This case illustrates the importance of the patient's early reporting of any toxic reactions, though we cannot know in this case whether this would have resulted in a happier outcome. It also demonstrates the seriousness of the risk of haemorrhage in marrow aplasia. No other reported serious toxic reaction to carbimazole has occurred more than two months after the beginning of therapy.

I wish to thank Dr. Russell Fraser for advice, assistance, and permission to publish this case; also the department of pathology, Postgraduate Medical School, in particular Dr. J. H. Little, for assistance.

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## SIGNIFICANCE OF *H. INFLUENZAE* IN BRONCHIECTASIS OF CHILDREN

BY

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The incidence of *Haemophilus influenzae* in bronchoscopic aspirations of 100 consecutive cases of bronchiectasis in young people in the Leeds area was reported in 1943 by Allison *et al.* The investigation was based on one examination per patient; *H. influenzae* was found in 63%.

As antibacterial agents became available their activity against capsulated and non-capsulated *H. influenzae* strains was investigated with a view to using them as tools in further investigations and in the therapy of chronic conditions due to *H. influenzae* (Gordon and Zinnemann, 1945; Zinnemann, 1950, 1953; Finland and Wilcox, 1950; Love and Finland, 1954). Franklin and Garrod (1953) reported on the effectiveness of systemic chloramphenicol in reducing the amount of sputum and at the same time eliminating pus and *H. influenzae* from the sputum of bronchiectatic children. Mulder *et al.* (1952), using the full range of antibiotic drugs then available, had previously described similar findings in adults. Results with chloramphenicol equally as good clinically as those obtained by Franklin and Garrod (1953), but without adequate bacteriological examinations, had also been reported by Wynn-Williams and Moyes (1951) and Harris *et al.* (1952).

### Present Investigation

In 1952, therefore, although precise evidence was lacking, it appeared probable that *H. influenzae* played a major part in maintaining chronic inflammatory processes in the bronchial tree, and that it could be suppressed by systematic use of antibacterial agents. We decided to investigate bronchiectatic children to see whether (1) findings identical with those obtained by Allison *et al.* (1943) could be repeated in younger age groups, which presumably represent an earlier stage of the disease, (2) by means of the therapeutic test to try to extend the evidence for the significance of *H. influenzae* in purulent bronchiectasis, and (3) to try to influence the condition therapeutically. Therapeutic results will be the subject of a separate clinical report.

*Patients Investigated.*—The group under investigation consisted of 32 children aged 4–15 years with purulent bronchiectasis, established clinically and radiologically. Before a bronchogram was taken under general anaesthesia, specimens for bacteriological investigation were collected by bronchoscopic aspiration. Apart from using general anaesthesia in place of local analgesia the technique was identical with that described by Allison *et al.* In addition, both maxillary antra were washed out with 5 ml. of physiological saline; the washings were centrifuged at 1,500 r.p.m. for 10 minutes and the deposit examined microscopically and by culture. The bacteriological technique used for culturing was that employed by Allison *et al.* In particular we should like to stress the importance of a very heavy inoculum; we used a ball of mucopus the size of a large pea (about 1–1.5 cm. diameter) which, without any washing

in saline, was thoroughly spread over an area of the culture plates from which secondary spreading was carried out in the usual way. This simple technique makes homogenization of sputum as advocated by May (1953) and Rawlins (1953) superfluous, and, moreover, avoids possible inhibition of organisms by trypsin.

**Sensitivity Tests.**—Strains of *H. influenzae* isolated were tested for their sensitivity to 1, 2, 5, and 10 international units or  $\mu\text{g./ml.}$  respectively of the following antibiotics as they became available in this country: penicillin, streptomycin, chloramphenicol, chlortetracycline, oxytetracycline, tetracycline, and erythromycin. In addition, sensitivity tests were carried out with a standard concentration of 10 mg. per 100 ml. of the following sulphonamides: sulphathiazole, "sulphatriad," sulphamerazine, and sulphadimidine. All sensitivity tests were done by the punch-hole technique on carefully prepared heated (chocolate) 10% horse-blood-agar plates, bearing in mind that sensitivities to sulphonamides might seem to be lower on this medium than on Levinthal agar (Zinnemann, 1950).

#### Therapeutic Test and Treatment

The success of Franklin and Garrod (1953) with chloramphenicol prompted us to use this drug first of all on its own. We found it necessary to give appreciably higher doses of this drug for the following reasons: (1) we failed to clear the sputum speedily and effectively unless we gave 1 g. four times daily; (2) we felt that with high doses the danger of breeding strains of *H. influenzae* and possibly other micro-organisms resistant to the antibiotic was minimized. Good results were obtained with 4 g. daily orally so far as disappearance of pus, reduction of amount of sputum, and elimination of *H. influenzae* were concerned. In view of reports of blood dyscrasias following repeated or prolonged therapy with chloramphenicol, the use of this antibiotic was discontinued. Chlortetracycline, oxytetracycline, tetracycline, and erythromycin given orally in doses of 4 g. daily over a period of several weeks failed to eliminate either pus or *H. influenzae*. However, as soon as an equal amount of one of the sulphonamides exerting good inhibitory action on *H. influenzae in vitro* was added to oxytetracycline, sputa could be rendered mucoid and *H. influenzae*-negative within a reasonably short period. The combination of chlortetracycline with sulphonamides was less effective than oxytetracycline. Later it was found that only 2 g. of erythromycin daily, combined with the same amount of sulphonamide, was sufficient to produce the desired effect.

In one case none of these combinations was able to clear the sputum, but high daily doses of intramuscular penicillin and streptomycin combined with a sulphonamide by mouth were finally effective.

The high daily doses were maintained until there was an appreciable reduction of the amount of sputum and conversion from the purulent *H. influenzae*-positive to the mucoid *H. influenzae*-negative state. The patients were then maintained as long as possible on intermittent treatment—namely, administration of the same high doses of antibacterial drugs on two consecutive days a week. For administrative reasons five of the children could not be included in the treatment schedule. They were reviewed periodically and thus served as controls. Most of the remaining 27 cases served as their own controls, as, for reasons of convenience or expediency, treatment had to be interrupted for longer or shorter periods. As reported by Franklin and Garrod (1953), a relapse to copious purulent *H. influenzae*-positive sputum was a regular occurrence during periods without treatment.

**Bacteriological Control During Therapeutic Test and Treatment.**—So far as possible one sputum specimen was examined microscopically and by culture from each patient on the day before treatment, on the second day of treatment, and a day or two after the second day of treatment, a total of three examinations per patient each week during the whole period of intermittent therapy. A total of 1,354

bronchoscopic aspirations and sputa specimens were examined microscopically and by culture from the 27 treated cases. All sputa for this purpose were collected by the physiotherapist during postural drainage. Microscopical recognition of *H. influenzae* was greatly facilitated by staining sputum smears with Leishman's as well as Gram's strain, as frequently the (Gram-negative) *H. influenzae* bacilli could not be discerned against the pink background or inside pus cells in Gram-stained smears.

**Bacterial Flora in Bronchial Secretions and Sputum.**—In our study (Allison *et al.*, 1943) we excluded, so far as possible, contamination of bronchial aspirates with the flora of the pharynx. In the present investigation it has been possible to compare cultural findings from bronchial secretions with those from sputa collected and cultured as indicated above. Provided the inoculum is copious and the mucopus is not washed in saline prior to plating out, cultural results from sputa are comparable to those from bronchial secretions. We now believe, therefore, that bronchoscopy is not required for bacteriological studies of inflammatory processes in the bronchi.

#### Results

**Incidence of *H. influenzae* in Bronchiectasis.**—*H. influenzae* was the predominant organism in cultures from bronchoscopic aspirations of the 32 children in this investigation. This corresponds to an isolation rate of 84.4% (see Table I), which is some 20% higher than in the three times larger adult series of Allison *et al.* (1943). However, if one takes into consideration the results from the culture of sputum samples before and after bronchoscopy it becomes clear that *H. influenzae* is present in the majority of these specimens. For this reason, we regard all 32 cases as having copious *H. influenzae* in their sputum during most of the time they are without antibacterial treatment—that is, *H. influenzae* is the predominant organism in 100% of the cases in the present investigation.

**Comparison of Microscopical and Cultural Findings.**—Out of a total of 1,354 specimens *H. influenzae* was found on 431 occasions. In 356 (82.6%) *H. influenzae* was recognized both microscopically and culturally. In 75 specimens (17.4%) the organism was cultured but could not be recognized in the smear, while in 14 examinations (3.3%) Gram-negative bacilli and coccobacilli were seen in the smear but could not be cultured. The discrepancies between microscopical and cultural findings are probably due to (a) a small number of Gram-negative bacilli not being clearly recognizable against a background not stainable with a contrast dye, and (b) by deficient batches of medium. From the results it is evident that microscopical examination is inferior to cultural isolation, as was to be expected. Yet we feel we cannot dispense with stained sputum smears, as they give a picture of inflammatory processes in the affected bronchi that cannot be obtained from cultures.

**Types of *H. influenzae* Strains Isolated.**—All cultures of *H. influenzae* isolated were observed carefully for indications of mucoid growth—that is, the presence of capsules. Mucoid cultures were typed with the *H. influenzae* agglutinating sera a-f by slide-agglutination and "capsule-swelling" tests. From two patients capsulated strains were recovered on primary isolation. These belonged to types b and c respectively. During prolonged observation *H. influenzae* type b appeared in one case and was isolated for four consecutive weeks. In another case, *H. influenzae* types d and e were present on two isolated occasions. In none of the cases from which capsulated strains were isolated were there any clinical signs that could have been attributed to the sudden appearance of capsulated strains. Thus the majority of *H. influenzae* strains isolated in this investigation, as in that during 1942-3, belonged to the non-capsulated heterogeneous group of *H. influenzae* usually found in the respiratory tract.

**Numerical Relationship Between the Presence of *H. influenzae* in the Bronchi and in the Maxillary Antra.**—Table I shows that at the time of their first examination

TABLE I.—*H. influenzae* Isolated from Bronchial Secretions and Saline Washings of One or Both Maxillary Antra

No. of Patients	<i>H. influenzae</i>	
	In Bronchial Secretion	In Antrum Washings
32	27* (84.4%)	19 (59.4%)

\* Five patients were free from *H. influenzae* at the time of bronchoscopy, but *H. influenzae* was isolated frequently from sputum before and/or after bronchoscopy.

nearly 60% of the children suffered from *H. influenzae* infection of their maxillary antra as well as of the bronchi. This bacteriological finding confirms the impression, gained clinically and radiologically, that a high proportion of bronchiectatic children suffer from simultaneous chronic infection of the upper respiratory tract. At the present state of our knowledge it is a matter for speculation whether the presence of *H. influenzae* in the antra is the origin of, a sequel to, or a part of the infection of the dilated bronchi with non-capsulated *H. influenzae*. It is conceivable that the invasion of both situations might start with the seasonal spread of this organism and of pneumococci from the nasopharynx upwards into the nose and downwards towards the bronchi (Straker *et al.*, 1939).

*Disappearance of Pus and H. influenzae from the Sputum Under the Influence of Antibacterial Treatment.*—In Table II details are given of the time required to bring about

TABLE II.—Response 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 "	1
5-6 "	1
12 "	1
None (failure)	1
	27

initial response to treatment—that is, conversion of purulent *H. influenzae*-positive to mucoid *H. influenzae*-negative sputum by means of all effective combinations of antibacterial drugs tried. The figures show that successful results can be obtained by persevering with continuous treatment for more than two and up to 12 weeks. The case recorded as a failure, as well as that requiring 12 weeks' continuous treatment, had saccular honeycomb-like bronchiectasis (? cystic lung), and the length or failure of continuous treatment may reasonably be ascribed to insufficient drainage. In 25 of the 27 cases *H. influenzae* could no longer be isolated from the sputum at about the same time as conversion from the purulent to the mucoid state took place. In the one remaining case (apart from the failure) conversion from the purulent to the mucoid state occurred, but microscopically and culturally the number of *H. influenzae* present was only reduced but not eliminated for two weeks before a relapse occurred. Continuous treatment for six weeks finally cleared the sputum by naked-eye appearance, culturally and microscopically. This process of clearance could be followed microscopically in all cases. In smears stained by Gram's and Leishman's methods numerous to abundant Gram-negative coccobacilli, free in the mucus and inside polymorphonuclear leucocytes, with few other organisms, could be seen. Under the influence of antibacterial treatment, intracellular and extracellular *H. influenzae* disappeared at the same time as, or more rapidly than, pus cells.

*Bacterial Flora in Sputum during Relapses.*—A return of purulent or yellowish sputum was regarded as a relapse even if amounts were small. Such relapses were apt to occur at intervals, particularly following coryza, during the winter months, in patients treated intermittently on two consecutive days a week. Altogether, 27 patients were treated over a total of 306 patient-months; during this time 204 relapses occurred, equivalent to an average of one relapse a patient every six weeks. Details of the bacteriological findings during these relapses are given in Table III.

TABLE III.—Relationship of Treatment Time (Total of Patient-Months) to Number of Relapses and Bacterial Flora Isolated

Months of Treatment	Total No. of Relapses	Relapses Due To		
		<i>H. influenzae</i>	Other Orgs.	No Apparent Bact. Cause
306	204	104 (51%)	19 (9.3%)	81 (39.7%)

Just over half of the relapses were due to *H. influenzae*. In about 10% other pathogenic organisms such as pneumococci, coliform bacilli, *Proteus vulgaris*, or, very rarely, a coarse species of *Neisseria pharyngis* could be regarded as the infecting organism. However, in nearly 40% of these relapses no bacterial cause could be recognized. In most of the relapses without apparent bacterial cause the sputum was rather more fluid and had a slightly yellow appearance which could not be mistakenly easily for that of the characteristically sticky greenish pus of bronchiectasis.

**Discussion**

The earlier observations of Allison *et al.* (1943), based as they were on single bacteriological examinations, gave a static impression of the bacterial flora in the bronchi of bronchiectatics. In the present investigation a smaller number of patients has been investigated over long periods, thus providing a dynamic picture of the changes occurring in the bacterial flora of the sputum in this disease, with and without antibacterial treatment.

Administration of antibacterial drugs has enabled us to extend our earlier observations and to confirm the findings in younger age groups. High doses of a suitable combination of antibacterial drugs bring about simultaneous disappearance of pus and *H. influenzae* in the sputum of bronchitics. Moreover, in the majority of relapses with marked purulence of the sputum, *H. influenzae* reappears in large numbers.

We cannot help being impressed by the obvious phagocytosis of *H. influenzae* in bronchiectatic sputa as well as by the close association of this organism with the presence, disappearance, or reappearance of pus before, during, and after suitable antibacterial treatment.

Our findings are in agreement with those of Mulder *et al.* (1952) in all essential points. One cannot do better than quote these authors' dictum: "In our opinion, the above observations lead with certainty to the conclusion that the group of non-encapsulated Hemophilus (*influenzae*) is a pathogen and not a saprophyte of the mucous membrane of the bronchial tree . . . we are entitled to say that the etiology of bronchitis and that of bronchiectasis cannot possibly be understood if the part played by Hemophilus infection is overlooked."

Although our therapeutic considerations and the clinical aspects will be dealt with more fully elsewhere, we should like to record here that in our opinion the very high doses of antibacterial drugs necessary for the suppression of *H. influenzae*, which are not always easily tolerated, make imperative the continued search for drugs effective against *H. influenzae* in low concentrations. These will be particularly valuable if they can be concentrated easily in the lungs and bronchi when given by mouth.

The cause of nearly 40% of abacterial relapses remains to be determined.

**Summary**

Thirty-two children with purulent bronchiectasis were investigated bacteriologically. Non-capsulated *H. influenzae* was present in bronchoscopic aspirations or sputum of all and in antrum washings of 19 (59.4%).

Twenty-seven of these children were treated with suitable combinations of antibiotic drugs and sulphonamides in high doses. As a result *H. influenzae* and pus disappeared from the sputum within two weeks of continuous treatment in 23 (85.1%) of the children. One

case required 3-4 weeks, another 5-6, and a third 12 weeks' continuous treatment before the sputum could be rendered mucoid and *H. influenzae*-negative. One case failed to respond.

A total of 204 relapses were observed during 306 patient-months of intermittent antibacterial treatment. The majority were associated with reappearance of *H. influenzae*.

In our view, the conclusion is inescapable that non-capsulated *H. influenzae* is responsible for keeping the chronic inflammatory process smouldering in bronchiectatic individuals.

We are indebted to the Antibiotics Clinical Trials (Non-Tuberculous Conditions) Committee of the Medical Research Council, Messrs. Pfizer Ltd., and Lederle Ltd. for supplies of oxytetracycline, chlortetracycline, and tetracycline; to Mr. A. J. Gunning for carrying out the antrum washings; and to Miss D. Peat for physiotherapeutic manipulations. One of us (K. Z.) has been in receipt of a grant from the M.R.C. in aid of expenses.

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## SERUM PROTEINS IN PULMONARY TUBERCULOSIS

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Assessment of disease activity in pulmonary tuberculosis can be very difficult and no test available is an adequate single guide. Many varied clinical factors have to be considered in order to gain a reasonably accurate assessment.

It has been known for some time, and is here confirmed, that there are alterations of serum proteins in the course of tuberculous infection. Such changes are not in themselves diagnostic of, or specific to, any particular disease, but probably reflect the clinical state of the patient.

The present study was undertaken to determine the nature of these serum protein changes in patients with pulmonary tuberculosis, with particular reference to the

possibility of a direct relationship to the extent, duration, and severity of the lesion, and the effect of the remedial chemotherapy now available. In each case a complete quantitative analysis of all protein fractions has been made.

### Clinical Method

A total of 327 adult patients with pulmonary tuberculosis were examined. They were selected without reference to their clinical condition, the first six attending each routine chest clinic session being invited to co-operate. To provide control values, 28 of the patients' adult "contacts," who were radiologically free from disease but living in a like domestic environment, were also examined. A clinical assessment was made of each patient at the time that the blood was withdrawn, and the erythrocyte sedimentation rate (Westergren) was also estimated. The assessment was made in relation to five main factors, recorded separately and uniformly for each patient, so that correlation with the biochemical findings was facilitated. These factors were:

1. *Extent of Disease.*—The assessment was entirely radiological, based upon the zone system (Ministry of Health, 1947). Presence of cavitation was also noted.

2. *Duration of Disease.*—This cannot be accurately assessed in the majority of cases, and so, for simplicity, three broad groups were chosen: (i) recent infection (under six months); (ii) intermediate (six months to three years); and (iii) disease present for over three years.

3. *Activity of Disease.*—In all cases bacteriological, radiological, and constitutional evidence of activity was recorded independently to provide additional categories for subsequent analysis. A separate note was made of the presence of severe constitutional disturbance as indicated by pyrexia. Tubercle bacilli demonstrated in sputum or laryngeal swabbing, changing radiological shadows, or the presence of an obvious cavity were necessary, either alone or in combination, before a patient was classed as "active." Separate categories were also provided for "quiescent" and "arrested" cases (Ministry of Health, 1947). A final category—"intermediate"—was used. In this group were placed those patients who could not yet be classified as "quiescent" but in whom there were no signs of activity.

4. *Treatment.*—Special reference was made here to chemotherapy (always the daily administration of two of the three commonly used drugs—streptomycin, isoniazid, and P.A.S.). Four separate categories were used for patients who (i) had not yet received chemotherapy; (ii) were on drugs at the time; (iii) had completed a course within the previous six months; or (iv) had received such treatment more than six months previously. In addition, patients were categorized according to whether they had received minor collapse therapy, resection, or thoracoplasty.

5. *Special Points.*—The majority of patients could be adequately assessed by appropriate placing in the categories outlined above, but a few presented special points of interest, which were noted. In particular, special categories were created for coexisting amyloidosis, pregnancy, or diabetes mellitus.

### Biochemical Method

Venous blood specimens were obtained with a minimum of stasis and allowed to clot. The serum was withdrawn on the same day, samples showing any trace of haemolysis being rejected. Most of the samples were analysed on the same day. A few were frozen at  $-4^{\circ}$  C. to be analysed on the next day.

The total protein concentration was measured by the specific-gravity method (Phillips *et al.*, 1944). Electrophoresis was carried out on Whatman No. 1 filter paper at pH 8.6 in an M/10 barbitone buffer. By means of a glass slide 0.01 ml. of serum was applied to the paper strip. A potential of 105 volts was applied to the paper for 15-16 hours. The strips were then dried in an oven at  $120^{\circ}$  C. for 30 minutes, and stained for 10 minutes in 0.2% lissamine

\*Participated in the work during the tenure of a scholarship from the Egyptian Government.